Contents

Contents of Monographs

Introduction

In March 2003, the Ministry of Health, Labour and Welfare established a panel to draft the eighth edition of the official compilation of food additives. The panel worked to enhance compositional specifications for existing food additives (additives derived from natural sources, so-called "natural additives") based on the 1995 revision of the Food Sanitation Law, to introduce new and improved test methods commensurate with the progress in science and technology, and to achieve international harmonization of standards. The panel also worked to reflect the advances in manufacturing and quality control technologies made since the publication of the seventh edition.

In November 2005, the Minister of Health, Labour and Welfare referred the review of the drafted compilation to the Pharmaceutical Affairs and Food Sanitation Council. In March 2007, the Council submitted the final report of the review to the Minister.

The eighth edition carries General Notices (43 items); General Tests (43 items); Reagents, Solutions, and Other Reference Materials (11 items); Monographs (507 substances); Standards for Manufacturing; Standards for Use; and Standards for Labeling.

The following sections outline the revision.

1. General Notices
(1) Units "mol/l" for mole per liter and "mmol/l" for millimole per liter have been replaced by "mol/L" and "mmol/L," respectively.
(2) The definitions of "cold place" and "cold water" have been changed.

2. General Tests
(1) For Gas Chromatography, a description on the use of a gas injection device, a flow regulator, and a headspace sample injection system has been added to the description of the apparatus. Also, the standard addition method has been added to the procedures.
(2) The names of tests have been changed: "Boiling Point and Amount of Distillate" to "Boiling Point and Distillation Range Tests," and "Spectrophotometry" to "Ultraviolet-Visible Spectrophotometry."
(3) The gas chromatographic assay has been added to the Flavor Substances Tests.
(4) Preparation methods for anolytes and catholytes for water determination have been added to the test Water Determination.
(5) For Infrared Spectrophotometry, the apparatus adjustment method and measurement method have been changed.
(6) For Thin-layer Chromatography, the use of commercial thin-layer plates has been made possible.
(7) Media for xerophilous fungi have been added to the Microbial Limit Tests. In addition, some modifications have been done to the procedures, such as in the serial dilution method.
(8) The iodine value test has been added to the Fats and Related Substances Tests.

3. Reagents, Solutions, and Other References Materials
(1) JIS (Japanese Industrial Standards) numbers have been added to reagents that meet the JIS standards.
(2) Some additions and modifications have been made to the description of reagents and test solutions, volumetric solutions, standard solutions, reference standards, and reference infrared spectra.
(3) Some requirements for thermometers with an immersion line have been changed.

4. Monographs
(1) Specifications for the following 63 existing food additives and 1 food ingredient have been newly established.*

N-Acetylglucosamine, Acid Clay, Activated Acid Clay, 5′-Adenylic Acid, L-Arabinose, Bacillus natto Gum, Bentonite, Betaine, Bone Charcoal, Calcinated Eggshell Calcium, Calcinated Shell Calcium, Chinese Bayberry Extract, Crude magnesium Chloride, Curdlan, Cyanocobalamin, α-Cyclodextrin, γ-Cyclodextrin, 5′-Cytidylic Acid, Dextran, Enju Extract, Enzymatically Decomposed Lecithin, Enzymatically Decomposed Rutin, Enzymatically Modified Hesperidin, Enzymatically Modified Isoquercitrin, Fukuronori Extract, Gardenia Blue, Gardenia Red, Gardenia Yellow, α-Glucosyltransferase Treated Stevia, Haematococcus Algae Color, Heme Iron, *myo*-Inositol, Lac Color, Lanolin, Licorice Extract, Luohanguo Extract, Lysozyme, Macrophomopsis Gum, Menaquinone, Microcrystalline Wax, Microfibrillated Cellulose, Milt Protein, Naringin, Paraffin Wax, ε-Polylysine, Psyllium Seed Gum, Pullulan, Purple Corn Color, Purple Sweet Potato Color, Red Cabbage Color, Rhamsan Gum, D-Ribose, Spirulina Color, Extract, Tamarind Seed Gum, Tara Gum, Taurine (Extract), Thujaplicin (extract), *d*-γ-Tocopherol, *d*-δ-Tocopherol, Tocotrienol, Tomato Color, Yeast Cell Wall, Yucca Foam Extract

* Red cabbage color is a food ingredient that is used as a food additive.

(2) Specifications and standards have been removed for substances the designation of which has been withdrawn since the publication of the seventh English edition, *Japan's Specification and Standards for Food Additives, Seventh Edition.* Also, specifications and standards have been added for those that have been designated during the same period of time.

Added (28 substances newly designated): Acetaldehyde, Ammonium Alginate, Amyl Alcohol, Biotin, Butanol, Calcium Alginate, Calcium Ferrocyanide, Calcium Stearate, 2-Ethyl-3,(5 or 6)-dimethylpyrazine, 2-Ethyl-3-methylpyrazine, Hydroxypropyl Cellulose, Hydroxypropyl Methylcellulose, Hypochlorous Acid Water, Isoamyl Alcohol, Isobutanol, Isopropanol, L-Ascorbic Acid 2-Glucoside, Magnesium Stearate, 5-Methylquinoxaline, Natamycin, Nitrous Oxide, Potassium Alginate, Potassium Ferrocyanide, Propanol, Sodium Ferrocyanide, 2,3,4,5-Tetramethylpyrazine, and Trimagnesium Phosphate, 2,3,5-Trimethylpyrazine.

Withdrawn (3 substances): Choline Phosphate, Ferrous Pyrophosphate, and Methyl Acetyl Ricinoleate.

(3) A test for hexachlorobenzene has been added to the purity tests for Food Colors No. 104 and No. 105.

(4) Some modifications have been made to the identification tests and purity tests to eliminate the use of harmful reagents, achieve international harmonization, and harmonize with the quality of commercially distributed food products.

(5) Scientific names have been added to existing food additives as far as possible, to define the source plants and microorganisms.

(6) For compounds having different degrees of hydration, CAS numbers have been added to individual hydrates. For hydrate salts without CAS numbers, the CAS number for the anhydrous salt is added as an identification aid.

(7) Infrared spectrophotometry has been introduced to the identification tests for some substances.

(8) The chemical names and chemical structural formulas of the principal components of the food additives in the Monographs have been reviewed, and some of them have been modified.

(9) This publication contains specifications and standards for the food additives that were published in the Official Gazette on or before March 31, 2007.

Explanatory Notes

General Notices This section gives general rules for performing tests in accordance with specifications and standards.

General Tests This section describes practical test methods common to certain additives.

Reagents, Solutions, and Other Reference Materials This section contains specifications concerning reagents, test solutions, and standards solutions in alphabetical order.

Monographs This section consists of "Definition," "Content," "Description," "Identification," "Purity," "Water Content," "Loss on Drying," "Residue on Ignition," "Assay," and "Storage Standards."

Standards for Manufacturing This section contains general and specific standards concerning the manufacture of food additives.

Standards for Use This section gives target foods, maximum use levels in each target food, and other restrictions for each of the additives with standards for use.

Standards for Labeling This section specifies items to be declared for additives and relevant requirements.

Historical Background

In 1878 Japan initiated the nationwide control of food safety. This was based on the Notice concerning the Control of the Use of Aniline Dyes and Mineral Pigments in Food (No. 35, April 18, 1878), which was issued from the Minister of Home Affairs to the local governments.

In 1900 Japan first enacted the general law concerning food safety (Law No. 15, the Law for the Control of Foods and Things Relating to Foods). This law was established to provide comprehensive provisions for the control of food and beverages. Based on this law, the Minister of Home Affairs established successive enforcement regulations. These regulations were specialized for individual product categories, like milk, and non-alcoholic drinks, and coloring gents. These regulations were promulgated under the approval of the Central Food Sanitation Council. The regulations related to food additives are given below.

Enforcement Regulations for the Control of Poisonous Coloring Matters
(Ministry of Home Affairs Ordinance No. 17, April 17, 1900)

Enforcement Regulations for the Control of Artificial Sweetening Agents
(Ministry of Home Affairs Ordinance No. 31, October 16, 1901)

Enforcement Regulations for the Control of Food Preservatives
(Ministry of Home Affairs Ordinance No. 10, August 28, 1903)

Enforcement Regulations for the Control of Methyl Alcohol
(Ministry of Home Affairs Ordinance No. 8, May 28, 1912)

With some revisions, the Japanese law and regulations for food had been mostly formulated by the early 1930s. However, they had not yet fully covered all matters related to food.

In 1938 the Ministry of Health and Welfare was founded, and the food safety responsibilities have come under its jurisdiction.

After World War II, Japan enacted the comprehensive food law, entitled the Food Sanitation Law (Law No. 233, December 24, 1947), in the wake of the enactment of the new Japanese constitution. The next year, the Minister of Health and Welfare established the Food Sanitation Law Enforcement Regulations (Ministry of Health and Welfare Ordinance No. 23, July 13, 1948), and the Specifications and Standards for Foods, Food Additives, Equipment, Containers, and Packages (Ministry of Health and Welfare Notification No. 54, July 13, 1948), based on Articles 7 and 10 of the Food Sanitation Law.[1]

In June 1957, the Food Sanitation Law was partially revised and a new provision (Article 13) concerning an official compilation of food additives was added. That revision was triggered by the arsenic-poisoning outbreaks in 1955, involving contaminated infant formula, resulting in a number of infant deaths.

Also, Article 25 was revised to include matters about the preparation of the official compilation of food additives in the scope of the matters on which the Food Sanitation Council shall deliberate upon request from the Minister.

> **Article 13.** The Minister of Health and Welfare shall prepare the official compilation of food additives, which will contain the specifications and standards for the additives established pursuant to the provisions of Article 7 Paragraph 1, and the standards for the additives established pursuant to the provisions of Article 11 Paragraph 1.[2]
>
> **Article 25 Paragraph 1.** The Minister of Health and Welfare shall establish the Food Sanitation Council under the supervision of the Minister of Health and Welfare, to deliberate matters relating to the prevention of food poisoning, matters relating to the preparation of the official compilation of food additives, and other important matters relating to food sanitation, in response to requests for consultation from the Minister of Health and Welfare.[3]

The Food Sanitation Council established the Subcommittee on the Official Compilation of Food Additives, in response to a request for opinion from the Minister of Health and Welfare, to draft and deliberate on a compilation. In November 1959, the subcommittee submitted its final report to the Minister of Health and Welfare.

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[1] The Food Sanitation Law has been revised several times. Article 7 and Article 10 correspond to Article 11 and Article 18, respectively, of the current law.

[2] Article 11 and Article 13 correspond to Article 19 and Article 21, respectively, of the current law.

[3] Article 25 Paragraph 1 was repealed, and the Cabinet Order of the Pharmaceutical Affairs and Food Sanitation Council (Cabinet Order No. 286) was enacted on June 7, 2000.

Based on the report, the Minister published the first Japanese edition of the official compilation of food additives in March 1960. That edition contained standards and specifications for 198 additives. Since the publication of the first edition, the official compilations have been prepared fairly regularly. In 1999 the seventh edition was published.

GENERAL NOTICES

A. GENERAL NOTICES

1. The title of this book is *Japan's Specifications and Standards for Food Additives, Eighth Edition,* which may be abbreviated as JSFA-VIII.

2. Unless otherwise specified, determination of the compliance of food additives shall be based on established provisions, specifications, and standards as directed under the headings "General Notices," "General Tests," and "Monographs." The physical form of each substance mentioned under *Description* in the Monographs is given merely as a reference, and not as a requirement for determination.

3. In this English translation, substances whose names are indicated with an initial capital letter and enclosed with quotation marks mean food additives that meet the requirements specified in the Monographs.

4. Any substance whose name is followed by its molecular formula in parentheses means a chemically pure substance. The atomic weights used in this publication comply with the Table of International Atomic Weights (2005). The molecular weights are expressed to two decimal places, rounded off to the nearest hundredth.

Units and Symbols

5. The following symbols are used as the main measurement units:

meter	m
centimeter	cm
millimeter	mm
micrometer	μm
nanometer	nm
square centimeter	cm^2
liter	L
milliliter	ml
microliter	μl
kilogram	kg
gram	g
milligram	mg
microgram	μg
newton	N
kilopascal	kPa
pascal	Pa
mol per liter	mol/L
millimol per liter	mmol/L
Per centimeter	cm^{-1}

6. The symbol "%" means percentage by weight, the symbol "% (w/v)" means the weight (g) of a substance in 100 ml of a solution, the symbol "% (v/w)" means the volume (ml) of a liquid in 100 g of a substance, and the symbol "% (vol)" means the volume (ml) of a substance in 100 ml of a solution or a gas. Unless otherwise specified, the substance content by weight (g) is expressed on an anhydrous basis.

7. The potency of a food additive shall be expressed using the unit specified in the individual monograph.

8. Temperatures are expressed in centigrade (Celsius) degrees with Arabic numerals followed by the symbol "ºC". Where a temperature is expressed at a point in the individual procedure, its acceptable error shall usually be ±5ºC, provided that this does not apply to the standards for Melting Point and Congealing Point.

9. "Standard temperature," "ordinary temperature," and "room temperature" mean 20ºC, 15–25ºC, and 1–30ºC, respectively. Also, temperatures indicated as "lukewarm" mean 30–40ºC. Unless otherwise specified, "cold place" means a place at a temperature of 1–15°C. "Cold water" means water at a temperature not higher than 10ºC, and "lukewarm water" means water at a temperature of 30–40ºC. "Warm water" means water at a temperature of 60–70ºC, and "hot water" means water at a temperature of about 100ºC. "To warm" usually means to raise the temperature to 60–70ºC.

10. The term "heated solvent" or "hot solvent" means a solvent heated almost to the boiling point of the solvent, and the term "warmed solvent" or "warm solvent" usually means a solvent heated to a temperature 60–70ºC.

Tests

11. An alternative testing method which is equal or superior in precision and accuracy to a method specified in JSFA-VIII may replace the specified method. However, if there are results that are doubtful, the final determination shall be made by the corresponding methods specified in JSFA-VIII.

12. Unless otherwise specified, the water to be used in the tests specified in JSFA-VIII shall be purified water.

13. To measure the number of drops of a liquid, a device which can supply 20 drops of purified water weighing 0.90–1.10 g at 20ºC shall be used.

14. Unless otherwise specified, the desiccant in a desiccator shall be silica gel.

15. The expression "after cooling" means when the temperature of a heated or warmed substance falls to room temperature unless any temperature is indicated. Unless otherwise specified, the expression "to heat on a water bath" means to heat the substance on a boiling water bath, and the expression "to heat in a water bath" means to heat the substance in a boiling water bath. A steam bath at about 100ºC may replace the boiling water bath. The expression "to heat a substance under a reflux condenser," unless otherwise specified, means to boil and reflux the solvent.

16. Unless otherwise specified, the term "reduced pressure" means a pressure not exceeding 2.0 kPa.

17. Unless otherwise specified, when the nature of a solution is indicated just as "acidic," "alkaline," or "neutral,"

the solution shall be tested using pH test paper. To indicate the nature of a solution more precisely, pH values shall be used. The terms "slightly acidic," "weakly acidic," "strongly acidic," "slightly alkaline," "weakly alkaline," and "strongly alkaline" indicate approximate degrees of acidity or alkali. The ranges of their pH values are given below.

Descriptive term	Range of pH
Slightly Acidic	About 5–6.5
Weakly Acidic	About 3–5
Strongly Acidic	About 3 or less
Slightly Alkaline	About 7.5–9
Weakly Alkaline	About 9–11
Strongly Alkaline	About 11 or higher

18. All solutions indicated with a solute name followed by the word "solution" are aqueous, unless a specific solvent name is given.

19. Where the name of a liquid reagent is expressed only with its concentration (for example, 1 mol/l hydrochloric acid, diluted sulfuric acid (1 in 10), or 50% (vol) ethanol), unless otherwise specified, it means that the reagent is diluted with water.

20. An expression such as "(1 in 5)" or "(1 in 100)" for the concentration of a solution means that 1 g of a solid substance or 1 ml of a liquid substance is dissolved in a solvent to make 5 ml or 100 ml, respectively. An expression such as "10:1" or "5:3:1" for a liquid mixture means a mixture of two different liquids in the ratio of 10 to 1 by volume, or a mixture of three different liquids in the ratio of 5 to 3 to 1 by volume, respectively.

21. Where judgment for conformity is made by comparing a value obtained by a test (experimental value) with a specified value (standard value), comparison shall be made between the standard value and the experimental value which is obtained to one more digit than required and rounded off to the nearest indicated digit. An expression "a–b" or "a to b" for a standard value means the standard value is not less than "a" and not more than "b."

22. When the quantity of the sample to be weighed or measured for assays or other tests is indicated with the word "about," the quantity actually weighed or measured may deviate within the range of ±10% of the indicated quantity. When the quantity of the sample to be weighed or measured is indicated without the word "about," the quantity actually weighed or measured shall be within the range of values where each value rounded off to the nearest indicated digit can make the indicated value.

23. The expression "to weigh accurately" means to weigh a substance down to one of the three accuracy ranges: the 0.1-mg range using a chemical balance, the 0.01-mg range using a semimicro chemical balance, or the 0.001-mg range using a micro chemical balance. The balance to be used shall be chosen from among these three types of chemical balances, taking into account the number of decimal digits of the standard value.

24. The expression "to weigh exactly" means to weigh the specified quantity to the given number of digits. For example, "to weigh 0.050 g, 0.10 g, 2.000 g, or 5.0 g of a substance" means to weigh it down within the range of 0.0495 –0.0504 g, 0.095–0.104 g, 1.9995–2.0004 g, or 4.95–5.04, respectively.

25. Unless otherwise specified, the expression "to measure exactly," means to measure the specified quantity using a whole pipet, buret, or other measuring device equal or superior in precision and accuracy to the aforementioned volumetric devices. Where the expression "to make exactly 100 ml" is given, unless otherwise specified, a volumetric flask shall be used.

26. Unless otherwise specified, all tests shall be performed at ordinary temperature, and for tests requiring observation, the observation shall be performed within 30 seconds after the specified procedure. However, tests which are affected by temperature shall be performed at standard temperature. A procedure described as "immediately" normally means that the procedure is performed within 30 seconds after the previous procedure is finished.

27. Unless otherwise specified, each test directed in the Monographs or other sections in JSFA-VIII shall be performed by the corresponding method specified in the General Tests, based on the directions given in the individual monograph or other corresponding section.

28. A substance described as "white" means that it is white or practically white, and a substance described as "colorless" means that it is colorless or practically colorless. When the tone of a color is tested, unless otherwise specified, in the case of a solid sample, 1 to 3 g of the sample, previously cut or ground unless it is a powder, shall be placed on a watch glass and observed against a white background; in the case of a liquid sample, the sample shall be placed into a colorless test tube with about 1.5-mm internal diameter to 3 mm in depth and observed from above and from the side against a white background. When the fluorescence of a liquid sample is observed, a black background shall be used.

29. A substance described as "odorless" means that it is odorless or practically odorless. Tests for odor shall, unless otherwise specified, be performed, in the case of a solid sample, by placing about 1g of the sample on an evaporating dish, or in the case of a liquid sample, by placing about 1 ml of the sample in a beaker.

30. The terms describing solubilities are given below. The solubility of a substance, unless specified otherwise, means the degree to which the substance dissolves within 30 minutes when the test is performed by placing the powdered sample into the specified solvent and shaking vigorously for 30 seconds at a time at 5-minute intervals at 20±5ºC.

Descriptive term	Volume (ml) of solvent required to dissolve 1 g or 1 ml of solute
Very soluble	less than 1
Freely soluble	from 1 to 10
Soluble	from 10 to 30
Sparingly soluble	from 30 to 100
Slightly soluble	from 100 to 1000

Very slightly soluble	from 1,000 to 10,000
Practically insoluble or insoluble	10,000 ml or more

31. Unless otherwise specified, filtration shall be done through filter paper.

32. Identification tests are useful to identify food additives, and these tests are performed for reaction of ions, reaction of functional groups, physical constants, and other related items.

33. Unless otherwise specified, identification tests shall be performed by placing 2 to 5 ml of the specified solution into a test tube having an internal diameter of 8.0 to 15 mm.

34. Under the heading "Identification," an expression "responds to the test for Carbonate" or "responds to the test for Sodium Salt" means that the specified reaction occurs when the test is performed for Carbonate or Sodium Salt as directed under the Qualitative Tests in the General Tests.

35. The aim of purity tests is to detect contaminants in food additives, and these tests usually specify the types of possible contaminants and their quantitative limits.

36. Unless otherwise specified, the clarity and color of a solution shall be examined for a solution obtained by placing the sample into the specified solvent and shaking the mixture for 30 seconds to 5 minutes.

37. Where the term "clear," "almost clear," "very slightly turbid," "slightly turbid," or "turbid" is used under the heading "Clarity and color of solution" in the purity tests, determination shall be based on the Turbidity Tests in the General Tests.

38. The requirement "no turbidity appears" means that the clarity of the solution does not change.

39. "A Nessler tube" shall be a colorless-glass and flat-bottom test tube with a ground-glass stopper, with the dimensions of 20 mm in internal diameter, 24 mm in external diameter, and 20 cm in height from the bottom of the tube to the bottom of the stopper. It shall be marked with 5-ml graduations up to 50 ml, and the difference of the height at the 50 ml graduation mark between tubes shall not exceed 2 mm.

40. Unless otherwise specified, the term "constant weight" in a drying or ignition procedure means that when an additional one hour of drying or ignition is performed, the difference in the two consecutive weighings (before and after the additional one hour) is not more than 0.1% of the preceding weighing of the dried substance or the residue on ignition. Where the difference in weighings is not more than 0.5 mg by a chemical balance or not more than 0.01 mg by a micro chemical balance, the difference is negligible and the weight obtained is deemed to be a constant weight.

41. An assay is a method to determine the contents of components of a food additive or their potency. The limit of the content of a component or the potency declared in each monograph indicates the value obtained by the assay. Unless the upper limit is given, the value shall not be more than 100.5%.

42. Where the instruction "to dry the sample" or "to ignite the sample" is given alone, the conditions for the drying or ignition shall be the same as those given in Loss on Drying or Residue on Ignition under the individual monograph.

Containers

43. Hermetic containers are those capable of protecting the contents from intrusion by extraneous air or other gases during handling and storage under ordinary conditions.

44. Light-resistant containers are those capable of preventing the transmission of light or those wrapped so that the transmission of light is prevented.

GENERAL TESTS

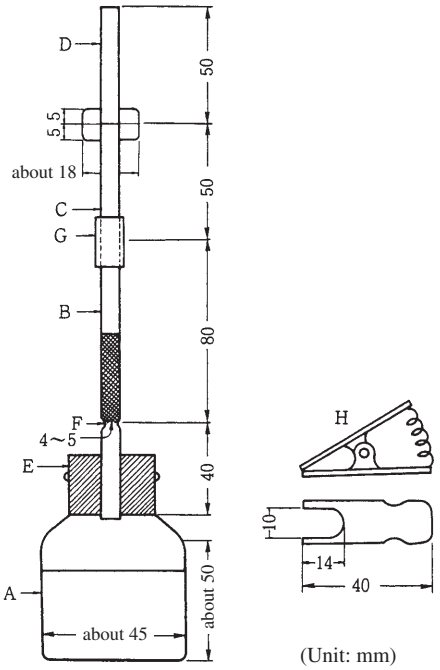
B. GENERAL TESTS

Arsenic Limit Test

The Arsenic Limit Test is designed to determine the allowable limit of arsenic in a sample. The limit is expressed in terms of arsenic trioxide (As_2O_3).

In the Monographs, such a specification as "not more than 4.0 μg/g as As_2O_3 (0.25 g, Method 1, Apparatus A)" for this test indicates that when determined as directed in the Method using Apparatus A, using the test solution prepared by weighing 0.25 g of the test substance and proceeding as directed in Method 1, the arsenic content of the substance is not more than 4.0 μg/g as As_2O_3.

Apparatus A Use the apparatus illustrated in Fig. 1.



A: Generator bottle (about 60 ml capacity, with a 40 ml mark)
B: Glass tube (about 6.5 mm internal diameter)
C and D: Glass tubes (with ground-glass joints of 6.5 mm internal diameter and about 18 mm external diameter; both circles have the same center.)
E: Rubber stopper
F: The position at which glass tube B is pinched to support a plug of glass fiber
G: Rubber tube
H: Clip

Fig. 1

Stuff a plug of glass fiber into glass tube B up to about 30 mm in height from dent F, moisten the fiber uniformly with a mixture of an equal volume of lead acetate TS and water, and then remove the excess of the mixture from the glass fiber and the wall of the tube by gentle suction from the lower end of the tube. Just before use, place a strip of mercuric bromide test paper between the joints of glass tubes C and D, and fix both tubes with clip H.

Apparatus B Use the apparatus illustrated in Fig. 2.

Stuff a plug of glass fiber F into exit tube B up to about 30 mm in height, moisten the glass fiber uniformly with a mixture of equal volumes of lead acetate TS and water, and remove the excess of the mixture by gentle suction from the lower end. Insert the tube vertically into the center of rubber stopper H, and attach stopper H with the tube to generator bottle A so that small perforation E in the lower end of B extends slightly below stopper H. To the upper end of tube B, attach rubber stopper J fitted with tube C vertically. Level the lower end of C with that of rubber stopper J in the exit tube.

Apparatus C Use the apparatus illustrated in Fig. 3.

Preparation of Test Solution Unless otherwise specified, proceed according to an appropriate method selected from among the following methods.

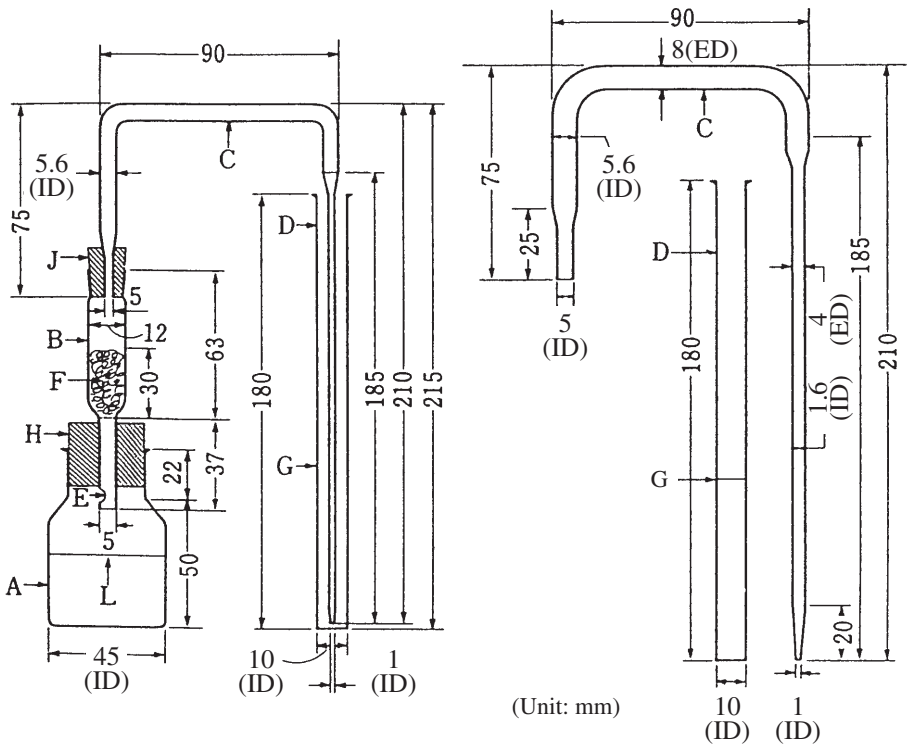
Method 1 Weigh the specified amount of the sample, add 5 ml of water, dissolve by heating if necessary, and use the resulting solution as the test solution.

Method 2 Weigh the specified amount of the sample, add 5 ml of water, and add 1 ml of sulfuric acid unless the samples are inorganic acids. Add 10 ml of sulfurous acid, transfer into a small beaker, and evaporate the mixture on a water bath until it is free from sulfurous acid and is reduced to about 2 ml. Dilute with water to make 5 ml, and use as the test solution.

Method 3 Weigh the specified amount of the sample, and place it in a crucible made of platinum, quartz, or porcelain. Add 10 ml of a solution of magnesium nitrate in ethanol (1 in 50), ignite the ethanol, and heat gradually to incinerate at 450–550°C. If carbonized matter still remains, moisten with a small quantity of a solution of magnesium nitrate in ethanol (1 in 50), ignite again, and heat again to incinerate at 450–550°C. After cooling, add 3 ml of hydrochloric acid, heat on a water bath to dissolve the residue, and use the resulting solution as the test solution.

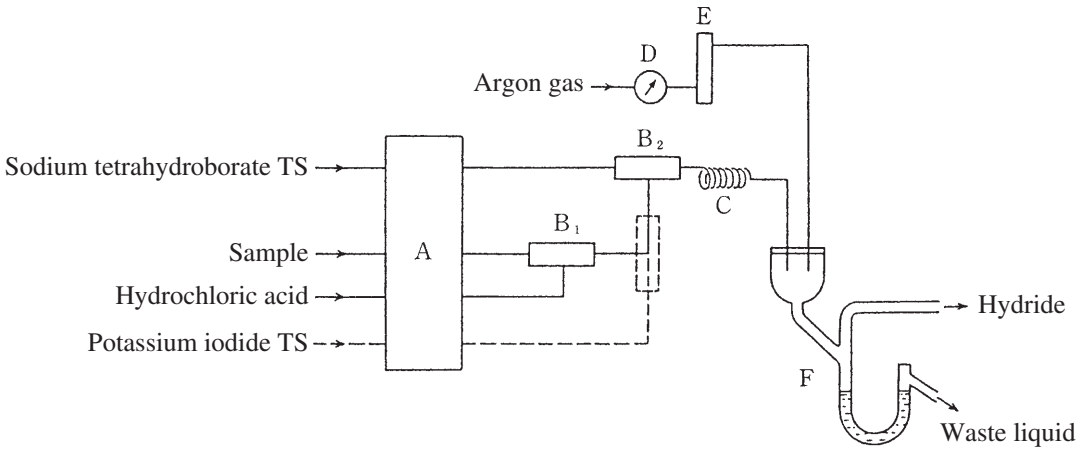
Method 4 Weigh the specified amount of the sample, and place it in a crucible made of platinum, quartz, or porcelain. Add 10 ml of a solution of magnesium nitrate in ethanol (1 in 10), ignite the ethanol, and heat gradually to incinerate at 450–550°C. If carbonized matter still remains, moisten with a small quantity of a solution of magnesium nitrate in ethanol (1 in 50), and ignite again to incinerate at 450–550°C. After cooling, add 3 ml of hydrochloric acid, heat on a water bath to dissolve the residue, and use the resulting solution as the test solution.

Procedure Unless otherwise specified, proceed according to an appropriate method selected from among the following



A: Generator bottle (about 70 ml capacity up to the shoulder)
B: Exit tube.
C: Glass tube (5.6 mm internal diameter, with an end stretched into a capillary of 1 mm internal diameter)
D: Absorber tube (10 mm internal diameter)
E: Small perforation
F: Glass fiber (about 0.2 g)
G: A 5 ml mark
H and J: Rubber stoppers
L: A 40 ml mark

Fig. 2



A: Pump
B_1, B_2: Mixing joints
C: Reaction tube
D: Pressure gauge
E: Flow meter
F: Gas-liquid separator

Fig. 3

methods.

Method using Apparatus A Transfer the test solution into the generator bottle, add 1 drop of bromophenol blue TS, neutralize with ammonia solution, ammonia TS, or diluted hydrochloric acid (1 in 4), add 5 ml of diluted hydrochloric acid (1 in 2) and 5 ml of potassium iodide TS, and allow to stand for 2 to 3 minutes. Add 5 ml of acidic stannous chloride TS, and allow to stand for 10 minutes. Add water to make 40 ml, add 2 g of arsenic-free zinc, and then immediately plug the generator bottle with rubber stopper E, connected to glass tubes B, C, and D. Immerse the generator bottle up to the shoulder in water of 25°C, and allow to stand for 1 hour. Immediately examine the color of the mercuric bromide test paper. The color is not deeper than the standard color.

The standard color is prepared in parallel with the test directed above. Unless otherwise specified, measure 1.0 ml of Arsenic Standard Solution, transfer into another generator bottle, and add 5 ml of diluted hydrochloric acid (1 in 2) and 5 ml of potassium iodide TS. Proceed in the same manner as for the test solution, and consider the color produced on the mercuric bromide test paper as the standard color.

Method using Apparatus B Transfer the test solution into the generator bottle, proceed as directed for the Method using Apparatus A, add 5 ml of acidic stannous chloride TS, and allow to stand for 10 minutes. Next, add water to make 40 ml, add 2 g of arsenic-free zinc, and immediately connect the rubber stopper H, fitted with B and C, to the generator bottle. Insert the tip of C to the bottom of absorber tube D containing 5 ml of the absorbing solution for arsine, immerse the generator bottle up to the shoulder in water maintained at 25°C, and allow to stand for 1 hour. Disconnect the absorber tube, add pyridine to make 5 ml, if necessary, and examine the color of the absorbing solution. The color produced is not deeper than the standard color.

The standard color is prepared in parallel with the test directed above. Measure 2.0 ml of Arsenic Standard Solution, transfer into another generator bottle, add 5 ml of diluted hydrochloric acid (1 in 2) and 5 ml of potassium iodide TS, and allow to stand for 2 to 3 minutes. Add 5 ml of acidic stannous chloride TS, and allow to stand at room temperature for 10 minutes. Proceed in the same manner as for the test solution, and consider the color of the absorbing solution as the standard color.

Method using Apparatus C To 4 ml of the test solution, add 1 ml of hydrochloric acid and 1 ml of potassium iodide solution (1 in 10), heat on a water bath at 70°C for 4 minutes, and add water to make 20 ml. Passing argon gas through the apparatus, introduce the test sample, an appropriate concentration of hydrochloric acid (1–6 mol/L), and sodium tetrahydroborate TS continuously into the apparatus at appropriate flow rates of 1–10 ml/min with pump A, and mix successively in the apparatus to form arsenic hydride. If the apparatus uses a system in which potassium iodide solution (1 in 10) is introduced continuously into the apparatus, introduce the test solution, if necessary, diluted with water, an appropriate concentration of hydrochloric acid (1–6 mol/L), potassium iodide solution (1 in 10), and sodium tetrahydroborate TS into the apparatus in the same manner as described above to mix in sequence, and generate arsine. The arsine obtained is separated from the waste liquid by gas-liquid separator F, and the gas containing arsenic hydride is introduced into an atomic absorption spectrophotometer equipped with a heating absorption cell. Measure the atomic absorbance of the test solution at 193.7 nm. The absorbance of the test solution does not exceed that of the following control solution.

The control solution is prepared in parallel with the test. Proceed in the same manner as for the test solution, using the specified Arsenic Standard Solution.

Notes on Procedure

(1) The apparatus, reagents, and test solutions used in the test should contain little or no arsenic. Perform a blank test if necessary.

(2) If Apparatus A is used, connect tightly the ground joints holding a mercuric bromide test paper strip so that the gas produced does not leak out.

(3) If Apparatus A is used, the color comparison should be performed promptly because the color of the mercuric bromide test paper fades when exposed to light, heat, or moisture. The colored test papers can be preserved for a while in a desiccator, while protected from light.

(4) If Apparatus C is used, the flow rates of the sample solution, diluted hydrochloric acid solution, sodium tetrahydroborate TS, and potassium iodide solution, and the concentrations of diluted hydrochloric acid and potassium iodide solution are dependent on the apparatus used. Furthermore, sodium tetrahydroborate solution with a concentration different from sodium tetrahydroborate TS may be used.

Ash and Acid-Insoluble Ash Limit Tests

1. Ash

The Ash Limit Test is designed to measure the amount of residual substances when a sample is ignited under the conditions specified in the individual monograph.

Procedure Ignite a platinum, quartz, or porcelain crucible at 500–550°C for 1 hour, allow to cool in a desiccator, and weigh accurately. Unless otherwise specified, place 2 to 4 g of the sample in the crucible and accurately weigh the crucible with the sample. Take off the lid of the crucible completely or halfway if necessary, heat the crucible gently first, then raise the temperature gradually, ignite at 500–550°C for not less than 4 hours to incinerate until it is free from any charred matter, cool in a desiccator, and accurately weigh the crucible with the residue. Repeat the procedure (incinerate, cool in the desiccator, and weigh accurately) until the residue reaches constant weight.

If charred matter still remains and a constant weight cannot be obtained with the above procedure, add hot water to the matter, filter through a filter paper for quantitative analysis, and ignite the insoluble residue on the filter paper together with the filter paper at 500–550°C until it is free from any charred matter. Add the filtrate to the residue, evaporate to dryness, ignite at 500–550°C, cool in a desiccator, and weigh accurately. If charred matter still remains, add a small amount of ethanol to moisten, break up the ash with a glass

rod, wash the glass rod with a small amount of ethanol, and carefully evaporate the ethanol. Proceed as directed previously, and weigh accurately.

2. Acid-insoluble Ash
The Acid-Insoluble Ash Limit Test is designed to measure the amount of ash insoluble in diluted hydrochloric acid (1 in 4).

Procedure Add carefully 25 ml of diluted hydrochloric acid (1 in 4) to the ash (obtained by the Ash Limit Test), boil gently for 5 minutes, collect the insoluble matter by filtration using a filter paper for quantitative analysis, wash thoroughly with hot water, and dry the residue together with the filter paper. Ignite the residue with the filter paper for 3 hours in a platinum, quartz, or porcelain crucible, previously treated as directed in the Ash Limit Test and accurately weighed. Cool it in a desiccator, and weigh accurately. If the value obtained is larger than the specified value, ignite to constant weight.

Atomic Absorption Spectrophotometry

Atomic Absorption Spectrophotometry is designed to determine the amount (concentration) of elements in a sample by virtue of the phenomenon that when a light beam passes through the atomic vapor layer of the element, the ground-state atoms absorb the light of the specific wavelength, characteristic to each element.

Apparatus The apparatus usually consists of a light source, a sample-atomizer, a spectroscopic system, a photometric system, and a recording system. Some are equipped with a background correction system. As the light source, a hollow cathode lamp or a discharge lamp is normally used.

There are three types of sample-atomizers: the flame type, electrothermal type, and the cold-vapor type. Cold-vapor atomizers are further categorized into two subtypes: one using the reduction vaporization method and the other using the thermal vaporization method. A flame atomizer is composed of a burner and a gas-flow regulator, an electrothermal atomizer is composed of an electric furnace and a power source, and a cold-vapor atomizer is composed of a mercury generator, such as a reduction-vaporizer and a thermal vaporizer, and an absorption cell.

For the spectroscopic system, a diffraction grating or interference filter is used. The photometric system mainly consists of a detector and a signal processing system. The recording system is composed of a display and a recording device. A background correction system is employed for the correction of background absorption on the measuring system. Several methods are utilized for background correction, including the Zeeman method, the self-reversal method, and other methods using a continuous spectrum source or a nearby nonresonance line.

Procedure Unless otherwise specified, proceed by either of the following methods.

(1) Flame Atomic Absorption Spectrophotometry Fit the light source lamp specified in the individual monograph to the lamp housing, and switch on the photometric system. Light the source lamp, set the spectrometer to the analytical wavelength specified in the individual monograph, and select an appropriate current value and slit-width. Ignite a mixture of the supporting gas and combustible gas specified, adjust the gas flow rate and pressure, and make the zero adjustment through nebulizing the solvent into the flame. Nebulize the test solution, prepared by the method specified, into the flame, and measure the absorbance. Follow the same procedure for the standard solutions and control solution prepared as specified.

(2) Electrothermal Atomic Absorption Spectrophotometry Fit the light source specified in the individual monograph to the lamp housing, and switch on the photometric system. Light the source lamp, set the spectrometer to the analytical wavelength specified in the individual monograph, and select an appropriate electric current and slit-width. Inject a suitable amount of the test solution, prepared by the method specified, into the furnace, and run an appropriate stream of the inert gas through the furnace. By heating at an appropriate temperature for an appropriate time in an appropriate mode, dry and incinerate the specimen, and atomize the element included in the specimen. Next, measure the absorbance. Follow the same procedure for the standard solutions and control solution prepared as specified.

(3) Cold-vapor Atomic Absorption Spectrophotometry Fit the light source lamp specified in the individual monograph to the lamp housing, and switch on the photometric system. Light the source lamp, set the spectrometer to the analytical wavelength specified in the individual monograph, and select an appropriate current value and a slit-width. If the reduction vaporizing method is used, transfer the test solution into a closed vessel, and reduce it to the element by addition of a proper reducing agent, and then vaporize. If the heat vaporizing method is used, vaporize the sample by heating. Next, measure the absorbance of the atomic vapor generated. Follow the same procedure for the standard solutions and control solution prepared as specified.

The determination can usually be performed using an appropriate method from among the methods given below. In the determination, the interference and background should be considered.

(1) Calibration Curve Method Prepare at least three standard solutions containing different concentrations of the element to be determined, measure the absorbances of these standard solutions, and prepare a calibration curve from the values obtained. Then measure the absorbance of the test solution, adjusted to be within the concentration range of the standard solutions, and determine the amount (concentration) of the element in the test solution from the calibration curve.

(2) Standard Addition Method To equal volumes of at least three test solutions, add suitable quantities of a standard solution containing a known concentration of the element to be determined so as to prepare a series of solutions containing stepwise increasing amounts of the element, and add a solvent to make a constant volume. Measure the absorbance of each solution, and plot the values obtained on a graph, with the added amount (concentration) of the element on the

abscissa and the absorbance on the ordinate. Extrapolate the regression line formed by joining the points on the graph, and determine the amount (concentration) of the element in the test solution from the distance between the origin and the intersection point of the regression line and the abscissa. This method is applicable only in the case that the calibration curve drawn as directed in (1) above passes through the origin.

(3) Internal Standard Method Prepare several standard solutions containing a constant amount of the internal standard element specified in the monograph, and known, graded amounts of the element to be determined. For these solutions, measure the atomic absorbances of the element to be determined and internal standard element at the analytical wavelength of each element under the same measuring conditions, and obtain the ratio of the absorbance of the element to be determined to the absorbance of the internal standard element for each solution. Prepare a calibration curve by plotting the values obtained, with the amount (concentration) of the element on the abscissa and the absorbance ratio on the ordinate. Next, prepare a test solution by adding the same amount of the internal standard element as contained in the standard solutions. Proceed under the same conditions as for the preparation of the calibration curve, obtain the absorbance ratio of the element to be determined to the internal standard element, and determine the amount (concentration) of the element in the test solution from the calibration curve.

Note: Avoid the use of reagents and test solutions that may interfere with determination.

Boiling Point and Distillation Range Tests

Unless otherwise specified, the boiling point and distillation range are determined by Method 1 or Method 2 below. Unless otherwise specified, the boiling point is expressed as the range between the minimum temperature, at which the first 5 drops of distillate leave the end of the condenser, and the maximum temperature, at which the liquid in the distillation flask is almost gone and a sufficient amount of vapor is no longer obtained. The distillation range test is conducted to determine the volume of the distillate collected in the temperature range specified in the individual monograph.

In the Monographs, such a specification as "55.5–57.0°C (Method 1)" for these tests indicates that when determined as directed in Method 1 under the Boiling Point and Distillation Range Tests, the boiling point of the substance is 55.5–57.0°C. Also, such a specification as "the amount of distillate at 64–70°C is not less than 95% vol. (Method 2)" indicates that when determined as directed in Method 2 under the Boiling Point and Distilling Tests, the amount of distillate of the substance at 64–70°C is not less than 95% vol.

Method 1 This method is used to determine the boiling point and amount of distillate of a liquid when the specified temperature range is less than 5°C.

Apparatus Use the apparatus illustrated on the next page.

Dry thoroughly all the glass instruments before use. Insert thermometer B into the distillation flask so that immersion line C is level with the lower end of cork stopper D and the upper end of its mercury bulb is located in the center of the delivery tube. Connect condenser E with distillation flask A and adapter F with E, and then insert the open end of F into the mouth of cylinder G so that air passes through slightly.

Place boiling chips or a capillary tube into A, use a hood with a height sufficient to shield A, and heat A with a suitable heat source. When an open flame is applied as the heat source, place A on a hole of a ceramic board (a 150 mm square wire gauze coated with a 6-mm thick ceramic layer with a 30-mm diameter round hole in its center), and then heat.

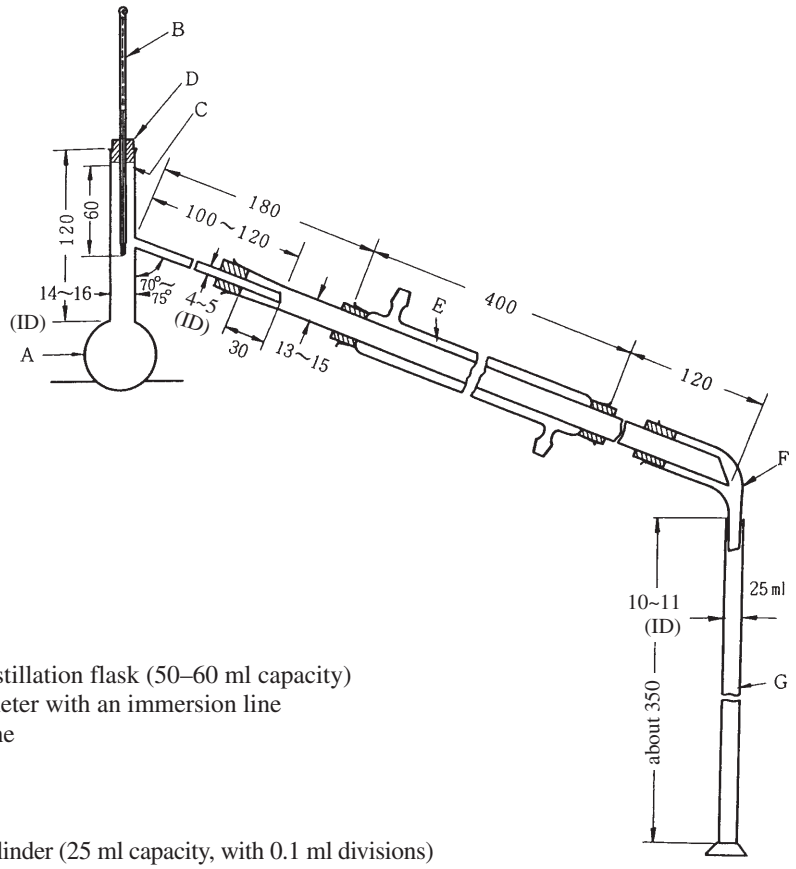
Procedure Measure 25 ml of the sample, whose temperature was previously measured, using G, and transfer into A. Use G as the receiver without washing. When the apparatus is set up, pass water through E, and heat A so that distillation starts after about 10 minutes. Unless otherwise specified, distill the liquid sample by the application of heat, at a rate of 4 to 5 ml per minute in the case of liquids whose boiling temperature to be determined is less than 200°C, or at a rate of 3 to 4 ml per minute in the case of liquids whose boiling temperature is not less than 200°C. Lower the temperature of distillate to the temperature at which the liquid was originally measured, and measure the volume of distillate. In the case of liquids that begin to distill at 80°C or below, cool to 10–15°C before measuring the volume, and keep receiving cylinder G immersed in ice up to a point 25 mm from the top during the distillation.

Correct the measured temperature for any variation in the barometric pressure from the normal (101 kPa) by allowing 0.1°C for each 0.36 kPa of variation, adding if the pressure is lower than 101 kPa, or subtracting if higher than 101 kPa.

Method 2 This method is used to determine the boiling point and amount of distillate of a liquid when the specified range of temperature is not less than 5°C.

Apparatus The same apparatus as described in Method 1 is used. The distillation flask (A) should be 200 ml in internal capacity and 18–24 mm in internal neck diameter, having a delivery tube of 5–6 mm in internal diameter. The ceramic board used for direct-flame heating should have a 50-mm diameter round hole in its center. Use a 100-ml volumetric cylinder graduated in 1-ml divisions as the receiver (G).

Procedure Measure 100 ml of the sample, whose temperature was previously noted, using G graduated in 1-ml divisions, and carry out the distillation in the same manner as Method 1.



A: Hard glass distillation flask (50–60 ml capacity)
B: Rod thermometer with an immersion line
C: Immersion line
D: Stopper
E: Condenser
F: Adapter
G: Volumetric cylinder (25 ml capacity, with 0.1 ml divisions)

(Unit: mm)

Calcium Salt Determination

Calcium Salt Determination is designed to determine the quantity of calcium salts contained in a sample by using disodium ethylenediaminetetraacetate (EDTA). There are two methods: the direct titration method (Method 1) using titration with the EDTA solution and the back-titration method (Method 2) using titration of an excess of EDTA added with zinc acetate solution.

Procedure Unless otherwise specified, proceed by Method 1 or Method 2, whichever is appropriate.

Method 1 Measure exactly 10 ml of the specified test solution, add 50 ml of water and 10 ml of potassium hydroxide solution (1 in 10), and allow to stand for 1 minute. Add about 0.1 g of NN indicator, and titrate immediately with 0.05 mol/L EDTA until the red-purple color of the solution completely disappears and a blue color develops.

Method 2 Measure exactly 20 ml of the specified test solution, add 25 ml of 0.02 mol/L EDTA, accurately measured, add 50 ml of water and 5 ml of ammonia–ammonium chloride buffer (pH 10.7), and allow to stand for 1 minute. Add 0.025 g of eriochrome black T–sodium chloride indicator, and titrate immediately the excess EDTA with 0.02 mol/L zinc acetate until the blue color of the solution changes to blue-purple. Perform a blank test in the same manner as the sample.

Chloride Limit Test

The Chloride Limit Test is designed to determine the allowable limit of chloride contained in a sample.

In the Monographs, such a specification as “not more than 0.041% as Cl (0.30 g, Control solution 0.01 mol/L hydrochloric acid 0.35 ml)” for this test indicates that when determined by weighing 0.30 g of the test substance and proceeding as directed in the following procedure, using 0.35 ml of 0.01 mol/L hydrochloric acid in the preparation of the control solution, the chloride content of the substance is not more than 0.041% as Cl.

Preparation of Test Solution and Control Solution Unless otherwise specified, proceed as directed below. Weigh the quantity of the sample specified in the individual monograph, transfer into a Nessler tube, and dissolve in about 30 ml of water. Neutralize the solution with diluted nitric acid (1 in 10) if the solution is alkaline. Add 6 ml of diluted nitric acid (1 in 10) and water to make 50 ml, and use this solution as the test solution. If the individual monograph requires the preparation of sample solution, transfer the solution into a Nessler tube, and add 6 ml of diluted nitric acid (1 in 10) and water to make 50 ml. Use this solution as the test solution.

Measure the specified amount of 0.01 mol/L hydrochloric acid, and transfer into another Nessler tube. Add 6 ml of diluted nitric acid (1 in 10) and water to make 50 ml. Use this solution as the control solution. If the test solution is not

clear, filter both solutions using the same procedure.

Procedure Unless otherwise specified, add 1 ml of silver nitrate solution (1 in 50) to each of the test and control solutions, mix thoroughly, and allow to stand for 5 minutes, protected from direct sunlight. Next, examine both Nessler tubes from the side and from above against a black background, and compare the turbidity. The turbidity developed in the test solution is not thicker than that of the control solution.

Coloring Matter Aluminum Lake Tests

The Coloring Matter Aluminum Lake Tests are used for the purity tests and assays of Coloring Matter Aluminum Lakes.

1. Hydrochloric Acid- and Ammonia-Insoluble Substances
In the Monographs, such a specification as "not more than 0.5% (Coloring Matter Aluminum Lake Tests)" for this test indicates that when determined as directed in the following procedure, the content of the hydrochloric acid- and ammonia-insoluble substance is not more than 0.5%.

Procedure Dry a crucible type glass filter (1G4) at 135°C for 30 minutes, allow to cool in a desiccator, and weigh accurately. Weigh accurately about 2 g of the sample into a beaker, mix with 20 ml of water, add 20 ml of hydrochloric acid, and stir well. Add 300 ml of boiling water, and shake well. Cover the beaker with a watch glass, heat on a water bath for 30 minutes, and cool. Filter the supernatant with the glass filter, previously prepared. Transfer the insoluble substances in the beaker into the filter with about 30 ml of water, wash twice the insoluble substances in the beaker and on the filter with 5 ml of water each time, further wash the insoluble substances on the glass filter with 1% ammonia solution until the washings become almost colorless, and wash with 10 ml of diluted hydrochloric acid (1 in 35), and then with water until the washings do not respond to silver nitrate solution (1 in 50). Dry the insoluble substances together with the glass filter at 135°C for 3 hours, allow to cool in a desiccator, and weigh accurately.

2. Iodide
In the Monographs, such a specification as "not more than 0.20% (Coloring Matter Aluminum Lake Tests)" for this test indicates that when determined as directed in the following procedure, the content of sodium iodide is not more than 0.20%.

Test Solution Weigh accurately about 0.06 g of the sample, add exactly 10 ml of water, shake occasionally for about 30 minutes, filter through a dry filter paper, and use the filtrate as the test solution.

Standard Solutions Transfer 0.5 ml, 1 ml, 10 ml, and 50 ml of Iodide Ion Standard Stock Solution into separate 100-ml volumetric flasks, and dilute each to volume with water to prepare standard solutions.

Procedure Proceed as directed under Ion Chromatography according to the following operating conditions, using 100 µl each of the test solution, standard solutions, and standard stock solution. Determine the peak heights or peak areas of the iodide ions of the standard solutions and the standard stock solution to make the calibration curve. Determine the peak height and peak area of the iodide ion of the test solution, and obtain the iodide ion content using the calibration curve. Multiply the iodide ion content by 1.18 to obtain the concentration of the sodium iodide of the test solution, and thus determine its content in the sample. Avoid direct sunlight during the procedure. Use a light-resistant container for the preparation of the test solution, and perform the test immediately after its preparation.

Operating Conditions
Detector: Electric conductivity detector.
Column: Stainless steel or plastic tube of 4.6–6.0 mm internal diameter and 5–10 cm length.
Column packing material: Porous anion exchanger.
Guard column: A column with the same internal diameter and the same packing material as the above column.
Mobile phase: A solution including 2.5 mmol/L phthalic acid and 2.4 mmol/L tris(hydroxymethyl)aminomethane (pH 4.0).
Column temperature: 40°C.
Flow rate: 1.5 ml/min.

3. Heavy Metals
In the Monographs, such a specification as "not more than 50 µg/g as Zn (Coloring Matter Aluminum Lake Tests, Heavy Metals (1))" for this test indicates that when determined as directed in the following procedure (1), the content of heavy metals is not more than 50 µg/g as Zn.

Preparation of Sample Solution Weigh 2.5 g of the sample, place in a quartz or porcelain crucible, moisten with a small quantity of sulfuric acid, heat gradually and incinerate almost completely at as low a temperature as possible, cool, add 1 ml of sulfuric acid, and heat gradually until the white fumes of sulfuric acid no longer evolve. Place in an electric furnace, ignite at 450–550°C to incinerate, and cool. Add 5 ml of hydrochloric acid and 1 ml of nitric acid, crush the lumps thoroughly, and evaporate to dryness on a water bath. Add 5 ml of hydrochloric acid, crush the lumps, and evaporate to dryness on a water bath. Add 10 ml of diluted hydrochloric acid (1 in 4) to the residue, dissolve by heating, cool, and filter through a filter paper (5C) for quantitative analysis. Wash the residue on the filter paper with about 30 ml of diluted hydrochloric acid (1 in 4), combine the filtrate and the washings, and evaporate to dryness on a water bath. Add 10 ml of diluted hydrochloric acid (1 in 4) to the residue, dissolve by heating, cool, and filter. Wash the crucible and the residue on the filter paper with 5 ml of diluted hydrochloric acid (1 in 4) and with 5 ml of water, combine the filtrate and the washings, and add water to make 50 ml. Prepare a blank test solution in the same manner as the sample solution.

(1) Zinc

Test Solution Measure 10.0 ml of the sample solution, add 10 ml of diluted hydrochloric acid (1 in 4), and then add water to make 50 ml.

Control Solution Measure 10.0 ml of the blank test solution, and add 2.5 ml of Zinc Standard Solution, 10 ml of diluted hydrochloric acid (1 in 4), and then water to make 50 ml.

Procedure Determine as directed under Atomic Absorption Spectrophotometry, using the test solution and the control solution, according to the following operating conditions. The absorbance of the test solution does not exceed that of the control solution.

Operating Conditions

Light Source: Zinc hollow cathode lamp.
Wavelength: 213.9 nm.
Supporting gas: Air.
Combustible gas: Acetylene.

(2) Iron

Test Solution Measure 4.0 ml of the sample solution, and add 10 ml of diluted hydrochloric acid (1 in 4) and water to make 50 ml.

Control Solution Measure 4.0 ml of the blank solution, and add 5 ml of Iron Standard Solution, 10 ml of diluted hydrochloric acid (1 in 4), and then water to make 50 ml.

Procedure Determine as directed under Atomic Absorption Spectrophotometry, using the test solution and the control solution, according to the following operating conditions. The absorbance of the test solution does not exceed that of the control solution.

Operating Conditions

Light source: Iron hollow cathode lamp.
Wavelength: 248.3 nm.
Supporting gas: Air.
Combustible gas: Acetylene.

(3) Other heavy metals

Test Solution Measure 20 ml of the sample solution, place into a Nessler tube, adjust the pH to about 4 with ammonium acetate solution (1 in 10), and add water to make 50 ml.

Control Solution Measure 20 ml of the blank test solution and 2.0 ml of Lead Standard Solution, place into a Nessler tube, and prepare the control solution in the same manner as the test solution.

Procedure Add 2 drops of sodium sulfide TS to each solution, shake well, and allow to stand for 5 minutes. The color of the test solution is not darker than that of the control solution.

4. Barium

In the Monographs, such a specification as "not more than 500 μg/g as Ba (Coloring Matter Aluminum Lake Tests)" for this test indicates that when determined as directed in the following procedure, the content of barium is not more than 500 μg/g as Ba.

Test Solution Weigh accurately about 1 g of the sample, place into a platinum crucible, moisten with a small quantity of sulfuric acid, and heat by gradually increasing the temperature until the sample is incinerated almost completely at as low a temperature as possible. Cool, add 1 ml of sulfuric acid, and heat gradually until the white fumes of sulfuric acid no longer evolve. Ignite the residue at 450–550°C for 3 hours in an electric furnace, and then cool. Add 5 g of anhydrous sodium carbonate to the residue, mix well, cover the crucible with a lid, and fuse by heating. Continue heating for 10 minutes, cool, add 20 ml of water, and heat on a water bath until the fused substance is dissolved. Cool, filter through a filter paper (5C) for quantitative analysis, and wash the residue on the filter paper with water until the washings are free from sulfate. Next, transfer the residue together with the filter paper into a beaker, add 30 ml of diluted hydrochloric acid (1 in 4), shake well, boil, and cool. Filter the content, wash the residue on the filter paper with 10 ml of water, combine the filtrate and the washings, and evaporate to dryness on a water bath. Dissolve the residue with 5 ml of water, filter if necessary, add 0.25 ml of diluted hydrochloric acid (1 in 4), and mix well. Add water to make 25 ml.

Control Solution To 0.5 ml of Barium Standard Solution, add 0.25 ml of diluted hydrochloric acid (1 in 20) and water to make 25 ml.

Procedure Determine as directed under Inductively Coupled Plasma-Atomic Emission Spectrometry using the test solution and the control solution. The emission intensity of the test solution is not stronger than that of the control solution.

5. Arsenic

In the Monographs, such a specification as "not more than 4.0 μg/g as As_2O_3 (Coloring Matter Aluminum Lake tests)" for this test indicates that when determined as directed in the following procedure, the content of arsenic as As_2O_3 is not more than 4.0 μg/g.

Test Solution Weigh 0.50 g of the sample, place into a quartz or porcelain crucible, add 20 ml of a solution of magnesium nitrate in ethanol (1 in 10), burn by igniting the ethanol, and heat gradually to incinerate at 450–550°C. If carbonized material still remains, moisten with a small quantity of nitric acid, and ignite again at 450–550°C to incinerate. Cool, add 6 ml of hydrochloric acid to the residue, add about 10 ml of water if necessary, and dissolve by heating on a water bath. Cool, and add water to make 25 ml.

Control Solution To 2.0 ml of Arsenic Standard Solution, add water to make 25 ml.

Procedure Determine as directed for the Method using Apparatus C under the Arsenic Limit Test, using the test solution and the control solution. The absorbance of the test solution is not more than that of the control solution.

6. Other Coloring Matter Lakes

In the Monographs, such a specification as "Coloring Matter Aluminum Lake Tests, Other Coloring Matter Lakes (1)" for this test indicates the test is to be performed as directed in (1) below.

Procedures Use an appropriate procedure from among the following procedures.

(1) Weigh the amount of the sample equivalent to 0.10 g as coloring matter, add 60 ml of diluted acetic acid (1 in 3), heat to boil, and cool. Add acetone to make 100 ml, mix well, and use the supernatant as the test solution. Analyze 2 μl of the test solution by paper chromatography without any control solution. Use a 6:3:2 mixture of 1-butanol/1% ammonia solution/absolute ethanol as the developing solvent. Use a No. 2 filter paper for paper chromatography. When the developing solvent ascends to a point about 15 cm above the original line, stop the development, and air-dry the filter paper. Place on a white plate, and examine from above in

daylight. Only one spot is observed.

(2) Proceed as directed in (1) using 1% ammonia solution instead of diluted acetic acid (1 in 3). Use a 1:1 mixture of 25% ethanol/5% ammonia solution as the developing solvent.

(3) Weigh the amount of the sample equivalent to 0.050 g of the color acid, and proceed as directed in (1).

(4) Proceed as directed in (1) using diluted acetic acid (1 in 20) instead of diluted acetic acid (1 in 3).

7. Assay
Use an appropriate procedure from among the following procedures.

(1) Weigh accurately the quantity of the sample specified in the individual monograph, transfer into a 500-ml wide-mouth Erlenmeyer flask, and add 20 ml of diluted sulfuric acid (1 in 20). Shake well, add 50 ml of boiling water, and dissolve by heating. Add 150 ml of boiling water and 15 g of sodium citrate. While passing carbon dioxide through the solution and boiling this solution vigorously, titrate with 0.1 mol/L titanium trichloride until the original color of the sample disappears.

(2) Proceed as directed in (1) using 15 g of sodium hydrogen tartrate instead of sodium citrate.

(3) Proceed as directed in (1) using 15 g of sodium hydrogen tartrate instead of sodium citrate. Use 10 ml of Light Green SF Yellow solution (1 in 1,000) as the indicator. Perform a blank test in the same manner as the test solution, and make any necessary correction.

Coloring Matter Tests

The Coloring Matter Tests are used for the purity tests and assays of Coloring Matters.

1. Water-insoluble Substances
In the Monographs, such a specification as "not more than 0.20% (Coloring Matter Tests)" for this test indicates that when determined as directed in the following procedure, the content of water-insoluble substances is not more than 0.20%.

Procedure Dry a crucible type glass filter (1G4) at 135°C for 30 minutes, cool in a desiccator, and weigh accurately. Weigh exactly 2.0 g of the sample, add 200 ml of boiling water, shake well, and cool. Filter the insoluble substances through the glass filter prepared as above, wash with water until the washings become colorless, dry together with the glass filter at 135°C for 3 hours, allow to cool in a desiccator, and weigh accurately.

2. Chloride and Sulfate
In the Monographs, such a specification as "not more than 5.0% as the total content (Coloring Matter Tests)" for this test indicates that when determined as directed in the following procedure, the combined total content of sodium chloride and sodium sulfate is not more than 5.0%.

Test Solution Weigh accurately about 0.1 g of the sample, and dissolve in water to make exactly 100 ml.

Standard Solutions Place exactly 0.2 ml, 1 ml, 10 ml, and 50 ml of each of the Chloride Ion Standard Stock Solution and Sulfate Ion Standard Stock Solution into separate 100-ml volumetric flasks, dilute each to volume with water to prepare four standard solutions for each stock solution.

Procedure Proceed as directed under Ion Chromatography according to the following operating conditions, using 20 µl each of the test solution, standard solutions, and standard stock solutions. Next, determine the peak heights or peak areas of the chloride ion and sulfate ion for each standard solution and each standard stock solution to make the calibration curves. Determine the peak heights or peak areas of chloride ion and sulfate ion for the test solution to obtain its chloride ion and sulfate ion contents from the calibration curves. Multiply the chloride content and the sulfate content by 1.65 and 1.48, respectively, to obtain the concentrations of sodium chloride and sodium sulfate in the test solution, and thus determine their contents in the sample.

Operating Conditions
Detector: Electric conductivity detector.
Column: A stainless steel or plastic tube of 4.6–6.0 mm internal diameter and 5–10 cm length.
Column packing material: Porous anion exchanger.
Guard column: A column with the same internal diameter and packing material as the above column.
Mobile phase: A solution containing 2.5 mmol/L phthalic acid and 2.4 mmol/L tris(hydroxymethyl)aminomethane (pH 4.0).
Column temperature: 40°C.
Flow rate: 1.5 ml/min.

3. Iodide
In the Monographs, such a specification as "not more than 0.40% (Coloring Matter Tests)" for this test indicates that when determined as directed in the following procedure, the content of sodium iodide is not more than 0.40%.

Test Solution Weigh accurately about 0.03 g of the sample, and dissolve in water to make exactly 10 ml.

Standard Solutions Place exactly 0.5 ml, 1 ml, 10 ml, and 50 ml of the Iodide Ion Standard Stock Solution into separate 100-ml volumetric flasks, and dilute each to volume with water to prepare standard solutions.

Procedure Proceed as directed under Ion Chromatography according to the same conditions specified under Chloride and Sulfate, using 100 µl each of the test solution, standard solutions, and standard stock solution. Determine the peak heights or peak areas of iodide ion for the standard solutions and the standard stock solutions to make the calibration curve. Determine the peak height or area of iodide ion for the test solution to obtain its content of iodide ion using the calibration curve. Multiply the content by 1.18 to obtain the concentration of sodium iodide in the test solution, and thus determine its content in the sample. Avoid direct sunlight during the procedure, use light-resistant containers for the preparation of the test solution, and perform the test imme-

diately after its preparation.

4. Bromides

In the Monographs, such a specification as "not more than 1.0% (Coloring Matter Tests)" for this test indicates that when determined as directed in the following procedure, the content of sodium bromide is not more than 1.0%.

Avoid direct sunlight in all the procedure. Use light-resistant equipment in preparing the test solution, and perform the test immediately after the preparation of the solution.

Test Solution Weigh accurately about 0.05 g of the sample, and dissolve in water to make exactly 10 ml.

Standard Solutions Place exactly 0.5 ml, 1 ml, 10 ml, and 50 ml of Bromide Ion Standard Stock Solution into separate 100-ml volumetric flasks, and dilute each to volume with water to prepare standard solutions.

Procedure Proceed as directed in Ion Chromatography according to the same conditions specified in Chloride and Sulfate, using 20 μl each of the test solution, standard solutions, and standard stock solution. Determine the peak heights or peak areas of bromide ion for the standard solutions and the standard stock solution to make the calibration curve. Determine the peak height or peak area of bromide ion for the test solution to obtain its content of bromide ion using the calibration curve. Multiply the content by 1.29 to obtain the concentration of sodium bromide, and thus determine its content in the sample.

5. Heavy Metals

In the Monographs, such a specification as "not more than 20 μg/g as Pb (Coloring Matter Tests, heavy metals (5))" for this test indicates that when determined as directed in (5) of the following procedures, the content of heavy metals as Pb is not more than 20 μg/g.

Preparation of Sample Solution Unless otherwise specified, weigh 2.5 g of the sample, transfer into a platinum, quartz, or porcelain crucible, moisten with a small quantity of sulfuric acid, and heat gradually until the contents is almost completely incinerated at as low a temperature as possible. Add 1 ml of sulfuric acid, and heat gradually until the white fumes of sulfuric acid no longer evolve. Place in an electric furnace, ignite at 450–550°C to incinerate, and cool. Add 3 ml of hydrochloric acid, stir add 7 ml of water, shake, and filter through a filter paper (5C) for quantitative analysis. Wash the residue on the filter paper with 5 ml of diluted hydrochloric acid (1 in 4) and 5 ml of water, combine the filtrate and the washings to prepare solution A, and add water to make 50 ml.

Proceed as follows to prepare the sample solution for chromium and manganese determination: Dry the residue obtained on the filter paper together with the filter paper at 105°C, place in a platinum crucible, and ignite at about 450°C to incinerate. Add 2 g of anhydrous sodium carbonate, heat to fuse, cool, add 10 ml of water, and add hydrochloric acid dropwise to acidify the solution. Transfer into a beaker, rinse the crucible with a small amount of water, add the rinsings to the beaker, and stir vigorously. Combine with solution A, and add water to make 50 ml.

Prepare a blank test solution in the same manner as the sample solution.

(1) Zinc

Test Solution Measure 2.5 ml of the sample solution, and add 10 ml of diluted hydrochloric acid (1 in 4) and water to make 50 ml.

Control Solution Measure 2.5 ml of the blank test solution, and add 2.5 ml of Zinc Standard Solution, 10 ml of diluted hydrochloric acid (1 in 4), and then water to make 50 ml.

Procedure Determine as directed under Atomic Absorption Spectrophotometry, using the test solution and the control solution, according to the following operating conditions. The absorbance of the test solution does not exceed that of the control solution.

Operating Conditions

Light Source: Zinc hollow cathode lamp.
Wavelength: 213.9 nm.
Supporting gas: Air.
Combustible gas: Acetylene.

(2) Chromium

Test Solution Unless otherwise specified, measure 5.0 ml of the sample solution, and add 5 ml of diluted hydrochloric acid (1 in 4) and water to make 25 ml.

Control Solution Measure 10.0 ml of the blank test solution, add 10.0 ml of Chromium Standard Solution, 10 ml of diluted hydrochloric acid (1 in 4), and then water to make 50 ml.

Procedure Determine as directed under Atomic Absorption Spectrophotometry, using the test solution and the control solution, according to the following operating conditions. The absorbance of the test solution does not exceed that of the control solution.

Operating Conditions

Light source: Chromium hollow cathode lamp.
Wavelength: 357.9 nm.
Supporting gas: Air.
Combustible gas: Acetylene.

(3) Iron

Test Solution Measure 2.0 ml of the sample solution, and add 10 ml of diluted hydrochloric acid (1 in 4) and water to make 50 ml.

Control Solution Measure 2.0 ml of the blank test solution, and add 5.0 ml of Iron Standard Solution, 10 ml of diluted hydrochloric acid (1 in 4) and then water to make 50 ml.

Procedure Determine as directed under Atomic Absorption Spectrophotometry, using the test solution and the control solution, according to the following operating conditions. The absorbance of the test solution does not exceed that of the control solution.

Operating Conditions

Light source: Iron hollow cathode lamp.
Wavelength: 248.3 nm.
Supporting gas: Air.
Combustible gas: Acetylene.

(4) Manganese

Test Solution Unless otherwise specified, measure 4.0 ml of the sample solution, and add 10 ml of diluted hydrochloric acid (1 in 4) and water to make 50 ml.

Control Solution Measure 4.0 ml of the blank test solu-

tion, and add 1.0 ml of Manganese Standard Solution, 10 ml of diluted hydrochloric acid (1 in 4), and then water to make 50 ml.

Procedure Determine as directed under Atomic Absorption Spectrophotometry, using the test solution and the control solution, according to the following operating conditions. The absorbance of the test solution does exceed that of the control solution.

Operating Conditions

Light source: Manganese hollow cathode lamp.
Wavelength: 279.5 nm.
Supporting gas: Air.
Combustible gas: Acetylene.

(5) Other heavy metals

Test Solution Measure 20 ml of the sample solution, transfer into a Nessler tube, add 1 drop of phenolphthalein TS, add ammonia TS dropwise until the color of the solution changes to pink, and add 2 ml of diluted acetic acid (1 in 4). Filter through a filter paper if necessary, wash the filter paper with water, and add water to make 50 ml.

Control Solution Measure 20 ml of the blank test solution, transfer into a Nessler tube, add 2.0 ml of Lead Standard Solution and 1 drop of phenolphthalein TS, and perform in the same manner as the test solution to prepare the control solution.

Procedure Add 2 drops of sodium sulfide TS to each solution, shake, and allow to stand for 5 minutes. The color of the test solution is not darker than that of the control solution.

6. Arsenic

In the Monographs, such a specification as "not more than 4.0 μg/g as As_2O_3 (Coloring Matter Tests)" for this test indicates that when determined as directed in the following procedure, the content of arsenic as As_2O_3 is not more than 4.0 μg/g.

Test Solution Weigh exactly 0.50 g of the sample, transfer into a quartz or porcelain crucible, add 20 ml of the solution of magnesium nitrate in ethanol (1 in 50), ignite the ethanol, and heat gradually to incinerate at 450–550°C. If a carbonized material still remains, moisten with a small quantity of nitric acid, re-ignite, and incinerate at 450–550°C. Cool, add 6 ml of hydrochloric acid to the residue, add about 10 ml of water if necessary, and dissolve by warming on a water bath. Cool, and then add water to make 25 ml.

Control Solution To 2.0 ml of Arsenic Standard Solution, add water to make 25 ml.

Procedure Determine as directed in the Method using Apparatus C under the Arsenic Limit Test, using the test solution and the control solution. The absorbance of Test Solution is not more than the absorbance of Standard Solution.

7. Other Coloring Matters

In the Monographs, such a specification as "(Coloring Matter Tests, Other Coloring Matters (1))" for this test indicates that the test is to be performed as directed in (1) below.

Procedures Use an appropriate procedure from among the following procedures.

(1) Weigh exactly 0.10 g of the sample, dissolve in water to make 100 ml, and use this solution as the test solution. Measure 2 μl of the test solution, and proceed as directed under Paper Chromatography, using a 6:3:2 mixture of 1-butanol/1% ammonia solution/absolute ethanol as the developing solvent, without any control solution. Use a No. 2 filter paper for chromatography. When the solvent ascends to a point about 15 cm above the original line, stop the development, air-dry the filter paper, place on a white plate, and examine from above in daylight. Only one spot is observed.

(2) Proceed as directed in (1) using a 1:1 mixture of 25% ethanol/5% ammonia solution as the developing solvent.

(3) Weigh 0.30 g of the sample, and dissolve in water to make 100 ml. Measure 10 ml of this solution, add water to make 100 ml, and use this solution as the test solution. Proceed as directed in (1) using a 20:13:5:5:2 mixture of acetone/isoamyl acetate/isoamyl alcohol/water/propionic acid as the developing solvent. When the developing solvent ascends to a point about 30 cm above the original line, stop the development.

(4) Weigh 0.10 g of the sample, and dissolve in water to make 200 ml. Using this solution as the test solution, proceed as directed in (1).

8. Subsidiary Colors

Test Solution Weigh accurately the specified amount of the sample, and dissolve in the specified solution to make exactly 100 ml.

Standard Solutions Dry the specified subsidiary colors for 24 hours in a vacuum desiccator, weigh 0.0100 g of each subsidiary color, and separately dissolve them in the specified solution to prepare standard stock solutions of exactly 100ml each. Prepare four standard solutions of different concentrations for each standard stock solution. Place exactly 1 ml, 2 ml, 5 ml, and 10 ml of each standard stock solution in separate 100-ml volumetric flasks, and dilute each to volume with the specified solution (the solution used to prepare the standard stock solution).

Procedure Analyze 20 μl portions of the test solution and the standard solution by liquid chromatography under the operating conditions given below. Next, measure the peak areas of the subsidiary colors for the standard solutions, and prepare a calibration curve for each subsidiary color. Measure the peak area of each subsidiary color for the test solution. Obtain the content of each color using the calibration curves, and calculate the total amount of the subsidiary colors.

Operating Conditions

Detector: Visible spectrophotometer (measurement wavelength: As specified in the individual monograph).
Column: A stainless steel tube of 4.6 mm internal diameter and 25 cm length.
Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.
Column temperature: 30°C.
Flow rate: 1 ml/min.

9. Unreacted Raw Materials and Products of Side Reactions

Test Solution Weigh accurately the specified amount of

the sample, and dissolve it in the specified solution to make exactly 100 ml.

Standard Solutions Dry the specified unreacted raw materials and products of side reactions for 24 hours in a vacuum desiccator, weigh 0.0100 g of each substance, and separately dissolve them in the specified solution to prepare standard stock solutions of exactly 100 ml each. Prepare four standard solutions of different concentrations for each standard stock solution. Place exactly 1 ml, 2 ml, 5 ml, and 10 ml of each standard stock solution in separate 100-ml volumetric flasks, and dilute each to volume the specified solution (the same solution as that used to prepare the standard stock solution).

Procedure Analyze 20 μl portions of the test solution and the standard solutions by liquid chromatography using the operating conditions given below. Next, measure the peak areas of the unreacted raw materials and products of side reactions for standard solutions, and prepare a calibration curve for each substance. Measure the peak area of each substance for the test solution. Obtain the contents of the unreacted raw materials and products of side reactions using the calibration curves.

Operating Conditions

Detector: Ultraviolet spectrophotometer (measurement wavelength: As specified in the individual monograph).

Column: A stainless steel tube of 4.6 mm internal diameter and 25 cm length.

Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 30°C.

Flow rate: 1 ml/min.

10. Unsulfonated Primary Aromatic Amines

(1) Aniline

In the Monographs, such a specification as "not more than 0.010% as aniline (Coloring Matter Tests)" for this test indicates that when determined as directed in the following procedure, the content of unsulfonated primary aromatic amines is not more than 0.010% as aniline.

Test Solution Weigh 2.0 g of the sample, transfer into a separating funnel containing 100 ml of water, and add 50 ml of water to dissolve. Add 5 ml of sodium hydroxide solution (4 in 100) and 50 ml of ethyl acetate, shake, and extract. Collect the ethyl acetate layer. Add 50 ml of ethyl acetate to the water layer, shake, and extract. Combine the two ethyl acetate layers, and wash with sodium hydroxide solution (4 in 1,000) until the color of the solution disappears. Extract three times from the washed ethyl acetate with 10 ml of diluted hydrochloric acid (3 in 10) each time. Combine the hydrochloric acid extracts, and add water to make exactly 100 ml. Use this solution as the sample solution. Transfer exactly 10 ml of the sample solution into a test tube, and cool in ice for 10 minutes. Add 1 ml of potassium bromide solution (1 in 2) and 0.05 ml of sodium nitrite solution (1 in 30), mix, and allow to stand in ice for 10 minutes. Wash the mixture down with water into a 25-ml volumetric flask containing 1 ml of 0.05 mol/L disodium 3-hydroxy-2,7-naphthalenedisulfonate and 10 ml of sodium carbonate solution (1 in 10), and add water to make exactly 25 ml. Allow this solution to stand for 15 minutes in a dark place.

Control Solution Weigh 0.010 g of aniline, dissolve it in 30 ml of diluted hydrochloric acid (3 in 10), and add water to make exactly 100 ml. Measure exactly 2 ml of this solution, and add 30 mL of diluted hydrochloric acid (3 in 10) and then water to make exactly 100 ml. Proceed in the same manner as directed for the test solution.

Reference Solution To measure the absorbance of the test solution, use the following reference solution: Transfer 10 ml of the sample solution into a 25-ml volumetric flask, add 1 ml of 0.05 mol/L disodium 3-hydroxy-2,7-naphthalenedisulfonate and 10 ml of sodium carbonate solution (1 in 10), and then add water to make exactly 25 ml. To measure the absorbance of the control solution, use the following reference solution: To 3 ml of diluted hydrochloric acid (3 in 10), add 1 ml of 0.05 mol/L disodium 3-hydroxy-2,7-naphthalenedisulfonate and 10 ml of sodium carbonate solution (1 in 10), and then add water to make exactly 25 ml.

Procedure Measure the absorbance for each solution at a wavelength of 510 nm. The absorbance of the test solution is not more than that of the control solution.

(2) α-Naphthylamine

In the Monographs, such a specification as "not more than 1.0 μg/g as α-naphthylamine (Coloring Matter Tests)" for this test indicates that when determined as directed in the following procedure, the content of α-naphthylamine is not more than 1.0 μg/g.

Test Solution Weigh accurately about 1 g of the sample, and transfer into a separating funnel containing 50 ml of water. Add 50 ml of water, and dissolve. Add 5 ml of sodium hydroxide solution (4 in 100) and 50 ml of ethyl acetate, shake, and extract. Collect the ethyl acetate layer. Add 50 ml of ethyl acetate to the aqueous layer, shake, and extract. Combine the two ethyl acetate layers, and wash with sodium hydroxide solution (4 in 1,000) until the color of the solution disappears. To the ethyl acetate layer, add 0.5 ml of diluted sulfuric acid (0.15 in 1,000). Dry under reduced pressure at 45°C, and immediately add 1.0 ml of a mixture of equal volumes of monosodium dihydrogen phosphate solution (0.3 in 100) and methanol.

Standard Solutions Weigh 0.010 g of α-naphthylamine, and dissolve it in 3 ml of diluted hydrochloric acid (3 in 10). Add water to prepare a standard stock solution of exactly 10 ml. To exactly 1 ml of the standard stock solution add ammonium acetate solution (1.54 in 1,000) to make exactly 100 ml. Place exactly 1 ml, 2 ml, 5 ml, and 10 ml of this solution into separate 100-ml volumetric flasks, and dilute each to volume with the mobile phase described in the operating conditions below.

Procedure Analyze 100 μl portions of the test solution and the standard solutions by liquid chromatography using the operating conditions given below. Measure the peak areas for α-naphthylamine for the standard solutions, and prepare a calibration curve. Next, measure the peak area of the peak corresponding to the retention time of α-naphthylamine for the test solution, and calculate the content of α-naphthylamine using the calibration curve.

Operating Conditions

Detector: Ultraviolet spectrophotometer (measuring wavelength: 304 nm).

Column: A stainless steel tube of 4.6 mm internal diameter and 15 cm length.

Column packaging material: 5-μm octadecylsilanized silica gel for liquid chromatography.
Column temperature: 40°C.
Mobile phase: To 500 ml of methanol, add ammonium acetate solution (1.54 in 1,000) to make 1000 ml.
Flow rate: 1 ml/min.

(3) *p*-Cresidine
In the Monographs, such a specification as "not more than 10 μg/g as *p*-cresidine (Coloring Matter Tests)" for this test indicates that when determined as directed in the following procedure, the content of *p*-cresidine is not more than 10 μg/g.

Test Solution Weigh accurately about 1 g of the sample, and transfer into a separating funnel containing 50 ml of water. Add 50 ml of water to dissolve. Add 5 ml of sodium hydroxide solution (4 in 100) and 50 ml of ethyl acetate, shake, and extract. Collect the ethyl acetate layer. Add 50 ml of ethyl acetate to the aqueous layer, shake, and extract. Combine the two ethyl acetate layers, and wash with sodium hydroxide solution (4 in 1,000) until the color of the solution disappears. To this ethyl acetate layer, add 0.5 ml of diluted sulfuric acid (0.15 in 1,000). Evaporate to dryness under pressure at 45°C, and immediately add 1.0 ml of a mixture of equal volumes of monosodium dihydrogen phosphate solution (0.3 in 100) and methanol.

Standard Solutions Weigh 0.100 g of *p*-cresidine, and dissolve it in 30 ml of diluted hydrochloric acid (3 in 10). Add water to prepare a standard stock solution of exactly 100 ml. To 10 ml of the standard stock solution, add ammonium acetate solution (1.54 in 1,000) to make exactly 100 ml. Place exactly 1 ml, 2 ml, 5 ml and 10 ml of this solution into separate 100-ml volumetric flasks, dilute each to volume with the mobile phase described in the operating conditions given below.

Procedure Analyze 100 μl portions of the test solution and the standard solutions by liquid chromatography using the operating conditions given below. Measure the peak areas for *p*-cresidine for standard solutions, and prepare a calibration curve. Measure the area of the peak corresponding to the retention time of *p*-cresidine to the test solution, and calculate the content of *p*-cresidine using the calibration curve.

Operating Conditions
Detector: Ultraviolet spectrophotometer (measuring wavelength: 290 nm).
Column: A stainless steel tube of 4.6 mm internal diameter and 15 cm length.
Column packaging material: 5-μm octadecylsilanized silica gel for liquid chromatography.
Column temperature: 40°C.
Mobile phase: To 400 ml of methanol, add ammonium acetate solution (1.54 in 1000) to make 1000 ml.
Flow rate: 1 ml/min.

11. Assay

(1) Titanium Trichloride Method Use an appropriate procedure from among the following procedures.

(i) Measure exactly the specified volume of the test solution, transfer into a 500-ml wide-mouthed Erlenmeyer flask, and add 15 g of sodium citrate and then water to make about 200 ml. Titrate with 0.1 mol/L titanium trichloride while passing carbon dioxide gas through the solution and vigorously boiling. The endpoint of the titration is when the original color of the sample disappears.

(ii) Proceed as directed in (i) using 15 g of sodium hydrogen tartrate instead of sodium citrate.

(iii) Proceed as directed in (i) using 15 g of sodium hydrogen tartrate instead of sodium citrate. Using 10 ml of diluted Light Green SF Yellow (1 in 1,000) as an indicator, perform a blank test in the same manner as for the sample to make any necessary correction.

(iv) Proceed as directed in (i) using 20 g of sodium tartrate instead of sodium citrate. The endpoint of the titration is when the original color of the sample disappears and an orange color appears.

(2) Mass Method Dry a crucible type glass filter (1G4) at 135°C for 30 minutes, allow to cool in a desiccator, and weigh accurately. Measure exactly the specified volume of the test solution, and transfer into a 500-ml beaker. Boil this solution, add 25 ml of diluted hydrochloric acid (1 in 50), and re-boil. Wash the inside of the beaker using about 5 ml of water, cover the beaker with a watch glass, heat on a water bath for about 5 hours, and then cool. Filter the precipitate through the glass filter prepared above, wash the beaker and precipitate three times with 10 ml of diluted hydrochloric acid (1 in 200) each time and then twice with 10 ml of water each time. Dry the precipitate together with the glass filter at 135°C for 3 hours, cool in the desiccator, and weigh accurately.

Color Value Test

The Color Value Test is designed to measure the concentration (Color Value) of a coloring agent in a food color by determining absorbance. The color value is the absorbance of a solution of the food color, in terms of the absorbance of a 10% (w/v) solution, that is obtained at the maximum absorption wavelength in the visible light region. It is generally expressed as the figure ($E^{10\%}_{1cm}$).

Procedure Unless otherwise specified, proceed as directed below. The test solution should be adjusted so that its absorbance falls within the range of 0.3–0.7.

Unless otherwise specified, weigh accurately the amount of the sample that corresponds to the labeled color value, shown in the table on the next page, transfer to a volumetric flask, dissolve in about 10 ml of the specified solvent, and add the solvent to make exactly 100 ml. Centrifuge or filter it if necessary, and use this solution as the sample solution. Dilute the solution according to the corresponding dilution factor given in the same table if necessary, and use this solution as the test solution. Unless otherwise specified, determine the absorbance (A) at the specified wavelength in a 1-cm cell using the same solvent used for preparing the test solution as the reference. Calculate the color value by the formula below. Determination of the color value should be done promptly after the preparation of the test solution to avoid the influence of fading after preparation.

Color value	Concentration for measurement (%)	Absorbance	Dilution method	Volume of dilution (ml)	F
20	0.025	0.5	0.25g → 100ml	100	1
50	0.10	0.5	0.1g → 100ml	100	1
100	0.05	0.5	0.5g → 100ml → 10ml → 100ml	1,000	10
200	0.03	0.6	0.6g → 100ml → 5ml → 100ml	2,000	20
400	0.015	0.6	0.3g → 100ml → 5ml → 100ml	2,000	20
500	0.01	0.5	0.2g → 100ml → 5ml → 100ml	2,000	20
700	0.01	0.7	0.2g → 100ml → 5ml → 100ml	2,000	20
800	0.00625	0.5	0.25g → 100ml → 5ml → 200ml	4,000	40
900	0.005	0.45	0.2g → 100ml → 5ml → 200ml	4,000	40
1,000	0.006	0.6	0.3g → 100ml → 5ml → 250ml	5,000	50
1,500	0.003	0.6	0.4g → 100ml → 5ml → 50ml → 5ml → 50ml	10,000	100
2,000	0.003	0.6	0.3g → 100ml → 5ml → 50ml → 5ml → 50ml	10,000	100
2,500	0.002	0.5	0.2g → 100ml → 5ml → 50ml → 5ml → 50ml	10,000	100

$$\text{Color value} = \frac{10 \times A \times F}{\text{Amount of sample (g)}}$$

A = absorbance of test solution,
F = dilution factor of test solution used to adjust the absorbance so that it can fall in the range of 0.3–0.7.

If the color value exceeds the maximum value in the above table, adjust the dilution factor.

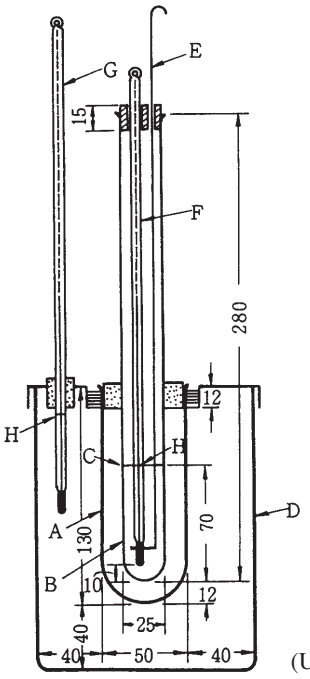
Congealing Point

The congealing point is measured as directed below.

Apparatus Use the apparatus illustrated in Fig. 1.

Procedure Fill glass or plastic cooling bath D almost to the top with water that is about 5°C lower than the expected congealing point. If the sample is a liquid at ordinary temperature, fill bath D with water whose temperature is 10–15°C lower than the expected congealing point. Transfer the sample into sample container B up to marked line C. If the sample is a solid, melt the sample carefully by warming to a temperature lower than 20°C above the expected congealing point, and transfer into B. Insert B into glass cylinder A, and adjust immersion line H of thermometer F so that it is level with the meniscus of the sample. After cooling the sample to a temperature 5°C higher than the expected congealing point, move stirrer rod E vertically at a rate of 60 to 80 strokes per minute, and take the temperature readings at 30-second intervals. The temperature falls gradually. Stop stirring when an appreciable amount of crystals is formed and the temperature becomes constant, or begins to rise.

Ordinarily, the temperature becomes constant for a short time after rising. Record the maximum temperature (reading on the thermometer) that is kept constant (Fig. 2). If the temperature does not increase, record the temperature that has remained constant for a while (Fig. 3). The congealing



(Unit: mm)

A: Glass cylinder (silicone oil is applied to the inner and outer surfaces of the tube to prevent clouding.)
B: Sample container (a hard-glass test tube. If necessary, apply silicone oil to the surfaces that do not come into contact with the sample to prevent clouding. Insert into cylinder A, and fix with a cork stopper.)
C: Mark
D: Glass or plastic cooling bath
E: Glass or stainless steel stirrer (a 3-mm diameter wire with the lower end bent into a loop of about 18 mm diameter)
F: Rod thermometer with an immersion line
G: Auxiliary thermometer
H: Immersion line

Fig.1

point is the average of four or more consecutive readings, between which the temperature difference is within 0.2°C.

If many impurities are included in the sample, the congealing point curve is not as shown in Fig. 2, but rather as shown in Fig. 3, 4, or 5. In the case of Figs. 4 and 5, extend each of the curves of the solid and liquid phases on the graph, and take their intersection as the congealing point. In the case of Fig. 3, proceed as in Fig. 2.

Note: If a state of supercooling is anticipated, rub the inner wall of bath B or put a small fragment of the solid sample into bath B to promote congealment when the temperature approaches the expected congealing point.

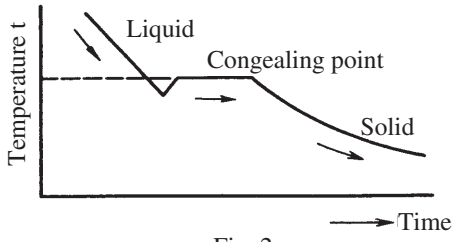


Fig. 2

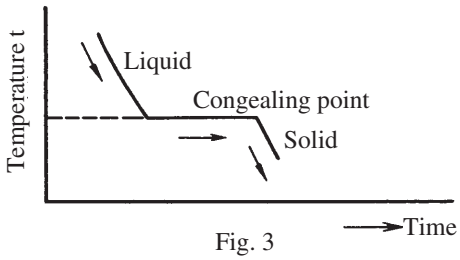


Fig. 3

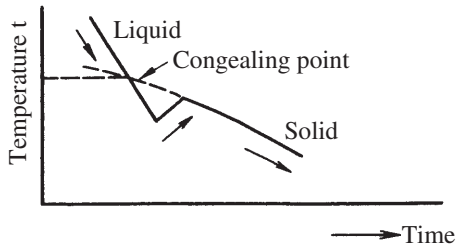


Fig. 4

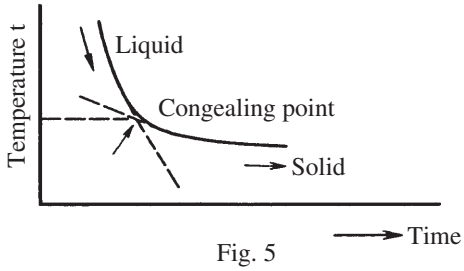


Fig. 5

Fats and Related Substances Tests

The Fats and Related Substances Tests are designed to determine the ester value, saponification value, acid value, hydroxyl value, and iodine value of fats and related substances, other than flavoring substances, such as fatty acids, higher aliphatic alcohols, and fatty acid esters.

1. Ester Value

The ester value is the number of mg of potassium hydroxide (KOH) required to saponify the esters in 1 g of a sample.

In the Monographs, such a specification as "125–164 (Fats and Related Substances Tests)" for this test indicates that when determined as directed in the procedure below, the ester value is 125–164.

Procedure Unless otherwise specified, determine the saponification value and the acid value, and calculate the ester value by the following formula:

$$\text{Ester value} = \text{Saponification Value} - \text{Acid Value}$$

2. Saponification Value

The saponification value is the number of mg of potassium hydroxide (KOH) required to saponify the esters and neutralize the free acids in 1 g of a sample.

Procedure Unless otherwise specified, proceed as follows: Weigh accurately about 1 g of the sample, transfer into an Erlenmeyer flask, add 40 ml of ethanol, and dissolve while warming if necessary. Add 20 ml of ethanolic potassium hydroxide TS, accurately measured, equip the flask with a reflux condenser, and heat in a water bath for 30 minutes while shaking occasionally. Cool, add a few drops of phenolphthalein TS, and immediately titrate the excess potassium hydroxide with 0.5 mol/L hydrochloric acid. Perform a blank test, and calculate the saponification value by the formula:

$$\text{Saponification value} = \frac{(a - b) \times 28.05}{\text{Weight (g) of the sample}}$$

a = volume (ml) of 0.5 mol/L hydrochloric acid consumed in the blank test,
b = volume (ml) of 0.5 mol/L hydrochloric acid consumed in the test.

3. Acid Value

The acid value is the number of mg of potassium hydroxide (KOH) required to neutralize 1 g of a sample.

In the Monographs, such a specification as "not more than 15 (Fats and Related Substances Tests)" for this test indicates that when determined as directed in the following procedure, the acid value is not more than 15.

Procedure Unless otherwise specified, proceed as follows: Weigh accurately the specified quantity of the sample given in the table on the next page, according to the acid value of the sample, and add 50 ml of a 1:1 mixture of ethanol/ether. Dissolve while heating if necessary, and use this solution as the test solution. Cool, add a few drops of phenolphthalein TS, titrate with 0.1 mol/L ethanolic potassium hydroxide to

the first pink color that persists for 30 seconds, and calculate the acid value using the formula below. To the solvent mixture used, previously add 0.1 mol/L ethanolic potassium hydroxide until its pink color persists for 30 seconds. Use phenolphthalein TS as the indicator.

$$\text{Acid value} = \frac{\left(\begin{matrix}\text{Volume (ml) of 0.1mol/L ethanolic}\\ \text{potassium hydroxide consumed}\end{matrix}\right) \times 5.611}{\text{Weight (g) of the sample}}$$

Table

Acid value	Weight (g) of sample
Less than 5	10
5 to less than 15	5
15 to less than 50	3
50 to less than 120	1
Not less than 120	0.5

4. Hydroxyl Value

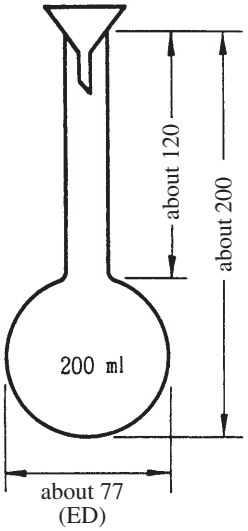
The hydroxyl value is the number of mg of potassium hydroxide (KOH) required to neutralize the acetic acid combined to hydroxyl groups, when 1 g of a sample is acetylated under the following conditions.

In the Monographs, such a specification as "155–187 (Fats and Related Substances Tests), provided the acid value is assumed to be 0" for this test indicates that when determined as directed in the following procedure by assuming the acid value to be 0, the hydroxyl value is 155–187.

Procedure Unless otherwise specified, proceed as follows: Weigh accurately 1 g of the sample, transfer into a round-bottom flask as shown in the right column, and add 5 ml of acetic anhydride–pyridine TS, accurately measured. Place a small funnel on the mouth of the flask, and heat for 1 hour while immersing the flask to a depth of about 1 cm from the bottom into an oil bath at 95–100°C. Cool, add 1 ml of water, shake well, and heat for 10 minutes. After cooling, rinse the funnel and the neck of the flask with 5 ml of ethanol, and titrate with 0.5 mol/L ethanolic potassium hydroxide (indicator: 1 ml of phenolphthalein TS). Perform a blank test, and calculate the hydroxyl value by the formula:

$$\text{Hydroxyl value} = \frac{(a - b) \times 28.05}{\text{Weight (g) of the sample}} + \text{acid value}$$

a = volume (ml) of 0.5 mol/L ethanolic potassium hydroxide consumed in the blank test,
b = volume (ml) of 0.5 mol/L ethanolic potassium hydroxide consumed in the test.



(Unit: mm)

5. Iodine Value

The iodine value is defined as the amount of halogens, in terms of the number of g of iodine (I), that are absorbed by 100 g of a sample under the following conditions.

Procedure Unless otherwise specified, weigh accurately the appropriate amount of the sample shown in the following table, according to the expected iodine value of the sample, in a small glass container. Place the container containing the sample in a 500-ml glass stoppered flask, add exactly 20 ml of cyclohexane to dissolve the sample, add 25 ml of Wijs TS, and mix well. Stopper the flask tightly, and allow to stand, protected from light, at 20–30°C for 30 minutes (when the expected iodine value is 100 or more, for 1 hour) with occasional shaking. Add 20 ml of potassium iodine solution, (1 in 10) and 100 ml of water, and shake. Next, titrate the freed iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 ml of starch TS). Perform a blank test. Obtain the iodine content by the formula:

$$\text{Iodine value} = \frac{(a - b) \times 1.269}{\text{Weight (g) of the sample}}$$

a = amount (ml) of 0.1 mol/L sodium thiosulfate consumed in the blank test,
b = amount (ml) of 0.1 mol/L sodium thiosulfate consumed in the test with the sample.

Table

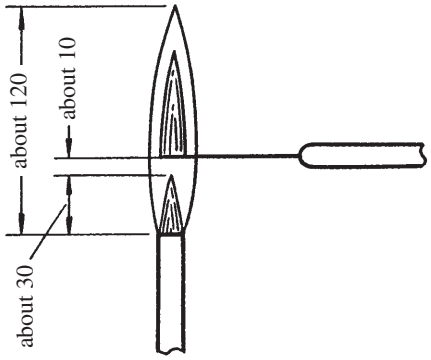
Acid value	Weight (g) of sample
Less than 30	1.0
30 to less than 50	0.6
50 to less than 100	0.3
Not less than 100	0.2

Flame Coloration Test

The Flame Coloration Test is designed to determine the identity of an element by means of the property that a certain type of element changes the colorless flame of a Bunsen burner to a characteristic color.

Procedure Use a straight platinum wire about 0.8 mm in diameter for the test. In the case of a solid sample, make it into a paste by adding a small quantity of hydrochloric acid. Apply a small amount of the sample to the end of the platinum wire to a length of about 5 mm from the end, and test immediately by putting the end into a colorless flame, keeping the platinum wire horizontal. In the case of a liquid sample, immerse the end of the platinum wire into the sample to about 5 mm below the liquid surface, remove it from the sample gently, and perform the test in the same manner as for the solid sample.

The description, "the flame coloration reaction persists," means that the reaction persists for 4 seconds.



(Unit: mm)

Flavoring Substances Tests

1. Alcohol Content

The alcohol content is the quantity of alcohols contained in a sample.

Procedure Unless otherwise specified, proceed as directed in Method 1 or Method 2, whichever is appropriate.

Method 1 Measure exactly 10 ml of the sample, transfer into a 100-ml flask, and add 10 ml of acetic anhydride and 1 g of anhydrous sodium acetate. Connect the flask to an air condenser, and boil the mixture gently on a hot-plate for 1 hour. Allow to cool for 15 minutes, and then add 50 ml of water. Heat the mixture in a water bath for 15 minutes while shaking occasionally. Cool, transfer the mixture into a separating funnel, and remove the aqueous layer. Wash the oil layer with anhydrous sodium carbonate solution (1 in 8) until the washings become alkaline, and wash further with sodium chloride solution (1 in 10) until the washings become neutral. Transfer the oil layer into a dry container, add about 2 g of anhydrous sodium sulfate, shake well, allow to stand for about 30 minutes, and filter. Weigh accurately the specified quantity of the acetylated oil thus obtained, and determine the ester value as directed under Ester Value in the Flavoring Substances Tests. This ester value, also referred to as the acetyl value, is calculated by the formula:

$$\text{Acetyl value} = \frac{(a - b) \times 28.05}{\text{Weight (g) of acetylated oil}}$$

Calculate the alcohol content by the formula:

$$\text{Alcohol content (\%)} = \frac{\left(\begin{matrix}\text{Molecular weight}\\ \text{of alcohol}\end{matrix}\right) \times (a - b) \times 0.5}{\left[\left(\begin{matrix}\text{Weight (g) of}\\ \text{acetylated oil}\end{matrix}\right) - 0.02102(a - b)\right] \times 1{,}000} \times 100$$

$$= \frac{\text{Acetyl value} \times \text{Molecular weight of alcohol}}{561.1 - (0.4204 \times \text{Acetyl value})}$$

a = volume (ml) of 0.5 mol/L hydrochloric acid consumed in the blank test,
b = volume (ml) of 0.5 mol/L hydrochloric acid consumed in the test.

Method 2 Weigh accurately the specified quantity of the sample, transfer into a 200-ml flask with a ground-glass stopper, and add 5 ml of acetic anhydride–pyridine TS, exactly measured. Moisten the ground-glass joint with 2 to 3 drops of pyridine, stopper lightly, and heat in a water bath for 1 hour. Cool, and then add 10 ml of water to wash the stopper and the inside of the flask. Stopper tightly, shake well, and cool to ordinary temperature. Wash the ground-glass joint and the inside of the flask with 5 ml of neutralized ethanol, and titrate with 0.5 mol/L ethanolic potassium hydroxide TS. Confirm the endpoint using a potentiometer or 2 to 3 drops of cresol red–thymol blue TS as the indicator. Perform a blank test in the same manner as for the test solution, and calculate the alcohol content by the formula:

$$\text{Alcohol content (\%)} = \frac{\text{Molecular weight of alcohol} \times (a - b) \times 0.5}{\text{Weight (g) of the sample} \times 1{,}000} \times 100$$

a = volume (ml) of 0.5 mol/L ethanolic potassium hydroxide TS consumed in the blank test,
b = volume (ml) of 0.5 mol/L ethanolic potassium hydroxide TS consumed in the test.

2. Aldehyde and Ketone Content

The aldehyde and ketone content is obtained using the property that aldehydes and ketones react with hydroxylamine (NH_2OH).

Procedure Unless otherwise specified, proceed as directed in Method 1 or Method 2, whichever is appropriate.

Method 1 Weigh accurately the specified quantity of the sample, add 50 ml of 0.5 mol/L hydroxylamine hydrochloride, exactly measured, and shake well. Either allow to stand for the time specified in the individual monograph, or boil gently under a reflux condenser in a water bath for the time

specified in the individual monograph, and then cool to room temperature. Titrate the liberated acid with 0.5 mol/L ethanolic potassium hydroxide. Confirm the endpoint using a potentiometer or observing the appearance of a green-yellow color. Perform a blank test, make any necessary correction, and calculate the content by the formula:

$$\text{Aldehyde or Ketone content (\%)} = \frac{\left(\begin{array}{c}\text{Molecular weight of}\\ \text{aldehyde or ketone}\end{array}\right) \times (a - b) \times 0.5}{\text{Weight (g) of the sample} \times 1{,}000} \times 100$$

a = volume(ml) of 0.5 mol/L ethanolic potassium hydroxide consumed in the blank test,
b = volume (ml) of 0.5 mol/L ethanolic potassium hydroxide consumed in the test.

Method 2 Weigh accurately the specified quantity of the sample, add 75 ml of hydroxylamine TS, exactly measured, and shake well. Either allow to stand for the time specified in the individual monograph, or boil gently under a reflux condenser in a water bath for the time specified in the individual monograph, and then cool to room temperature. Titrate excess hydroxylamine with 0.5 mol/L hydrochloride acid. Confirm the endpoint using a potentiometer or macroscopically observing the change in the color of the solution from purple to green-yellow. Perform a blank test, make any necessary correction, and calculate the content by the formula:

$$\text{Aldehyde or Ketone content (\%)} = \frac{\left(\begin{array}{c}\text{Molecular weight of}\\ \text{aldehyde or ketone}\end{array}\right) \times (a - b) \times 0.5}{\text{Weight (g) of the same} \times 1{,}000} \times 100$$

a = volume (ml) of 0.5 mol/L hydrochloric acid consumed in the blank test,
b = volume (ml) of 0.5 mol/L hydrochloric acid consumed in the test.

3. Ester Value

The ester value is the number of mg of potassium hydroxide (KOH) required to saponify the esters contained in 1 g of a sample.

In the Monographs, such a specification as "not more than 3.0 (5 g, Flavoring Substances Tests)" for this test indicates that when determined by weighing about 5 g of the test substance and proceeding as directed in the following procedure, the ester value is not more than 3.0.

Procedure Unless otherwise specified, proceed as follows: Weigh accurately the specified quantity of the sample, transfer into a 200-ml flask, add 10 ml of ethanol and 3 drops of phenolphthalein TS, and neutralize with potassium hydroxide solution (1 in 250). Add 25 ml of 0.5 mol/L ethanolic potassium hydroxide, exactly measured, , and boil gently under a reflux condenser in a water bath for 1 hour. Cool, and then titrate the excess potassium hydroxide with 0.5 mol/L hydrochloride acid. Confirm the endpoint using a potentiometer or 2 to 3 drops of phenolphthalein TS as the indicator. Perform a blank test in the same manner, and calculate the ester value by the formula:

$$\text{Ester value} = \frac{(a - b) \times 28.05}{\text{Weight (g) of the sample}}$$

a = volume (ml) of 0.5 mol/L hydrochloric acid consumed in the blank test,
b = volume (ml) of 0.5 mol/L hydrochloric acid consumed in the test.

4. Ester Content

The content of ester of monobasic acid is obtained by performing the test as directed under Ester Value in Flavoring Substances Tests and then calculating by the formula:

$$\text{Ester content (\%)} = \frac{\left(\begin{array}{c}\text{Molecular weight}\\ \text{of ester}\end{array}\right) \times (a - b) \times 0.5}{\text{Weight (g) of the sample} \times 1{,}000} \times 100$$

$$= \frac{\text{Ester value} \times \text{Molecular weight of ester}}{561.1}$$

in which a and b are the a and the b, respectively, indicated in Ester Value.

5. Halogenated Compounds

This test identifies halogenated compounds by utilizing the flame coloration of copper chloride.

Procedure Use a copper wire that is 1.5 cm wide and 5 cm long, and has about 1-mm mesh copper gauze wound around its edge. Heat the copper gauze in the colorless flame of a burner until no green color is observed in the flame, and cool. Repeat this procedure a few times. After cooling, moisten the copper gauze with 2 drops of the sample, and burn. Repeat this procedure three times, and burn the gauze in the outer edge of a colorless flame adjusted to a height of about 4 cm. No green color develops in the flame.

6. Saponification Value

The saponification value is the number of mg of potassium hydroxide (KOH) required to saponify the ester and neutralize the free acid contained in 1 g of a sample.

Procedure Unless otherwise specified, proceed as follows: Weigh accurately the specified quantity of the sample, and transfer into a 200-ml flask. Add 25 ml of 0.5mol/L ethanolic potassium hydroxide, exactly measured, and boil gently under a reflux condenser in a water bath for 1 hour. Cool, and then titrate the excess alkali with 0.5 mol/L hydrochloric acid. Confirm the endpoint using a potentiometer or 1 ml of phenolphthalein TS as the indicator. Perform a blank test in the same manner, and calculate the saponification value by the formula:

$$\text{Saponification value} = \frac{(a - b) \times 28.05}{\text{Weight (g) of the sample}}$$

a = volume (ml) of 0.5 mol/L hydrochloric acid consumed in the blank test,
b = volume (ml) of 0.5 mol/L hydrochloric acid consumed in the test.

7. Acid Value

The acid value is the number of mg of potassium hydroxide (KOH) required to neutralize 1 g of a sample.

In the Monographs, such a specification as "not more than 6.0 (Flavoring Substances Tests)" for this test indicates that when determined as directed in the following procedure, the acid value is not more than 6.0.

Procedure Unless otherwise specified, proceed as follows: To about 10 g of the sample, accurately weighed, add about 50 ml of neutralized ethanol, dissolve by warming if necessary, and add several drops of phenolphthalein TS. Using a microburet, titrate while shaking occasionally, with 0.1 mol/L potassium hydroxide to the first light pink color that persists for 30 seconds. To confirm the endpoint, a potentiometer can be used. Calculate the acid value by the following formula:

$$\text{Acid value} = \frac{\left(\begin{array}{c}\text{Volume (ml) of 0.1 mol/L potassium}\\ \text{hydroxide consumed}\end{array}\right) \times 5.611}{\text{Weight (g) of the sample}}$$

8. Phenol Content

The phenol content is the quantity of alkali hydroxide-soluble substances contained in a sample.

Procedure Unless otherwise specified, proceed as follows: Transfer exactly 10 ml of the sample into a 150-ml cassia flask, and add 75 ml of 1 mol/L potassium hydroxide in 3 additions while shaking well. Shake well for an additional 5 minutes, allow to stand for 30 minutes, and gradually add 1 mol/L potassium hydroxide to raise the insoluble oil layer to the graduated part of the cassia flask. Allow to stand for 1 hour, read the volume of the insoluble oil (ml), and calculate the content by the following formula:

$$\text{Phenol content (\%(vol))} = 10 \times (10 - \text{Volume (ml) of insoluble oil})$$

9. Gas Chromatographic Assay of Flavoring Agents

Apparatus Follow the directions given under Gas Chromatography in the General Tests.

Procedure Unless otherwise specified, proceed as directed below. If the sample is a solid, dissolve it in the specified solvent before the test.

Peak Area Percentage Method

This method applies to samples that do not produce any involatile component during storage and whose all components are adequately separated from one another in the chromatogram. The sum of the peak areas of all peaks that appear between 0 and 40 minutes after the injection of the test solution is normalized to 100. The peak area percentage of the component being sought to the sum is determined as the content. When a solid sample is dissolved in a solvent, the same test should be conducted on the solvent to confirm the peak from the solvent, and the sum of peak areas of the peaks, other than the peak of the solvent, is normalized to 100.

Operating conditions (1)

These conditions apply to samples whose boiling point is 150°C or higher.

Detector: Flame ionization detector or thermal conductivity detector.

Column: Use a silicate glass capillary column (30–60 m length and 0.25–0.53 mm internal diameter) coated with a 0.25- to 1-μm thick layer of dimethylpolysiloxane or polyethylene glycol for gas chromatography.

Column temperature: 50–230°C. Raise the temperature from 50°C to 230°C at a rate of 5°C/min, and maintain at 230°C for 4 minutes.

Inlet temperature: 225–275°C.

Detector temperature: 250–300°C.

Split ratio: 30:1–250:1. Adjust the ratio so that no component exceeds the capacity of the column.

Carrier gas: Helium or nitrogen.

Flow rate: Adjust the flow rate so that the peak of the component being sought appears 5–20 minutes after injection.

Operating conditions (2)

These conditions apply to samples whose boiling point is lower than 150°C.

Detector: Flame ionization detector or thermal conductivity detector.

Column: Use a silicate glass capillary column (30–60 m length and 0.25–0.53 mm internal diameter), coated with a 0.25- to 1-μm thick layer of dimethyl polysiloxane or polyethylene glycol for gas chromatography.

Column temperature: 50–230°C. Maintain the temperature at 50°C for 5 minutes, and then raise the temperature to 230°C at a rate of 5°C/min.

Inlet temperature: 125–175°C.

Detector temperature: 250–300°C.

Split ratio: 30:1–250:1. Adjust the ratio so that no component exceeds the capacity of the column.

Carrier gas: Helium or nitrogen.

Flow rate: Adjust the flow rate so that the peak of the component being sought appears 5–20 minutes after injection.

Gas Chromatography

Gas Chromatography is designed to develop in the gaseous state a mixture injected into a column by passing a gaseous mobile phase (carrier gas) through the column, to separate the mixture into individual components by virtue of differences in retention capacity on the stationary phase, and to determine those components. This method is applicable to gases, liquids, and other samples that can be made into vapor. The method is used for identification tests, purity tests, and assays.

Apparatus The apparatus consists generally of a carrier gas introducing port, a sample injection port, a column built in a thermostatic chamber, a detector, and a recorder. If necessary, a gas injection device and a flow regulator are used for a combustion gas, a combustion supporting gas, and an

accessory gas, and a headspace sample injection system is also used. The carrier gas introducing port serves to deliver a carrier gas into the column at a constant flow rate. The detector is a device to detect components with properties different from the carrier gas. Generally, thermal conductivity, flame-ionization, electron-capture, nitrogen-phosphorous, or flame photometric detectors are used. The recorder is a device to record the intensity of the signal produced by the detector.

Procedure Unless otherwise specified, proceed as directed below.
Condition the apparatus in advance, and adjust the detector, the column, the temperature, and the flow rate of the carrier gas to the operating conditions specified in the individual monograph. Using a microsyringe, inject a specified amount of the test solution prepared as specified in the individual monograph into the sample injection port. The separated components are detected by the detector, and the detector output is recorded as a chromatogram by the recorder. Follow the same procedure for the standard solutions and control solution prepared as specified in the same monograph. The identification of the test substance is carried out by confirming that the retention time ("retention time" refers to the time from the injection of the test solution to the emergence of the peak maximum of the component) corresponds to that of the standard sample, or that the retention time does not change, nor does the peak width widen when the standard sample is added.

Determination is performed according to either of the following methods, generally using the peak height or peak area.

(1) Internal Standard Method Prepare several standard solutions containing a constant amount of the internal standard specified in the individual monograph and known, graded amounts of the compound to be determined. From each of the chromatograms obtained by injecting a constant volume of each standard solution, calculate the ratio of the peak height or peak area of the compound to be determined to that of the internal standard. Prepare a calibration curve by plotting the values obtained on a graph, with the peak height (peak area) ratio on the ordinate and the ratio of the amount of the compound to be determined to that of the internal standard, or the added amount of the compound on the abscissa. The calibration curve is usually a straight line through the origin. Next, prepare a test solution containing the same amount of the internal standard as contained in the standard solutions, record the chromatogram under the same conditions as for the preparation of the calibration curve, calculate the peak height (peak area) ratio of the compound to be determined to the internal standard, and then determine the concentration of the compound in the test solution from the calibration curve.

(2) Absolute Calibration Curve Method Prepare standard solutions containing graded amounts of the compound to be determined, and inject a constant volume of each standard solution, exactly measured. Using each of the chromatograms obtained, prepare a calibration curve by plotting the values obtained on a graph, with the peak height or peak area of the compound on the ordinate and the amount of the compound on the abscissa. The calibration curve is usually a straight line through the origin. Next, prepare a test solution as directed in the individual monograph, record the chromatogram under the same conditions as for the preparation of the calibration curve, measure the peak height or peak area of the compound to be determined, and determine the concentration of the compound in the test solution from the calibration curve.

(3) Standard Addition Method Prepare at least four volumetric flasks. Place a constant volume of the sample solution in each flask, and add to all but one of the flasks suitable quantities a standard solution of containing a known concentration of the compound to be determined so as to prepare a series of solutions containing stepwise increasing concentrations of the compound. Dilute exactly all the solutions to a definite volume, and use these solutions as the test solutions. From each of the chromatograms obtained by exactly injecting a constant volume of individual test solutions so that high reproducibility is obtained, measure the peak areas for the individual test solutions. Calculate the concentration of the compound to be determined added to each test solution, and prepare a regression line by plotting the values obtained on a graph, with the added amount (concentration) of the compound on the abscissa and the peak area on the ordinate. Extrapolate the linear regression, and determine the amount of the compound in the test solution from the distance between the coordinate origin and intersection point of the regression line and the abscissa. Generally, the relative standard deviation (variation coefficient) is calculated to confirm the reproducibility of the peak areas of the compound to be determined from individual chromatograms obtained by repeatedly injecting a constant volume of a standard solution. This method is applicable only in the case that the calibration curve is a straight line passing through the coordinate origin when prepared by the absolute calibration curve method. When performing this method, constant conditions must be strictly followed.

In any of the above methods, the peak height or peak area is measured generally by method (i) or (ii), whichever is appropriate.

(i) Method using peak height Use either of the two methods
Peak height method Measure the distance between the peak maximum and the intersection of a perpendicular line drawn from the peak maximum to the horizontal axis of the recording paper and a line linking both side inflection points of the lower end of the peak.
Automatic peak height method Measure the signal obtained from the detector using a data processor device, and determine the peak height.

(ii) Method using peak area Use either of the two methods
Width at half-height method Multiply the peak width at half-height by the peak height.
Automatic integration method Measure the signal obtained from the detector using a data processor device, and determine the peak area.

Heavy Metals Limit Test

The Heavy Metals Limit Test is designed to determine the allowable total limit of heavy metals contained as impurities in a sample. In this test, the "heavy metals" mean the metallic substances that are darkened with sodium sulfide TS in its acidic solution, and their total content is expressed in terms of the quantity of lead (Pb).

In the Monographs, such a specification as "not more than 20 µg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml)" for this test indicates that when determined by weighing 1.0 g of the test substance and proceeding as directed in Method 1, using 2.0 ml of Lead Standard Solution for the preparation of the control solution, the content of heavy metals in the substance is not more than 20 µg/g as Pb.

Preparation of Test Solution and Control Solution Unless otherwise specified, proceed by one of the following methods, as appropriate.

Method 1

Test Solution Weigh the specified amount of the sample, transfer into a Nessler tube, dissolve in about 40 ml of water, add 2 ml of diluted acetic acid (1 in 20), and add then water to make 50 ml.

Control Solution Measure the specified amount of Lead Standard Solution, transfer into another Nessler tube, add 2 ml of diluted acetic acid (1 in 20), and then add water to make 50 ml.

Method 2

Test Solution Weigh the specified amount of the sample, transfer into a quartz or porcelain crucible, place a cover on loosely, and carbonize by gently heating. Cool, add 2 ml of nitric acid and 5 drops of sulfuric acid, heat until white fumes no longer evolve, and ignite at 450–550°C to incinerate. Cool, add 2 ml of hydrochloric acid, evaporate to dryness on a water bath, add 3 drops of hydrochloric acid to the residue, add 10 ml of boiling water, and warm for 2 minutes. Cool, add 1 drop of phenolphthalein TS, and add ammonia TS until the solution becomes slightly red. Next, transfer the solution into a Nessler tube using water, add 2 ml of diluted acetic acid (1 in 20), and then add water to make 50 ml.

Control Solution Place 2 ml of nitric acid, 5 drops of sulfuric acid, and 2 ml of hydrochloric acid into a crucible of the same quality as used for the sample, heat to evaporate to dryness, and add 3 drops of hydrochloric acid to the residue. Next, proceed as directed in the preparation for the test solution, transfer the resulting solution into another Nessler tube, add the specified amount of Lead Standard Solution and 2 ml of diluted acetic acid (1 in 20), and then add water to make 50 ml.

If the test solution is not clear, filter both the test solution and the control solution under the same conditions.

Method 3

Test Solution Weigh the specified amount of the sample, place into a quartz or porcelain crucible, heat gently and carefully, and then ignite to incinerate. Cool, add 1 ml of aqua regia, and evaporate to dryness on a water bath. Moisten the residue with 3 drops of hydrochloric acid, add 10 ml of boiling water, and warm for 2 minutes. Next, add 1 drop of phenolphthalein TS, ammonia TS until the solution becomes slightly red, and2 ml of diluted acetic acid (1 in 20), Filter the solution into a Nessler tube if necessary, wash with 10 ml of water, add washings to the Nessler tube, and add water to make 50 ml. If filtration is not necessary, directly transfer the resulting solution into a Nessler tube, and add water to make 50 ml.

Control Solution Place 1 ml of aqua regia into a crucible of the same quality as for the sample, and evaporate on a water bath. Proceed as directed in the preparation for the test solution, transfer the resulting solution into a Nessler tube, and add specified amount of Lead Standard Solution and water to make 50 ml. If filtration is conducted, combine the filtrate and washings into a Nessler tube, and add the specified amount of Lead Standard Solution and water to make 50 ml.

Method 4

Test Solution Weigh the specified amount of the sample, place into a platinum, quartz, or porcelain crucible, add 10 ml of a solution of magnesium nitrate in ethanol (1 in 10), and mix. Ignite and burn the ethanol, and carbonize by heating gradually. Cool, add 1 ml of sulfuric acid, heat carefully, and ignite at 500–600°C to incinerate. Moisten with a small amount of sulfuric acid if any carbonized residue is present, and ignite to incinerate. Cool, dissolve the residue with 3 ml of hydrochloric acid, and evaporate to dryness on a water bath. Moisten the residue with 3 drops of hydrochloric acid, add 10 ml of water, and dissolve by warming. Add 1 drop of phenolphthalein TS, add ammonia TS until the solution becomes slightly red, and transfer the solution into a Nessler tube using water. Add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Place 10 ml of a solution of magnesium nitrate in ethanol (1 in 10) into a crucible of the same quality as for the sample, ignite, and burn the ethanol. Cool, add 1 ml of sulfuric acid, proceed as directed in the preparation for the test solution, and transfer the resulting solution into another Nessler tube. Add the specified amount of Lead Standard Solution and 2 ml of diluted acetic acid (1 in 20), and then add water to make 50 ml.

If the test solution is not clear, filter both the test solution and the control solution under the same conditions.

Procedure Unless otherwise specified, add 2 drops of sodium sulfide TS to each of the test solution and the control solution, mix thoroughly, and allow to stand for 5 minutes. Next, examine the tubes from above and from the side against a white background to compare the colors of the two solutions. The color of the test solution is not darker than that of the control solution.

Inductively Coupled Plasma-Atomic Emission Spectrometry

Inductively Coupled Plasma-atomic Emission Spectrometry is designed to determine the amount (concentration) of the analyte element in a sample from the emission intensity of the atomic spectral line that is obtained by atomizing and

exciting the element in inductively coupled plasma (ICP).

Apparatus Normally, the apparatus consists of an excitation source, a sample injection port, a light emitting device, a spectroscopic system, a photometric system, and an indicating and recording system. The excitation source is composed of an electric power source, a control system, and circuit to supply and control the electric energy which excites and emits the element. The source also includes a gas source and a cooling system. The sample injection port is composed of a nebulizer and a spray chamber. The light emitting device is composed of a torch and a high-frequency induction coil. The spectroscopic system is composed of a light-converging device and a spectroscope, such as a diffraction grating. The photometric system is composed of a detector and a signal processing system. The indication and recording system is composed of a display and a recording device. There are three analytical types: single-element sequential analysis, multi-element sequential analysis (these two types use a wavelength scanning spectroscope), and a multiple simultaneous analysis (using a wavelength-fixed polychromator).

Procedure Confirm that all live parts are normal. Switch on the excitation source and the cooling system. When a vacuum-type spectroscope is used to measure the emission line in the vacuum-ultraviolet region, purge the air in the light-path between the light emitting device and the spectroscope with argon or nitrogen gas. Adjust the flow rate of argon or nitrogen gas to the specified rate, switch on the high frequency power, and generate the plasma. Correct the wavelength of the spectroscope with the spectral line of a mercury lamp. Introduce a specified amount of the test solution prepared as specified in the individual monograph, and measure the emission intensity of an appropriate emission line of the analyte element. Follow the same procedure for the standard solutions and the control solution prepared as specified.

Usually, determination is done using one of the following methods. In the determination, the interference and background should be considered.

(1) Calibration Curve Method Prepare at least three standard solutions of different concentrations of the element to be determined, measure the emission intensities of these standard solutions, and prepare a calibration curve from the values obtained. Next, measure the emission intensity of the test solution, adjusted to be within the concentration range of the standard solutions, and determine the amount (concentration) of the element from the calibration curve.

(2) Standard Addition Method To equal volumes of at least three test solutions, add suitable quantities of a standard solution containing a known concentration of the element to be determined so as to prepare solutions containing stepwise increasing amounts of the element, and then add a solvent to make a constant volume. Measure the emission intensity of each solution, and plot the values obtained on a graph, with the added amount (concentration) of the element to be determined on the abscissa and the emission intensity on the ordinate. Extrapolate the regression line formed by joining the points on the graph, and determine the amount (concentration) of the element in the test solution from the distance between the origin and the intersection point of the regression line and the abscissa. This method is applicable only when the calibration curve drawn as directed in method (1) above is a straight line passing through the origin.

(3) Internal Standard Method Prepare several standard solutions containing a constant amount of the specified internal standard element, and known graded amounts of the element to be determined. For these solutions, measure the emission intensities of the element to be determined and internal standard element at the analytical wavelength of each element under the same measuring conditions, and obtain the ratio of the emission intensity of the element to be determined to that of the internal standard element for each solution. Prepare a calibration curve by plotting the values obtained on a graph, with the amount (concentration) of the element on the abscissa and the emission intensity ratio on the ordinate. Next, prepare a test solution by adding the same amount of internal standard element as contained in the standard solutions. Proceed under the same conditions as for the preparation of the calibration curve, obtain the emission the intensity ratio of the element to be determined to the internal standard element, and determine the amount (concentration) of the element in the test solution from the calibration curve.

Note: Avoid the use of reagents, test solutions, and gases that may interfere with the determination.

Infrared Spectrophotometry

Infrared Spectrophotometry is designed to identify or determine a sample by making use of the property that the infrared absorption spectrum of each substance is specific to the chemical structure of the substance. This method measures the amounts of infrared radiation absorbed by an analyte in the sample at various wavenumbers when infrared radiation in the range of 4000–600 cm^{-1} passes through the sample. An infrared spectrum is represented as a graph plotted with the wavenumber (cm^{-1}) on the abscissa and the transmittance (%) or absorbance on the ordinate.

Unless otherwise specified, in a comparison between the spectrum of a sample and the spectrum of the Reference Standard of the substance to be identified or the Reference Spectrum, which is given in section C.11 of this publication, when both spectra exhibit almost the same intensities of absorption at the same wavenumbers, the sample is confirmed as identical to the substance. When the spectrum of the sample measured in the solid-state is different from the Reference Standard spectrum or the Reference Spectrum, measurement should be performed again, using the sample and the Reference Standard that are treated according to the conditions specified in the individual monograph.

When comparing the sample spectrum with the Reference Spectrum, any difference in resolution between the instruments used should be taken into consideration because these two spectra are usually measured by different instruments. The wavenumber variation based on the difference in resolution between two instruments is greatest in the wavenumber range between 4000 cm^{-1} and 2000 cm^{-1}. When Fourier-transform infrared spectrophotometers are used, the wave-

number accuracy is invariable through the scanning range because their resolution is constant, regardless of the wavenumber.

Section C.11 of JSFA-VIII contains Infrared Reference Spectra (in the range between 4000 cm^{-1} and 600 cm^{-1}) of substances for which the individual monographs specify identification tests by infrared spectrophotometry. These substances exclude those for which identification by absorption wavenumbers is specified.

Apparatus and Adjustment Use a dispersive infrared spectrophotometer or a Fourier-transform infrared spectrophotometer. Adjust the spectrophotometer as indicated for the instrument, and confirm that the resolution, transmittance reproducibility, and wavenumber reproducibility comply with the test given below. When the spectrum is measured on a 0.04-mm thick polystyrene film, the transmittance difference of the obtained spectrum should be 18% or more between the minimum absorption at about 2870 cm^{-1} and the maximum absorption at about 2850 cm^{-1}. Also, it should be 12% or more between the minimum at about 1589 cm^{-1} and the maximum at about 1583 cm^{-1}.

The wavenumber scale is usually calibrated by the use of some of the characteristic absorption wavenumbers (cm^{-1}) of a polystyrene film, shown below. The numbers in parentheses indicate the acceptable range of these values.

3060.0 (± 1.5) 2849.5 (± 1.5) 1942.9 (± 1.5) 1601.2 (± 1.0)
1583.0 (± 1.0) 1154.5 (± 1.0) 1028.3 (± 1.0)

When a dispersive infrared spectrophotometer is used, the acceptable range is ± 2.0 for 1601.2 cm^{-1} and ± 2.0 for 1028.3 cm^{-1}.

The transmittance reproducibility and wavenumber reproducibility should satisfy the following requirements when the absorption of a polystyrene film is measured twice at several wavenumbers in the range of 3000–1000 cm^{-1}: The difference in transmittance is within 0.5%, and the wavenumber difference is within 5 cm^{-1} at about 3000 cm^{-1} and within 1 cm^{-1} at about 1000 cm^{-1}.

Preparation of Samples and Measurement Unless otherwise specified, when individual monographs require the test to be conducted using a dried sample, dry the sample before use, according to the conditions specified for the Loss on Drying Test in the corresponding monograph. Prepare the sample for measurement according to an appropriate preparation method among those given below so that the transmittance of major absorption bands of the sample is in the range of 5–80%. Sodium chloride and potassium bromide are usually used for optical plates. Generally, for double-beam instruments, the reference cell or material is placed in the reference beam and the spectrum is measured at the same time as the specimen. For single-beam instruments, it is placed in the same optical path as the specimen and the spectrum is measured separately from the specimen under the same operation conditions. What reference should be used depends on the sample preparation methods. The background absorption of the atmosphere may be utilized.

Unless otherwise specified in the individual monographs, the spectrum is usually determined between 4,000–600 cm^{-1}. The spectrum should be scanned under the same operating conditions as when the accuracy of resolution, wavenumber scale, and wavenumbers was confirmed.

(1) Potassium Bromide Disk Method Powder 1 to 2 mg of a solid sample with an agate mortar, add 0.10–0.20 g of potassium bromide for infrared spectrophotometry, rapidly triturate the mixture while being careful to prevent moisture absorption, and transfer into a die (disk forming container). Press it at 50 to 1,00 kN (5,000–10,000 kg)/cm^2 under reduced pressure of not more than 0.67 kPa for 5 to 8 minutes to make a transparent disk. Prepare a potassium bromide reference disk in the same manner as the sample disk.

(2) Solution Method Place the sample solution prepared by the method directed in the individual monographs in a fixed cell for liquid, and measure the spectrum against the reference. Normally, the solvent used for preparing the sample solution is used as the reference. The solvent used in this method should be one that does not interact or chemically react with the sample to be examined and that does not damage the optical plate. The thickness of the fixed cell is generally 0.1 mm or 0.5 mm.

(3) Paste Method Powder 5–10 mg of a solid sample with an agate mortar, and unless otherwise specified, triturate the sample with 1–2 drops of liquid paraffin to produce a paste. Spread the paste on the center of an optical plate, place another optical plate on top of the paste, making sure no air is trapped under the plate, and measure the spectrum.

(4) Liquid Film Method Hold 1 to 2 drops of a liquid sample between two optical plates, and examine the liquid layer between the plates. If the liquid layer needs to be thicker, place aluminum foil or a similar material between the two optical plates to produce a thicker space for the sample between the plates.

(5) Thin Film Method Use a thin film as is or use a thin film sample prepared as directed in the individual monographs.

(6) Gas Sample Measurement Put a sample gas in a gas cell with a light path of 5 to 10 cm in length, previously evacuated, under pressure specified in the individual monograph, and measure its spectrum. A long cell with a light path not shorter than 1 m can also be used if necessary.

Ion Chromatography

Ion chromatography is designed to develop a mixture injected into a column packed with a suitable stationary phase, such as ion exchanger, by passing an eluent as a mobile phase through the column, to separate it into individual components by virtue of differences in the ion-exchange capacity of components, and to determine those components. This method is applicable to liquids or substances that can be made into solutions, and is usually used for identification tests, purity tests, and assays.

Apparatus The apparatus consists generally of a pumping system to deliver the mobile phase, a sample injection port,

a column, a detector, and a recorder. The column is maintained at a constant temperature, using a thermostatic chamber or other appropriate equipment. The pump is capable of delivering the mobile phase into the column, connecting tube, and other devices at a constant flow rate.

The detector detects components with different properties from the mobile phase. It generally produces electric signals proportional to the concentration of substances at amounts of a few micrograms or less. Electric conductometers or UV spectrophotometers are commonly used as detectors.

The recorder records the intensities of the signals produced by the detector. When an electrical conductometer is used as the detector, a suppressor can be placed in front of the conductometer. The suppressor is used to reduce the electric conductivity of the mobile phase and amplify the ratio of the signals to the noises.

Procedure Condition the apparatus in advance, adjust the mobile phase, the column, the detector, and the flow rate of the mobile phase to the operating conditions specified in the individual monograph, and equilibrate the column at a specified temperature. Using a microsyringe or sample valve, inject a specified amount of the test solution prepared as specified in the individual monograph into the sample injection port. The separated components are detected by the detector, and the detector output is recorded as a chromatogram on the recorder. Follow the same procedure for the standard solutions and control solution prepared as specified in the individual monograph.

Identification of the substance is carried out by confirming that the retention time corresponds to that of the standard sample, or that the retention time does not change nor does the peak width widen when the standard sample is added

Determination is usually performed by method (1) or (2) using the peak height or peak area.

(1) Internal Standard Method Prepare several standard solutions containing a constant amount of the internal standard specified in the individual monograph and known, graded amounts of the compound to be determined. From each of the chromatograms obtained by injecting a constant volume of each standard solution, calculate the ratio of the peak height or peak area of the compound to be determined to that of the internal standard. Prepare a calibration curve by plotting the values obtained on a graph, with the peak height (peak area) ratio on the ordinate and the ratio of the amount of the compound to that of the internal standard, or the added amounts of the compound on the abscissa. The calibration curve is usually a straight line through the origin. Next, prepare a test solution containing the same amount of the internal standard as contained in the standard solutions, record the chromatogram under the same conditions as for the preparation of the calibration curve, calculate the peak height (peak area) ratio of the compound to be determined to the internal standard, and determine the concentration of the compound in the test solution from the calibration curve.

(2) Absolute Calibration Curve Method Prepare several standard solutions containing graded amounts of the compound to be determined, and inject a constant volume of each standard solution. Using the chromatograms obtained, prepare a calibration curve by plotting the values obtained on a graph, with the peak height or peak area of the compound on the ordinate and the amount of the compound on the abscissa. The calibration curve is usually a straight line through the origin. Next, prepare a test solution as specified in the individual monograph, record the chromatogram under the same conditions as for the preparation of the calibration curve, and measure the peak height or peak area of the compound to be determined. Determine the concentration of the compound in the test solution from the calibration curve.

Unless there is a particular problem, when an anion standard solution is prepared, use its sodium or potassium salt, and when a cation standard solution is prepared, use its chloride or nitrate.

For either method above, the peak height or peak area is usually measured using method (i) or (ii), whichever is appropriate.

(i) Method using peak height Use either of the two methods.

Peak height method Measure the distance between the peak maximum and the intersection of a perpendicular line drawn from the peak maximum to the horizontal axis of the recording paper and a line linking both side inflection points of the lower end of the peak.

Automatic peak height method Measure the signal from the detector, and then determine the peak height using a data processor system.

(ii) Method using peak area Use either of the two methods.

Width at half-height method Multiply the peak width at half-height by the peak height.

Automatic integration method Measure the signal from the detector, and determine the peak area using a data processing system.

Iron Limit Test

The Iron Limit Test is designed to determine the allowable limit of iron compounds contained as impurities in a sample.

In the Monographs, such a specification as “not more than 10 μg/g as Fe (1.0 g, Method 1, Control solution Iron Standard Solution 1.0 ml)” for this test indicates that when determined by weighing 1.0 g of the test substance and proceeding as directed in Method 1, using 1.0 ml of Iron Standard Solution in the preparation of the control solution, the iron content of the substance is not more than 10 μg/g as Fe.

Preparation of Test Solutions and Control Solutions Unless otherwise specified, proceed according to Method 1 or Method 2, whichever is appropriate.

Method 1

Test Solution Weigh the quantity of the sample specified in the individual monograph, add 30 ml of acetic acid-sodium acetate buffer solution for iron limit test (pH 4.5), and dissolve by warming if necessary.

Control Solution To the amount of Standard Iron Solution specified in the individual monograph, add 30 ml of acetic-sodium acetate buffer solution for iron limit test (pH 4.5).

Method 2

Test Solution Weigh the quantity of the sample specified in the individual monograph, add 10 ml of dilute hydrochloric acid (1 in 4), and dissolve by warming if necessary. Dissolve 0.5 g of tartaric acid, and add one drop of phenolphthalein TS. Add ammonia TS until the solution develops a pale red color. Add 20 ml of acetic acid-sodium acetate buffer solution for iron limit test (pH 4.5).

Control Solution To the amount of Standard Iron Solution specified in the individual monograph, add 10 ml of dilute hydrochloric acid (1 in 4), and proceed as directed for the test solution.

Procedure Unless otherwise specified, transfer the test solution and the control solution into separate Nessler tubes, add 2 ml of a solution of ascorbic acid for iron limit test (1 in 100) to each, mix well, and allow to stand for 30 minutes. Add 1 ml of a solution of α,α′-dipyridyl in ethanol (1 in 200), add water to make 50 ml, and allow to stand for 30 minutes. Finally, compare the colors of both solutions against a white background. The color of the test solution is not deeper than the control solution.

Lead Limit Test (Atomic Absorption Spectrophotometry)

The Lead Limit Test is designed to determine the allowable limit of lead contained in a sample by atomic absorption spectrophotometry.

Method 1

Preparation of Test Solution and Control Solution Unless otherwise specified, proceed as directed below.

Test Solution Weigh the quantity of the sample specified in the individual monograph, transfer into a platinum or quartz crucible, moisten with a small amount of sulfuric acid, and ignite slowly at a temperature as low as possible until the sample is almost completely incinerated. Cool, add 1 ml of sulfuric acid, heat slowly, and ignite at 450–550°C to incinerate. Dissolve the residue in a small amount of diluted nitric acid (1 in 150), and add diluted nitric acid (1 in 150) to make 10 ml.

Control Solution Measure 1.0 ml of Lead Standard Solution, and add diluted nitric acid (1 in 150) to make 10 ml.

Procedure Unless otherwise specified, proceed as directed below.

Measure the absorbance of the test solution and the control solution as directed under Atomic Absorption Spectrophotometry (Flame Atomic Absorption Spectrophotometry) using the conditions given below. The absorbance of the test solution does not exceed that of the control solution.

Operating Conditions
Light source: Lead hollow cathode lamp.
Analytical line wavelength: 283.3 nm.
Supporting gas: Air.
Inflammable gas: Acetylene.

Method 2

Preparation of Test Solution Unless otherwise specified, proceed as directed below.

Weigh the quantity of the sample specified in the individual monograph, transfer into a polytetrafluoroethylene decomposition-vessel, add 0.5 ml of nitric acid to dissolve, seal the vessel, and heat at 150°C for 5 hours. After cooling, add water to make exactly 5 ml.

Procedure Unless otherwise specified, perform the test as directed below.

Prepare at least 3 solutions containing the same volume of the test solution, and perform the test as directed in the standard addition method under Atomic Absorption Spectrophotometry (Electrothermal Atomic Absorption Spectrophotometry) using the operating conditions below. The standard solution is prepared by measuring exactly a suitable volume of Standard Lead Solution and adding water. Add the same volume of palladium nitrate TS to the sample solution, and mix well. Perform a blank determination with a solution prepared by adding water to exactly 10 ml of nitric acid to make exactly 100 ml, and make any necessary correction.

Operating Conditions
Light source: Lead hollow cathode lamp.
Analytical line wavelength: 283.3 nm.
Temperature for drying: 110°C.
Temperature for incineration: 600°C.
Temperature for atomizing: 2,100°C.

Liquid Chromatography

Liquid Chromatography is designed to develop a mixture injected into a column packed with a suitable stationary phase by passing a liquid mobile phase through the column under pressure using a pump, to separate the mixture into individual components by virtue of differences in retention capacity on the stationary phase, and to determine the components. This method is applicable to liquids or substances that can be made into solutions, and is used for identification tests, purity tests, assays, and other tests.

Apparatus The apparatus consists generally of a pumping system, a sample injection port, a column, a detector, and a recorder. The chromatographic column is maintained at a constant temperature by a thermostat if necessary. The pumping system serves to deliver the mobile phase into the column and connecting tube at a constant flow rate.

The detector detects components with different properties from the mobile phase, and produces signals proportional to the concentration of substances at amounts of a few micrograms or less. Ultraviolet-visible spectrophotometers, differential refractometers, or fluorescence spectrophotometer are commonly used as detectors. The recorder records the intensities of the signals produced by the detector.

Procedure Condition the apparatus in advance, adjust the mobile phase, the column, the detector, and the flow rate of the mobile phase to the specified operating conditions as

directed in the individual monograph, and equilibrate the column at the specified temperature. Using a sample valve or a microsyringe, inject a specified amount of the test solution prepared as directed in the individual monograph into the sample injection port. The separated components are detected by the detector and the detector output is recorded as a chromatogram on the recorder. Follow the same procedure for the standard solutions and control solution.

Identification of the substances is carried out by confirming that the retention time corresponds to that of the standard sample, or that the retention time does not change nor does the peak width widen when the standard sample is added. Determination is generally performed according to either of the methods below using the peak height or peak area.

(1) Internal Standard Method Prepare several standard solutions containing a constant amount of the internal standard specified in the individual monograph and known, graded amounts of the compound to be determined. From each of the chromatograms obtained by injecting a constant volume of each standard solution, calculate the ratio of the peak height or peak area of the compound to be determined to that of the internal standard. Prepare a calibration curve by plotting the values obtained on a graph, with the peak height (peak area) ratio on the ordinate and the ratio of the amount of the compound to that of the internal standard, or the added amount of the compound on the abscissa. The calibration curve is usually a straight line through the origin. Next, prepare a test solution containing the same amount of the internal standard as contained in the standard solutions, record the chromatogram under the same conditions as for the preparation of the calibration curve, calculate the peak height (peak area) ratio of the compound to be determined to the internal standard, and determine the concentration of the compound in the test solution from the calibration curve.

(2) Absolute Calibration Curve Method Prepare standard solutions containing graded amounts of the compound to be determined. Inject a constant volume of each standard solution. Using the chromatograms obtained, prepare a calibration curve by plotting the values obtained on a graph, with the peak height or peak area of the compound on the ordinate and the amount of compound on the abscissa. The calibration curve is usually a straight line through the origin. Next, prepare a test solution as specified in the individual monograph, record the chromatogram under the same conditions as for the preparation of the calibration curve, measure the peak height or peak area of the component to be determined, and determine the concentration of the compound in the test solution from the calibration curve.

For either method above, the peak height or peak area is generally measured using method (i) or (ii), whichever is appropriate.

(i) Method using peak height Use either of the two methods.

Peak height method Measure the distance between the peak maximum and the intersection of a perpendicular line drawn from the peak maximum to the horizontal axis of the recording paper and a line linking both side inflection points of the lower end of the peak.

Automatic peak height method Determine the signal from the detector as the peak height using a data processor system.

(ii) Method using peak area Use either of the following methods.

Width at half-height method Multiply the peak width at half-height by the peak height.

Automatic integration method Measure the signal from the detector and, then determine the peak area using a data processing system.

Loss on Drying

The Loss on Drying Test is designed to measure the amount of water and volatile matter in a sample when the sample is dried under the conditions specified in the individual monograph.

In the Monographs, such a specification as "not more than 0.50% (105°C, 3 hours)" for this test indicates that when determined by drying 1 to 2 g of the sample, accurately weighed, at 105°C for 3 hours, the loss in weight is not more than 0.50% of the sample. Also such a specification as "not more than 0.50% (0.5 g, not more than 1.3 kPa, 24 hours)" indicates that when determined by placing about 0.5 g of the sample, accurately weighed, in a desiccator with silica gel as the desiccant and drying under pressure at 1.3 kPa or less for 24 hours, the loss in weight is not more than 0.50% of the sample.

Procedure Dry a weighing bottle for about 30 minutes under the specified conditions, allow to cool in a desiccator if heated, and weigh accurately. If the sample consists of large crystals or lumps, promptly ground into particles not larger than about 2 mm in diameter. Unless otherwise specified, place 1 to 2 g of the prepared sample into the weighing bottle, spread to a layer of not more than 5 mm thick, and weigh the bottle containing the sample accurately. Place the bottle in a drying oven, remove the stopper (placing it nearby), dry under the specified conditions, stopper again, take the bottle out of the oven, and weigh again. If heated, unless otherwise specified, allow to cool in a desiccator, and weigh accurately. If the sample melts at a temperature lower than the specified drying temperature, dry the bottle with the sample at a temperature 5–10°C lower than the melting temperature for 1 to 2 hours, and then dry under the specified conditions.

Loss on Ignition

The Loss on Ignition Test is designed to measure the amount of moisture and other impurities lost when a sample is ignited under the conditions specified in the individual monograph.

In the Monographs, such a specification as "18.0–24.0%" for this test indicates that when determined by igniting 1 to 2 g of the sample, accurately weighed, at 450–550°C for 3 hours, the loss in weight is 18.0–24.0% of the sample. Also, such a specification as "not more than 10% (0.5 g,

1,000°C, 30 minutes)" indicates that when determined by igniting about 0.5 g of the sample, accurately weighed, at 1,000°C, for 30 minutes, the loss in weight is not more than 10% of the sample. When the requirement "dried sample" is given in the Monographs, the sample to be used for the test should be previously dried under the conditions specified for the Loss on Drying Test in the individual monograph.

Procedure Ignite a platinum, quartz, or porcelain crucible under the conditions specified in the individual monograph for about 30 minutes, allow to cool in a desiccator, and weigh accurately.

If the sample consists of large crystals or lumps, rapidly grind them into particles not larger than about 2 mm in diameter. Unless otherwise specified, place 1 to 2 g of the sample in the crucible previously ignited, and accurately weigh the crucible containing the sample. Next, transfer the crucible into an electric furnace, and unless otherwise specified, ignite at 450–550°C for 3 hours, allow to cool in a desiccator, and weigh accurately.

Melting Point

The melting point means the temperature at which or the temperature range within which a solid completely melts when determined by either of the methods given below. For convenience of measurement, solid samples are classified into the following two types:

Class 1 substances: Those that can easily be reduced into a powder.

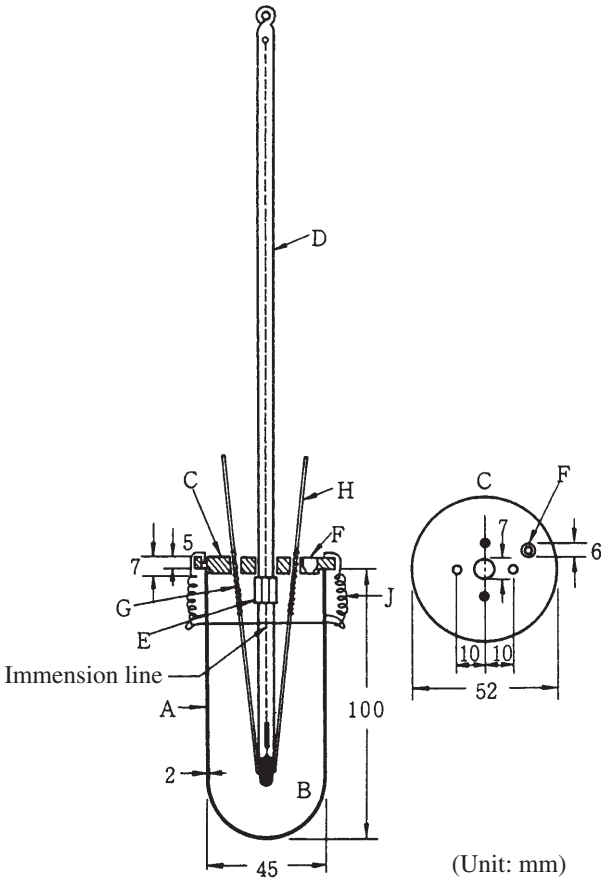
Class 2 substances: Those that cannot easily be reduced into a powder, such as fats, fatty acids, paraffins, and waxes.

(1) Class 1 Substances

Apparatus Use the apparatus illustrated in the right column.

Procedure Finely pulverize the sample, and unless otherwise specified, dry in a desiccator for about 24 hours. When the stipulation"dried sample"is given in the Monographs, dry the sample under the conditions specified for the Loss on Drying Test in the individual monograph. Charge capillary tube H with the sample as tightly as practical to form a layer of 2.5 to 3.5 mm in thickness. When the stipulation "sealed tube"is given in the Monographs, close one open end of the tube. When the stipulation"vacuum sealed tube" is given, close one open end of the tube by heating gently under reduced pressure not exceeding 0.67 kPa.

Heat bath fluid B slowly until the temperature rises to about 10°C below the expected melting point, adjust the immersion line of thermometer D at the same level as the meniscus of the bath fluid, and insert capillary tube H containing the sample into coil spring G so that the position of the sample in H is level with the middle of the mercury bulb of thermometer D. Next, continue heating so that the temperature rises at a rate of about 3°C per minute until the temperature rises to about 5°C below the expected melting point, and continue heating at a rate of increase of 1°C per minute.



A: Heating vessel (hard glass)
B: Bath fluid (use clear silicone oil with a viscosity of 50 to 100 mm²/s at ordinary temperature.)
C: Teflon stopper
D: Rod thermometer with an immersion line
For a melting point lower than 50°C, use Type 1;
for not less than 40°C and less than 100°C, Type 2;
for not less than 90°C and less than 150°C, Type 3;
for not less than 140°C and less than 200°C, Type 4;
for not less than 190°C and less than 250°C, Type 5;
for not less than 240°C and less than 320°C, Type 6.
F: Vent for adjustment of the bath fluid volume
G: Coiling spring
H: Capillary tube (a 120 mm long hard glass tube, one end of which is sealed, with an internal diameter of 0.8 to 1.2 mm and with walls 0.2 to 0.3 mm thick)
J: Spring to fasten the Teflon stopper

The temperature at which slight wetting or disintegration is observed in the contact surface of the sample and the inside wall of H is defined as the temperature at the start of melting. The temperature at which the sample melts completely and becomes transparent is defined as the temperature at the end of melting, which is referred to as the melting point.

(2) Class 2 Substances

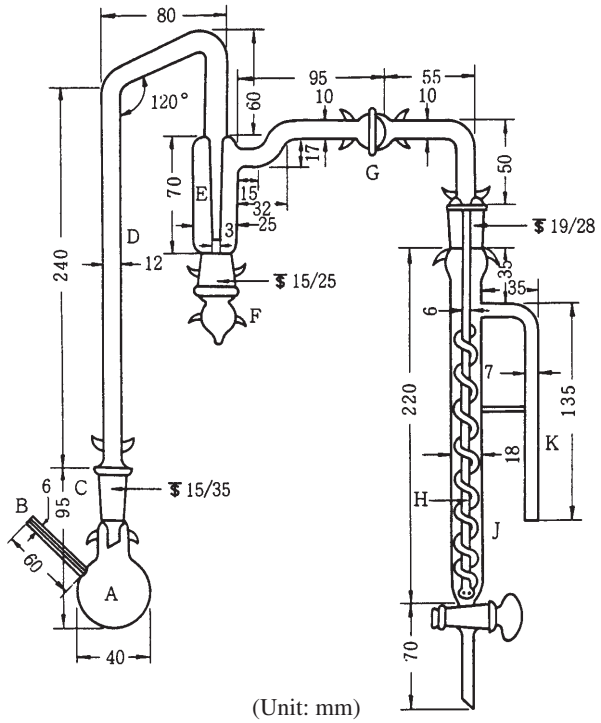
Procedure Melt the sample at as low a temperature as practical, and then aspirate it up into the capillary tube (the

same one as used in (1) except that both ends are open) to a depth of about 10 mm. Allow to stand at about 10°C for 24 hours or cool for at least 2 hours with ice. Attach the tube to the thermometer by a rubber band at the position where the part of the tube containing the sample is level with the middle part of the mercury bulb. Place it in a beaker of water to such a position that the upper end of the sample is about 10 mm below the water surface. Heat the water with constant stirring until the temperature rises to about 5°C below the expected melting point, and continue heating at a rate of increase of 1°C per 2 minutes. The melting point is defined as the temperature at which the sample is observed to rise in capillary tube H.

Methoxy Determination

Methoxy Determination is designed to quantify the methoxy group in a sample by heating the sample with hydriodic acid, oxidizing the methyl iodide produced with bromine, and titrating the iodide acid produced with sodium thiosulfate solution.

Apparatus Use the apparatus illustrated in the figure below.



(Unit: mm)

A: Decomposition flask
B: Gas-inlet tube
C: Ground-glass joint
D: Air condenser
E: Gas scrubber
F: Glass stopper
G: Ground-glass ball joint
H: Gas duct
J: Absorption tube
K: Gas-exhaust tube

Preparation of Scrubbing Solution and Absorbing Solutions

Rinsing solution (*Scrubbing Solution*) Weigh 1 g of red phosphorus, and suspend in 100 ml of water.

Absorbing solution Weigh 15 g of potassium acetate, and dissolve in 150 ml of a 9:1 mixture of acetic acid/acetic anhydride. To 145 ml of this solution, add 5 ml of bromine. Prepare fresh before use.

Procedure Put the rinsing solution into gas scrubber E up to about half the height of the scrubber, and transfer about 20 ml of the absorbing solution into absorption tube J. Weigh accurately an amount of the sample, equivalent to about 6.5 mg as methoxy group (CH_3O: 31.03), transfer to decomposition flask A, and add boiling chips and about 6 ml of hydriodic acid. Moisten ground-glass joint C of A with 1 drop of hydriodic acid, and connect A to condenser D. Assemble the apparatus by connecting ground-glass ball joint G using a suitable silicone resin. Pass nitrogen or carbon dioxide through gas-inlet tube B, and adjust the flow rate using a suitable pressure-regulating device so that bubbles appear in E at a rate of 2 bubbles per second. Place A in an oil bath, heat the flask so that the temperature of the bath reaches 150°C in 20 or 30 minutes, and continue to boil for another 60 minutes. Remove the oil bath, allow the flask to cool with the gas passing through it, and remove G after cooling. Drain the contents of J into a 500-ml Erlenmeyer flask with a ground-glass stopper containing 10 ml of sodium acetate solution (1 in 5), wash the tube with water several times, add the washings to the flask, and dilute to about 200 ml with water. Add formic acid dropwise while shaking until the red color of the bromine disappears, and add another 1 ml of formic acid. Next, add 3 g of potassium iodide and 15 ml of diluted sulfuric acid (1 in 20), stopper, shake gently, allow to stand for 5 minutes, and titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: 1 ml of starch TS). Perform a blank test in the same manner, and make any necessary correction.

1 ml of 0.1 mol/L sodium thiosulfate = 0.5172 mg of CH_3O

Microbial Limit Tests

The Microbial Limit Tests are used for the qualitative and quantitative estimations of specific viable microorganisms present in samples. They include tests for total viable count (bacteria and fungi) and *Escherichia coli*. Great care must be taken when performing the tests to avoid microbial contamination from the outside. When test samples have antimicrobial activity or contain antimicrobial substances, the antimicrobial properties must be eliminated by dilution, filtration, neutralization, inactivation, or other appropriate means. Samples should be prepared by mixing multiple portions randomly chosen from individual ingredients or products. When samples are diluted with fluid medium, the tests must be conducted immediately. Due attention must be paid to ensure effective quality control and prevent biohazard accidents.

1. Total Viable Aerobic Count
This test determines mesophilic bacteria and fungi that can grow in aerobic conditions. Psychrophilic, thermophilic, basophilic, and anaerobic bacteria, and other microorganisms that require specific ingredients for growth may give a negative result, even if they exist in samples in a significant number. There are four methods for this test: membrane filtration method, pour plate method, spread plate method, and serial dilution method (most probable number method). An appropriate method should be used, depending on objectives. Automated methods may be used if they are comparable or superior in sensitivity and accuracy to the methods given in the Microbial Limit Tests. Different culture media and incubation temperatures are needed for the growth of bacteria and fungi (molds and yeasts). The serial dilution method is applicable only to bacteria.

Preparation of Test Fluids To dissolve or dilute the sample, use phosphate buffer (pH 7.2), sodium chloride–peptone buffer solution, or fluid medium used for the test. Unless otherwise specified, use 10 g or 10 ml of the sample. However, a different quantity or volume of the sample may be used, depending on the nature of the sample. Adjust the test fluid to pH 6–8. Use the test fluid within one hour of preparation.

Fluid Samples or Soluble Solid Samples Take 10 g or 10 ml of the sample and mix with the buffer or fluid medium given above to make 100 ml. Use this mixture as the test fluid. For a fluid sample containing insoluble substances, shake well just before mixing to make it homogeneous.

Insoluble Solid Samples Take 10 g of the sample, grind to a fine powder, and suspend in the buffer or fluid medium given above to make 100 ml. Use this suspension as the sample fluid. A larger volume of the buffer or fluid medium than specified here may be used to make a suspension, depending on the nature of the sample. If necessary, a blender may be used to disperse the insoluble particles uniformly in the suspension. Also, an appropriate surfactant (e.g., 0.1% (w/v) polysorbate 80) may be added to help dissolve the sample.

Fatty Samples For semisolid samples and liquids consisting mainly of lipid, take 10 g or 10 ml of the sample, emulsify in the buffer or fluid medium given above using a surfactant, such as polysorbate 20 or polysorbate 80, and make to 100 ml. Use this emulsified sample as the sample fluid. If necessary, warm at a temperature not exceeding 45°C to emulsify the sample. Avoid warming for 30 minutes or longer.

Procedure
(1) Membrane Filtration Method This method is applied to samples that contain antimicrobial substances.

Use membrane filters of an appropriate material with a pore size of 0.45 μm or less. Filters of about 50 mm diameter are recommended, but other sizes may also be used. The filters, filtration apparatus, media, and other things to be used for the test should be well sterilized. Usually, 20 ml of the test fluid (two 10-ml portions) is used, each portion is filtered through a separate filter. If necessary, the test fluid may be diluted. If the bacteria concentration is high, the pretreated test fluid should be diluted; it is desirable that 10–100 colonies are produced for each filter. After filtration, wash each filter at least three times with an appropriate wash solution, such as phosphate buffer, sodium chloride–peptone buffer, or fluid medium to be used. The volume of the wash solution should be about 100 ml each time. If the diameter of the filter used is greatly different from 50 mm, use an appropriate volume of wash solution, depending on the size of the filter. If the sample includes lipid, polysorbate 80 or other appropriate emulsifier may be added to the wash solution. After filtration, place the two filters on separate plates of agar medium. For bacteria detection, use soybean-casein digest agar medium, and for fungi detection, use either Sabouraud glucose agar, potato-dextrose agar, or GP agar medium, to which an antibiotic is usually added. When xerophilous fungi (fungi adaptable to a dry environment), which are likely to grow in foods with low water activity, are examined, use M40Y agar medium, dichloran-glycerol (DG18) agar medium, or their equivalent medium. Incubate the plates for at least 5 days at 30–35°C for bacteria detection and at 20–25°C for fungi detection, and count the number of colonies. If the counts are considered to be reliable, the counts obtained in an incubation time shorter than 5 days may be adopted for calculation of the viable count.

(2) Pour Plate Method Use petri dishes of 9–10 cm diameter. Use at least 2 agar media for each dilution. Take 1 ml of the test fluid or its dilution into each petri dish aseptically, add to each dish 15–20 ml of sterilized agar medium, previously melted and kept below 45°C, and mix. For bacteria detection, use soybean-casein digest agar medium, and for fungi detection, use either Sabouraud glucose agar, potato-dextrose agar, or GP agar medium, to which an antibiotic is usually added. When xerophilous fungi (fungi adaptable to a dry environment), which are likely to grow in foods with low water activity, are examined, use M40Y agar medium or dichloran-glycerol (DG18) agar medium, or another appropriate medium. After the agar solidifies, incubate for at least 5 days at 30–35°C for bacteria detection and at 20–25°C for fungi detection. If a large number of colonies develop, calculate viable counts based on counts obtained from plates with not more than 300 colonies per plate for bacteria detection and from plates with not more than 100 colonies per plate for fungi detection. If the counts are considered to be reliable in an incubation time shorter than 5 days, these counts may be adopted.

(3) Spread Plate Method Place 0.05–0.2 ml of the test fluid on the solidified and dried surface of the agar medium and spread it uniformly using a spreader. Proceed under the same conditions as for the Pour Plate Method, especially with respect to petri dishes, agar media, incubation temperature and time, and calculation method.

(4) Serial Dilution Method (Most Probable Number Method) Set up 10 test tubes with each containing 10 ml of soybean-casein digest medium. Using the previously prepared test fluid, prepare three different dilutions (10-fold, 100-fold, and 1,000-fold dilutions). For each dilution, use three test tubes, with another tube being used for the control. Add 1 ml of the test fluid to each of the three test tubes to make 10-fold dilution fluids. Using a 10-fold dilution, prepare 100-fold dilution fluids, and then 1000-fold dilution fluids in the same manner. Incubate all 10 test tubes for at least 5 days at 30–35°C. No microbial growth should

be observed in the control test tube. If the determination of the result is difficult or if the result is not reliable, take 0.1 ml fluid from each test tube, and place it into an agar medium or fluid medium. Incubate all media for 24–72 hours at 30–35°C, and check for the absence or presence of microbial growth. Using the table given below, calculate the most probable number of microorganisms per g or ml of the sample.

The number of test tubes in which microbial growth is observed, when the amount of the sample given below is added per test tube			The most probable number (MPN) of microorganisms per g or ml	95% confidence limit of MPN
0.1 g/0.1 ml	0.01 g/0.01 ml	1 mg/1 µl		
0	0	0	<3	0–9.4
0	0	1	3	0.1–9.5
0	1	0	3	0.1–10
0	1	1	6.1	1.2–17
0	2	0	6.2	1.2–17
0	3	0	9.4	3.5–35
1	0	0	3.6	0.2–17
1	0	1	7.2	1.2–17
1	0	2	11	4–35
1	1	0	7.4	1.3–20
1	1	1	11	4–35
1	2	0	11	4–35
1	2	1	15	5–38
1	3	0	16	5–38
2	0	0	9.2	1.5–35
2	0	1	14	4–35
2	0	2	20	5–38
2	1	0	15	4–38
2	1	1	20	5–38
2	1	2	27	9–94
2	2	0	21	5–40
2	2	1	28	9–94
2	2	2	35	9–94
2	3	0	29	9–94
2	3	1	36	9–94
3	0	0	23	5–94
3	0	1	38	9–104
3	0	2	64	16–181
3	1	0	43	9–181
3	1	1	75	17–199
3	1	2	120	30–360
3	1	3	160	30–380
3	2	0	93	18–360
3	2	1	150	30–380
3	2	2	210	30–400
3	2	3	290	90–990
3	3	0	240	40–990
3	3	1	460	90–1,980
3	3	2	1,100	200–4,000
3	3	3	>1,100	

Effectiveness of Culture Media and Confirmation of Antimicrobial Substances Use the following strains or their equivalents for tests: *Escherichia coli* (NBRC 3972, ATCC 8 Use the following strains or their equivalents for tests: *Escherichia coli* (NBRC 739, or NCIMB 8545), *Bacillus subtilis* (NBRC 3134, ATCC 6633, or NCIMB 8054), *Staphylococcus aureus* subsp. *aureus* (NBRC 13276, ATCC 6538, or NCIMB 9518), *Candida albicans* (NBRC 1594, ATCC 2091, or ATCC 10231), and *Aspergillus niger* (NBRC 9455, or ATCC 16404). Incubate the above strains in the following media under the following conditions: for bacteria, soybean-casein digest medium or soybean-casein digest agar medium at 30–35°C for 18–24 hours; for *Candida albicans*, soybean-casein digest medium, Sabouraud glucose broth, or Sabouraud glucose agar medium at 20–25°C for 2–3 days; and for *A. niger*, Sabouraud glucose agar medium or potato-dextrose agar medium at 20–25°C for 5–7 days.

Dilute each of the prepared cultures with sodium chloride –peptone buffer or phosphate buffer to prepare test suspensions with each containing 50–200 viable microorganisms (10–100 for *A. niger*) per ml. To suspend *A. niger* spores, 0.05% polysorbate 80 may be added. The test suspensions should be used within 2 hours or 24 hours if stored in a refrigerator. For *B. subtilis* and *A. niger*, stable spore suspensions may be used. Examine the effectiveness of the media to be used for the test. The media are satisfactory if clear evidence of growth is observed and good recovery of the microorganisms is obtained, when 1 ml of each test suspension is inoculated into each medium and incubated for 5 days at a specified temperature. If a count of test microorganisms in the presence of the sample is one fifth or less of that in the absence of the sample, then any effect must be eliminated using appropriate means, such as dilution, filtration, neutralization, and inactivation. Use the sodium chloride–peptone buffer or phosphate buffer used for dilution as the control in order to verify the sterility of the medium and diluent and the aseptic performance of the test.

2. *Escherichia coli* Test

This test determines *Escherichia coli. Escherichia coli*, the target strain for this test, becomes an important index to evaluate microbial contamination of ingredients and intermediate products as well as of the finished products. *Escherichia coli* should not be present in any of them.

Preparation of Test Fluid Unless otherwise specified, proceed as directed in the preparation of test fluid for total viable aerobic count. When a liquid medium is used to dissolve or dilute the sample, use lactose broth medium or BGLB medium unless otherwise specified.

Procedure Take 10 ml (equivalent of 1 g or 1 ml of the sample) of the sample, add lactose broth medium or BGLB medium to make 100 ml, and incubate at 30–35°C for 24–72 hours. Examine the medium for growth. If growth is present, shake gently, take a portion of the culture fluid using an inoculating loop, such as a platinum loop, streak it on MacConkey agar medium, and incubate at 30–35°C for 18–24 hours. Examine the plate for suspicious colonies. If red-brick colonies of Gram-negative rod-shaped bacteria surrounded by a reddish precipitation zone are not found, the sample is determine to be *E. coli* negative. If colonies meeting the above description are found, then transfer the suspect colonies individually onto the surface of EMB agar medium and incubate at 30–35°C for 18–24 hours. Upon examination, if no colonies exhibit a metallic sheen or a blue-black color under transmitted light, the sample is determined to be negative. Confirm suspect colonies on the plate by conducting the IMViC tests (Indole production test, Methyl red reaction test, Voges-Proskauer test, and Citrate utilization test) and the growth test at 44.5°C. When the colonies exhibit the pattern [+ + − −] for these four IMViC tests (in the order listed above) and a positive response for the growth test, they are determined to be *Escherichia coli*. Rapid detection kits for *Escherichia coli* may be used.

Effectiveness of Culture Media and Confirmation of Antimicrobial Substances For the confirmation test, use an appropriate *Escherichia coli* strain (NBRC 3972, ATCC 8739, or NCIMB 8545) or one of their equivalents. Incubate it in lactose broth medium, soybean-casein digest agar medium, or fluid soybean-casein digest medium at 30–35°C for 18–24 hours. Dilute the incubated cultures with a sodium chloride–peptone buffer solution, phosphate buffer, or lactose broth agar medium to make suspensions with each containing 1,000 viable microorganisms per ml. If necessary, using 0.1 ml of *E. coli* suspensions (1,000/ml), examine the effectiveness of the media to be used for the test and the presence of antimicrobial substances both in the presence and absence of the sample.

Confirmation If uncertain or doubtful results are obtained, conduct the test again with an amount of sample 2.5 times that used in the original test, as directed in Procedure. Use additional amounts of medium and reagents in proportion to the increase of the sample.

3. Buffer Solutions and Media

Use the buffer solutions and media given below for the Microbial Limit Test. Other media may be used if they include similar nutritive ingredients and have similar selectivity and growth-promoting ability for the microorganism to be tested.

(1) Buffer Solutions

(i) *Phosphate buffer* (pH 7.2)

Stock solution: Dissolve 34 g of monopotassium phosphate in about 500 ml of water, add about 175 ml of sodium hydroxide TS to adjust to pH 7.1–7.3, and add water to make 1,000 ml. Use this solution as the stock solution. Autoclave and cool this solution, and store in a cool place. Before use, dilute the stock solution with water to 800 times its original volume, and autoclave at 121°C for 15–20 minutes.

(ii) *Sodium chloride-peptone buffer (pH 7.0)*

Monopotassium phosphate	3.56 g
Disodium phosphate	18.23 g
Sodium chloride	4.30 g
Peptone	1.0 g
Water	1,000 ml

Mix all the ingredients, and autoclave at 121°C for 15–20 minutes. The pH is 6.9–7.1 after sterilization. To this buffer, 0.1–1.0% (w/v) polysorbate 20 or polysorbate 80 may be added.

(2) Media

(i) *Soybean-casein digest agar medium*

Casein peptone	15.0 g

Soybean peptone	5.0	g
Sodium chloride	5.0	g
Agar	15.0	g
Water	1,000	ml

Mix all the ingredients, and autoclave at 121°C for 15–20 minutes. The pH is 7.1–7.5 after sterilization.

(ii) *Fluid soybean-casein digest medium*

Casein peptone	17.0	g
Soybean peptone	3.0	g
Sodium chloride	5.0	g
Dipotassium phosphate	2.5	g
Glucose	2.5	g
Water	1,000	ml

Mix all the ingredients, and autoclave at 121°C for15–20 minutes. The pH is 7.1–7.5 after sterilization.

(iii) *Sabouraud glucose agar medium with antibiotics*

Peptone (derived from meat and casein)	10.0	g
Glucose	40.0	g
Agar	15.0	g
Water	1,000	ml

Mix all the ingredients, and autoclave at 121°C for15–20 minutes. The pH is 5.4–5.8 after sterilization. Immediately before use, add 0.10 g of benzylpenicillin potassium and 0.10 g of tetracycline per liter of medium as a sterile solution, or alternatively, 0.050 g of chloramphenicol per liter of medium.

(iv) *Sabouraud glucose broth*

Peptone (derived from meat and casein)	10.0	g
Glucose	20.0	g
Water	1,000	ml

Mix all the ingredients, and autoclave at 121°C for 15–20 minutes. The pH is 5.4–5.8 after sterilization.

(v) *Potato-dextrose agar medium with antibiotics*

Potato extract	4.0	g
Glucose	20.0	g
Agar	15.0	g
Water	1,000	ml

Mix all the ingredients, and autoclave at 121°C for15–20 minutes. The pH is 5.4–5.8 after sterilization. Immediately before use, add 0.10 g of benzylpenicillin potassium and 0.10 g of tetracycline per liter of medium as a sterile solution, or alternatively, add 0.050 g of chloramphenicol per liter of medium.

(vi) *GP (glucose-peptone) agar medium with antibiotics*

Glucose	20.0	g
Yeast extract	2.0	g
Magnesium sulfate	0.5	g
Peptone	5.0	g
Monopotassium phosphate	1.0	g
Agar	15.0	g
Water	1,000	ml

Mix all the ingredients, and autoclave at 121°C for15–20 minutes. The pH is 5.6–5.8 after sterilization. Immediately before use, add 0.10 g of benzylpenicillin potassium and 0.10 g of tetracycline per liter of medium as a sterile solution, or alternatively, add 0.050 g of chloramphenicol per liter of medium.

(vii) *M40Y agar medium*

Malt extract	20.0	g
Yeast extract	2.5	g
Sucrose	400.0	g
Agar	20.0	g
Water	1,000	ml

Mix all the ingredients, dissolve while warming, and autoclave at 121°C for 15–20 minutes.

(viii) *Dichloran glycerol (DG18) agar medium*

Peptone	5.0	g
Glucose	10.0	g
Monopotassium phosphate	1.0	g
Magnesium sulfate	0.5	g
Dichloran	2.0	mg
Glycerol	220.0	g
Agar	15.0	g
Chloramphenicol	0.10	g
Water	1,000	ml

Mix all ingredients excluding glycedrol and chloramphenicol, and dissolve while warming. Add glycerol and chloramphenicol, previously dissolved in 6 ml of ethanol, and autoclave at 121°C for15–20 minutes. The pH is 5.4–5.8 after sterilization.

(ix) *Fluid lactose broth medium*

Meat extract	3.0	g
Gelatin peptone	5.0	g
Lactose	5.0	g
Water	1,000	ml

Mix all the ingredients, and autoclave at 121°C for15–20 minutes. The pH is 6.7–7.1 after sterilization. Cool immediately after autoclaving.

(x) *BGLB (brilliant green lactose bile) medium*

Peptone	10.0	g
Lactose	10.0	g
Powdered Cattle Bile	20.0	g
Brilliant green	0.0133	g
Water	1,000	ml

Mix all the ingredients, and autoclave at 121°C for15–20 minutes. The pH is 7.0–7.4.

(xi) *MacConkey agar medium*

Gelatin peptone	17.0	g
Casein peptone	1.5	g
Meat peptone	1.5	g
Lactose	10.0	g
Sodium de(s)oxycholate	1.5	g
Sodium chloride	5.0	g
Agar	13.5	g
Neutral red	0.03	g
Crystal violet	1.0	mg
Water	1,000	ml

Mix all the ingredients, boil for 1 minute, and autoclave at 121°C for15–20 minutes. The pH is 6.9–7.3 after sterilization.

(xii) *EBM (Eosin-methylene blue) agar medium*

Gelatin peptone	10.0	g
Dipotassium phosphate	2.0	g
Lactose	10.0	g
Agar	15.0	g

Eosine	0.40 g
Methylene blue	0.065 g
Water	1,000 ml

Mix all the ingredients, and autoclave at 121°C for15–20 minutes. The pH is 6.9–7.3 after sterilization.

Nitrogen Determination

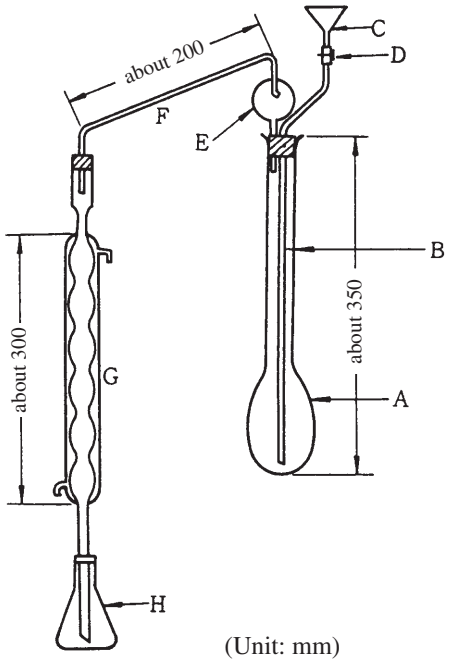
Nitrogen Determination is designed to quantify ammonia in ammonium sulfate obtained by decomposing organic compounds containing nitrogen with sulfuric acid.

(1) Kjeldahl Method

Apparatus Use the apparatus illustrated below. Ground-glass may be used for joints.

Procedure Unless otherwise specified, proceed as directed below.

Weigh accurately a quantity of the sample equivalent to about 0.02 to 0.03 g of nitrogen, place it into Kjeldahl flask A, and add 5 g of powdered potassium sulfate, 0.5 g of cupric sulfate, and 20 ml of sulfuric acid. Tilt flask A at about 45°, heat gently until the effervescence almost stops, and raise the temperature to boiling. After the contents become a clear, blue solution, heat for another 1 to 2 hours. Cool, add gradually 150 ml of water, and cool again. Add 2 or 3 granules of boiling chips or granulated zinc, and assemble the apparatus.



(Unit: mm)

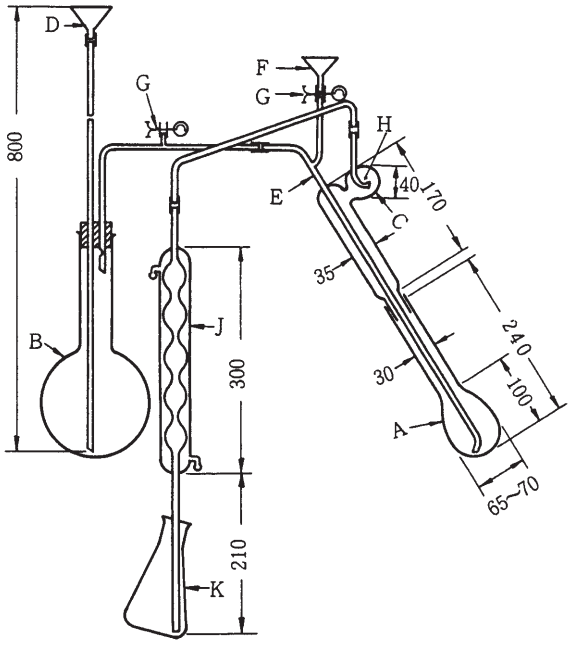
A: Kjeldahl flask (made of hard glass, about 300 ml capacity)
B: Glass tube
C: Funnel for addition of alkaline solution
D: Rubber tube (connecting B and C, with a pinch cock attached)
E: Spray trap
F: Delivery tube
G: Condenser
H: Absorption flask (about 300 ml capacity)

Measure exactly 25 ml of 0.05 mol/L sulfuric acid, transfer into absorption flask H, add about 50 ml of water, and immerse the lower end of condenser G into this solution. Add gradually 85 ml of sodium hydroxide solution (2 in 5) to flask A through funnel C, rinse the funnel with a small quantity of water, close the pinch cock on rubber tube D, mix the contents by lightly shaking flask A, and heat gently. When the solution starts to boil, turn up the heat, and distill until about two thirds of the contents are distilled. Lower H until the lower end of G is above the solution surface in H, continue the distillation for a short time, wash the lower end of G with a small quantity of water, and titrate the excess acid in the solution in H with 0.1 mol/L sodium hydroxide. The endpoint is usually confirmed using a potentiometer. When 3 drops of bromocresol green–methyl red mixture TS are used as the indicator, the endpoint is when the color of the solution changes from red-purple through pale grayish yellow to pale grayish green. Perform a blank test and make any necessary correction.

1 ml of 0.05 mol/L sulfuric acid = 1.401 mg of N

(2) Semi-micro Kjeldahl Method

Apparatus Use the apparatus illustrated below, which is made of hard glass. Ground glass may be used for joints. All rubber parts used in the apparatus should be boiled in sodium hydroxide solution (1 in 25) for 10 to 30 minutes and then in water for 30 to 60 minutes, and finally washed thoroughly with water before use.



(Unit: mm)

A: Kjeldahl flask
B: Steam generator (containing boiling chips to prevent bumping, filled with water containing 2 to 3 drops of sulfuric acid)
C: Spray trap
D: Water supply funnel
E: Steam tube
F: Funnel for addition of alkaline solution
G: Rubber tube with a pinch cock
H: Small hole (with the diameter approximately equal to the internal diameter of the tube)
J: Condenser (with a beveled lower end)
K: Absorption flask

Procedure Unless otherwise specified, proceed as directed below.

Weigh accurately or pipet a quantity of the sample equivalent to 2 to 3 mg of nitrogen, and place in Kjeldahl flask A. Add 1 g of a powdered mixture of 10 g of potassium sulfate and 1 g of cupric sulfate. Wash down the sample adhering to the neck of flask A with a small quantity of water. Add 7 ml of sulfuric acid, allowing it to flow along the inside wall of A.

Next, while shaking flask A, carefully add 1 ml of hydrogen peroxide drop by drop along the inside wall of A. Heat A on a ceramic gauze or ceramic plate, over a free flame until the solution exhibits a clear blue color and the inside walls of A are free from carbonaceous material, and heat for another 1 to 2 hours. If necessary, cool, add a small quantity of hydrogen peroxide, and heat again. Cool, add carefully 20 ml of water, cool the solution, and connect A to the distillation apparatus, washed in advance by passing steam through it. To absorption flask K, add 15 ml of boric acid solution (1 in 25), and sufficient water to immerse the lower end of condenser tube J. Add 30 ml of sodium hydroxide solution (2 in 5) through funnel F, carefully wash the funnel with 10 ml of water, immediately close the pinch cock attached to rubber tube G, and distill with steam until the distillate measures 80 to 100 ml. Remove the lower end of J from the solution, continue the distillation for a short time, wash the lower end of J with a small quantity of water, and titrate the distillate with 0.005 mol/L sulfuric acid. The endpoint is usually confirmed using a potentiometer. When an indicator (3 drops of bromocresol green–methyl red mixture TS) is used the endpoint is when the color of the solution changes from red –purple through pale grayish yellow to pale grayish green. Perform a blank test in the same manner, and make any necessary correction.

1 ml of 0.005 mol/L sulfuric acid = 0.1401 mg of N

Paper Chromatography

Paper Chromatography is designed to develop a mixture in the mobile phase and to separate it into individual components using a sheet of filter paper. This method is applicable to identification tests and purity tests.

Procedure Unless otherwise specified, proceed as directed below.

Draw a line horizontally with a pencil across a sheet of the filter paper for chromatography specified in the individual monograph at a distance about 40 mm from one end of the filter paper. Using a micropipet or capillary tube, apply the specified quantity of the test solution and the control solution on the line. The distance between the centers of the two spots applied should be about 25 mm. Air-dry the filter paper. Next, suspend it vertically from the stopper with a thread or wire in a developing container of about 500-mm height that is filled beforehand with the specified developing solvent and saturated with the vapor of the solvent. This procedure should be done with care to avoid contact with the walls. Immerse the lower end of the paper 10 mm into the developing solvent, seal the container, and allow to stand. When the solvent front has ascended from the sample spot to the specified position, remove the paper from the container, and air-dry. Using the specified method, examine the location, color, etc. of the spots obtained from the test solution and the control solution.

pH Determination

pH is measured using a glass electrode pH meter. pH is fundamentally a value representing hydrogen ion activity in a solution. It is defined by the equation given below. This value corresponds well to the logarithm of the reciprocal of hydrogen-ion concentration in dilute solutions.

$$pH = pHs + \frac{E - Es}{2.3026\,RT / F}$$

pHs = pH value of a pH standard solution,
E = electromotive force (volts) of the following galvanic cell, composed of a glass electrode and a suitable reference electrode in a sample solution:

Glass electrode | sample solution | reference electrode

Es = electromotive force (volts) of the following galvanic cell, composed of a glass electrode and a suitable reference electrode in a pH standard solution:

Glass electrode | pH standard solution | reference electrode

R = gas constant,
T = absolute temperature,
F = Faraday constant.

The values of 2.3026 RT/F (volts) at various temperatures are as follows:

Temperature of solution	2.3026 RT/F	Temperature of solution	2.3026 RT/F
5°C	0.05519	35°C	06114
10°C	0.05618	40°C	0.06213
15°C	0.05717	45°C	0.06313
20°C	0.05817	50°C	0.06412
25°C	0.05916	55°C	0.06511
30°C	0.06015	60°C	0.06610

In the Monographs, such a specification as "pH 6.0–7.5 (1.0 g, water 20 ml)" for this test indicates that the pH of the solution is 6.0–7.5, when determined on a solution of 1.0 g of the test substance, weighed accurately, in 20 ml of water.

Preparation of pH Standard Solutions The pH standard solutions are used as standards of pH. Water used for preparation of the pH standard solutions is prepared as follows: Distill purified water, boil the distillate for 15 minutes or more to purge the carbon dioxide, and cool in a container fitted with a carbon dioxide-absorbing tube (soda lime). Store the pH standard solutions in hard glass or polyethylene bottles. As the pH value may change during long-term storage, acidic standard solutions should normally be used within 3 months. Also, basic standard solutions should be stored in containers fitted with a carbon dioxide absorbing tube and used within 1 month.

Oxalate pH Standard Solution Weigh exactly 12.71 g of potassium tetraoxalate for pH determination, previously pulverized and dried in a desiccator, and dissolve in water to make exactly 1,000 ml.

Phthalate pH Standard Solution Weigh exactly 10.21 g of potassium hydrogen phthalate for pH determination, previously pulverized and dried at 110°C to constant weight, and dissolve in water to make exactly 1,000 ml.

Phosphate pH Standard Solution Weigh exactly 3.40 g (0.025 mol) of monopotassium phosphate for pH determination and 3.55 g of anhydrous disodium phosphate for pH determination, each previously pulverized and dried at 110°C to constant weight, and dissolve together in water to make exactly 1,000 ml.

Borate pH Standard Solution Weigh exactly 3.81 g of sodium borate for pH determination, left to stand in a desiccator (sodium bromide moistened with water) to constant weight, and dissolve in water to make exactly 1,000 ml.

Carbonate pH Standard Solution Weigh exactly 2.10 g of sodium hydrogen carbonate for pH determination, previously dried in a desiccator to constant weight, and 2.65 g of sodium carbonate for pH determination, previously dried at 300 to 500°C to constant weight. Mix together, and dissolve in water to make exactly 1,000 ml.

Calcium Hydroxide pH Standard Solution Transfer 5 g of calcium hydroxide for pH determination, previously pulverized, into a flask, add 1,000 ml of water, and shake well. Maintain the flask at 23–27°C to saturate thoroughly, and filter the supernatant at the same temperature. Use the clear filtrate (about 0.02 mol/L).

The pH values of these pH standard solutions at various temperatures are shown in the table below. pH values at temperatures not indicated in the table are calculated from the value in the table by the interpolation method.

pH Meter A pH meter generally consists of a detecting unit that is made up of a glass electrode and a reference electrode, and an display unit, which displays the pH value corresponding to the electromotive force detected. The display unit is usually fitted with dials for zero point adjustment and for temperature compensation, and some units have a dial for sensitivity adjustment.

The reproducibility of five consecutive measurements should be within ±0.05 when the pH is measured for an arbitrary pH standard solution after the detecting unit is washed well with water at each measurement.

Procedure

Calibration Immerse the glass electrode in water for several hours before measurement. Start the measurement at least 5 minutes after switching on the pH meter. Rinse the detecting unit well with water, and blot the water gently with a piece of filter paper. When the pH meter is calibrated at one point, rotate the temperature compensation dial to set it to the temperature of the pH standard solution, immerse the detecting unit in a pH standard solution with a pH value approximate to that of the sample solution, and after at least 2 minutes, adjust the zero point adjustment dial so that the reading of the pH meter is set to the pH of the pH standard solution corresponding to that temperature (see the table). When the meter is calibrated at two points, rotate the temperature compensation dial to set it to the temperature of the solutions, immerse the detecting unit in an appropriate pH standard solution (usually, Phosphate pH Standard Solution is used), adjust the pH by means of the zero point adjustment, immerse in a pH standard solution with a pH value approximate to that of the sample solution, and proceed in the same manner as directed above, by means of a sensitivity adjustment dial or a temperature compensation dial irrespective of the temperature of the pH standard solution.

Measurement After calibration, rinse the detecting unit

pH values of pH standard solutions

Temperature	Oxalate pH Standard Solution	Phthalate pH Standard Solution	Phosphate pH Standard Solution	Borate pH Standard Solution	Carbonate pH Standard Solution	Calcium Hydroxide pH Standard Solution
0°C	67	4.01	6.98	9.46	10.32	13.43
5°C	1.67	4.01	6.95	9.39	10.25	13.21
10°C	1.67	4.00	6.92	9.33	10.18	13.00
15°C	1.67	4.00	90	9.27	10.12	12.81
20°C	1.68	4.00	6.88	9.22	10.07	12.63
25°C	1.68	4.01	6.86	9.18	10.02	12.45
30°C	1.69	4.01	6.85	9.14	9.97	12.30
35°C	1.69	4.02	6.84	10	9.93	12.14
40°C	1.70	4.03	6.84	9.07		11.99
50°C	1.71	4.06	6.83	9.01		11.70
60°C	1.73	4.10	6.84	8.96		11.45

well with water, and blot the water gently with a piece of filter paper. Immerse the detecting unit in the sample solution, and read the pH value.

Notice on Procedure

(1) The structure and operating procedure are different for different pH meters.

(2) Because solutions above pH 11 containing alkali metal ions may give rise to large measurement errors, use an electrode with less alkali error, and make any necessary correction.

(3) It is desirable that the temperature of the sample solution be the same as that of the pH standard solution that is used for pH determination of the sample solution.

Qualitative Tests

The Qualitative Tests are mainly applied to identification tests. Unless otherwise specified, the concentration of the sample solutions used is about 1%.

Acetate

(1) Solutions of acetates, when warmed with sulfuric acid, evolve the odor of acetic acid.

(2) Acetates, when heated with ethanol and a small quantity of sulfuric acid, evolve the odor of ethyl acetate.

(3) With ferric chloride solution (1 in 10), neutral solutions of acetates (1 in 20) produce a red-brown color, and yield a red-brown precipitate by boiling. On the addition of hydrochloric acid, the precipitate dissolves, and the color of the solution changes to yellow

Aluminum Salt

(1) With ammonium chloride solution (1 in 10) and ammonia TS, solutions of aluminum salts (1 in 20) yield a white, gelatinous precipitate that does not dissolve in an excess of ammonia TS.

(2) With sodium hydroxide solution (1 in 25), solutions of aluminum salts (1 in 20) yield a white, gelatinous precipitate that dissolves in an excess of sodium hydroxide solution (1 in 25).

(3) When ammonia TS is added to a solution of an aluminum salt until a precipitate is slightly formed, and then 5 drops of alizarin S solution (1 in 1,000) are added, the color of the precipitate changes to red.

Ammonium Salt

When warmed with an excess of sodium hydroxide solution (1 in 25), ammonium salts evolve a gas with the odor of ammonia that changes red litmus paper moistened with water to blue.

Benzoate

(1) When acidified by diluted hydrochloric acid (1 in 4), solutions of benzoates (1 in 20) produce a crystalline precipitate. The separated precipitate, when washed well with cold water and dried, melts at about 121–123°C.

(2) With ferric chloride solution (1 in 10), neutral solutions of benzoates (1 in 20) yield a light yellow-red precipitate. The color of the precipitate changes to white on the addition of diluted hydrochloric acid (1 in 4).

Bicarbonate

(1) Bicarbonates effervesce with diluted hydrochloric acid (1 in 4), evolving a gas that yields a white precipitate immediately when passed into calcium hydroxide TS (common with carbonates).

(2) With magnesium sulfate solution (1 in 10), solutions of bicarbonates (1 in 20) yield no precipitate at ordinary temperature but yield a white precipitate when boiled.

(3) By the addition of phenolphthalein TS, solutions of bicarbonates remain unchanged or exhibit a faint pink color (distinction from carbonates).

Bromate

(1) With 2 to 3 drops of silver nitrate solution (1 in 50), solutions of bromates (1 in 20) acidified with nitric acid yield a white crystalline precipitate that dissolves when heated. The resulting solution yields a light yellow precipitate on the addition of 1 drop of freshly prepared sodium nitrite solution (1 in 10).

(2) Solutions of bromates (1 in 20) acidified with nitric acid exhibit a yellow to red-brown color on the addition of 5 to 6 drops of freshly prepared sodium nitrite solution (1 in 10).

Calcium Salt

(1) When the Flame Coloration Test is performed, calcium salts impart a yellow-red color to a colorless flame.

(2) With ammonium oxalate solution (1 in 30), solutions of calcium salts yield a white precipitate. The separated precipitate does not dissolve in diluted acetic acid (1 in 20) but dissolves on the subsequent addition of diluted hydrochloric acid (1 in 4).

Carbonate

(1) Carbonates effervesce with diluted hydrochloric acid (1 in 4), evolving a gas that yields a white precipitate immediately when passed into calcium hydroxide TS (common with bicarbonate).

(2) With magnesium sulfate solution (1 in 10), solutions of carbonates (1 in 20) yield a white precipitate that dissolves in diluted acetic acid (1 in 20).

(3) With phenolphthalein TS, solutions of carbonates produce an extremely pink color (distinction from bicarbonates).

Chloride

(1) When heated with sulfuric acid and potassium permanganate, solutions of chlorides (1 in 20) evolve a gas with the odor of chlorine that changes the color of potassium iodide–starch paper moistened with water to blue.

(2) With silver nitrate solution (1 in 50), solutions of chlorides yield a white precipitate. The separated precipitate does not dissolve in diluted nitric acid (1 in 10), but dissolves in an excess of ammonia TS.

Chlorite

(1) When treated with 5 ml of diluted hydrochloric acid (1 in 4), 5 ml of a solution of a chlorite (1 in 20) produces a yellow-brown color, forming a yellow gas.

(2) When 0.1 ml of potassium permanganate solution (1 in 300) is added to 5 ml of a solution of a chlorite (1 in 20),

and then 1 ml of diluted sulfuric acid (1 in 20) is added to the mixture, the red-purple color of the solution disappears.

Citrate

(1) When 20 ml of a 3:1 mixture of pyridine/acetic anhydride is added to 1 to 2 drops of a solution of a citrate (1 in 20), the mixture produces a red-brown color.

(2) Neutral solutions of citrates (1 in 10), when mixed with an equal volume of diluted sulfuric and about two-thirds volume of potassium permanganate solution (1 in 300), heated until the color of solution disappears, and then treated dropwise with bromide TS, yield a white precipitate.

Cupric Salt

(1) When a well polished iron fragment is immersed in solutions of cupric salts acidified with hydrochloric acid and is allowed to stand in it, a yellow-red metal is deposited on the surface of the fragment.

(2) With a small quantity of ammonia TS, solutions of cupric salts yield a light blue precipitate. The precipitate dissolves in an excess of ammonia TS, producing a dark blue solution.

(3) With freshly prepared potassium ferrocyanide solution (1 in 10), solutions of cupric salts yield a red-brown precipitate. The separated precipitate does not dissolve in diluted acetic acid (1 in 20), but dissolves in ammonia TS, producing a dark blue solution.

Ferric Salt

(1) With freshly prepared potassium ferrocyanide solution (1 in 10), weakly acidic solutions of ferric salts yield a blue precipitate. The precipitate does not dissolve in diluted hydrochloric acid (1 in 4) or in diluted nitric acid (1 in 10).

(2) With sodium hydroxide solution (1 in 25) or ammonia TS, solutions of ferric salts yield a red-brown gelatinous precipitate. The color changes to black on the addition of sodium sulfide TS. The precipitate dissolves in diluted hydrochloric acid (1 in 4), producing a white turbidity.

(3) With ammonium thiocyanate solution (2 in 25), neutral to weakly acidic solutions of ferric salts produce a red color that remains unchanged by the addition of hydrochloric acid.

Ferrous Salt

(1) With freshly prepared potassium ferricyanide solution (1 in 10), weakly acidic solutions of ferrous salts yield a blue precipitate that does not dissolve in diluted hydrochloric acid (1 in 4) or in diluted nitric acid (1 in 10).

(2) With sodium hydroxide solution (1 in 25) or ammonia TS, solutions of ferrous salts yield a white, gelatinous precipitate (the color changes rapidly to grayish green and then gradually to red-brown when shaken). On the subsequent addition of sodium sulfide TS, a black precipitate is produced. The separated precipitate dissolves in diluted hydrochloric acid (1 in 4).

Glycerophosphate

(1) With ammonium molybdate TS, solutions of glycerophosphates yield no precipitate in the cold, but yield a yellow precipitate when boiled for a long time.

(2) Glycerophosphates, when gently heated over an open flame with an equal volume of powdered potassium hydrogen sulfate, evolve the pungent odor of acrolein.

Hypochlorite

(1) When 2 ml of hydrochloric acid is added to 5 ml of a solution of a hypochlorite, effervescence occurs with the evolution of a gas.

(2) When 1 ml of sodium hydroxide solutions (1 in 2,500) and 0.2 ml of potassium iodide TS are added to 5 ml of a solution of a hypochlorite (1 in 1,000), a yellow color develops. The resulting solution produces a deep blue color on the subsequent addition of 0.5 ml of starch TS.

(3) When 5 ml of a solution of a hypochlorite (1 in 4) is treated with 0.1 ml of potassium permanganate solution (1 in 300) and then with 1 ml of diluted sulfuric acid (1 in 20), the red-purple color of solution does not fade (distinction from chlorites).

Lactate

When solutions of lactates (1 in 20) are acidified with sulfuric acid, potassium permanganate solution (1 in 50) is added, and the mixture is heated, the odor of acetaldehyde is evolved.

Magnesium Salt

With ammonium chloride solution (1 in 10) and ammonium carbonate TS, solutions of magnesium salts yield no precipitate, but a white crystalline precipitate is formed on the subsequent addition of disodium phosphate solution (1 in 10). The separated precipitate does not dissolve in ammonia TS.

Nitrate

(1) When a solution of nitrate is mixed well with an equal volume of sulfuric acid, the mixture is cooled, and ferrous sulfate TS is superimposed, a dark brown ring is produced at the junction of the two liquids.

(2) Solutions of nitrates acidified with sulfuric acid do not discolor the red-purple color of potassium permanganate solution (1 in 300) (distinction from nitrites).

Nitrite

(1) Solutions of nitrites (1 in 20), when acidified with diluted sulfuric acid (1 in 20), evolve a yellow-brown gas with a characteristic odor. The solutions produce a dark brown color on the subsequent addition of a small amount of ferrous sulfate crystals.

(2) When 2 to 3 drops of potassium iodide TS are added and then diluted hydrochloric acid (1 in 4) is added dropwise, solutions of nitrites produce a yellow-brown color, and then yield a black-purple precipitate. On the subsequent addition of starch TS, the solutions exhibit a deep blue color.

Peroxide

(1) When solutions of peroxides are mixed with an equal volume of ethyl acetate and 1 or 2 drops of potassium dichromate solution (3 in 40), and then the mixture is acidified with diluted sulfuric acid (1 in 20), the water layer exhibits a blue color. When the mixture is shaken immediately and allowed to stand, the blue color is transferred to the ethyl acetate layer.

(2) Solutions of peroxides acidified with sulfuric acid decolorize potassium permanganate solution (1 in 300) added dropwise, producing effervescence.

Phosphate (Orthophosphate)

(1) With silver nitrate solution (1 in 50), neutral solutions

of phosphates yield a yellow precipitate that dissolves in diluted nitric acid (1 in 10) and in ammonia TS.

(2) With ammonium molybdate TS, neutral solutions of phosphates or solutions of phosphates acidified with nitric acid yield a yellow precipitate on warming. The precipitate dissolves in sodium hydroxide solution (1 in 25) and in ammonia TS.

Potassium Salt

(1) When the Flame Coloration Test is performed, potassium salts impart a pale purple color to a colorless flame. When the flame exhibits a yellow color, a red-purple color is seen through cobalt glass.

(2) With freshly prepared sodium hydrogen tartrate solution (1 in 10), neutral solutions of potassium salts (1 in 20) yield a white crystalline precipitate (the formation of the precipitate is accelerated by rubbing the inside wall of the test tube with a glass rod) that dissolves in ammonia TS, in sodium hydroxide solution (1 in 25), and in anhydrous sodium carbonate solution (1 in 8).

Sodium Salt

(1) When the Flame Coloration Test is performed, sodium salts impart a yellow color to a colorless flame.

(2) With potassium hydrogen pyroantimonate TS, neutral solutions of sodium salts (1 in 20) yield a white crystalline precipitate (the formation of the precipitate is accelerated by rubbing the inside wall of the test tube with a glass rod).

Succinate

When 1 ml of ferric chloride solution (1 in 10) is added to 5 ml of a solution of a succinate (1 in 20), previously adjusted to pH 6 to 7, a brown precipitate is formed.

Sulfate

(1) With barium chloride solution (3 in 25), solutions of sulfates yield a white precipitate that does not dissolve in hydrochloric acid or in diluted nitric acid (1 in 10).

(2) With lead acetate TS, neutral solutions of sulfates yield a white precipitate that dissolves in ammonium acetate solution (1 in 10).

(3) An equal volume of diluted hydrochloric acid (1 in 4) produces no white turbidity nor odor of sulfur dioxide when added to solutions of sulfates (distinction from sulfites).

Sulfite and Bisulfite

(1) When iodine–potassium iodide TS is added dropwise to solutions of sulfites or bisulfites acidified with acetic acid, the color of the TS disappears.

(2) When an equal volume of diluted hydrochloric acid (1 in 4) is added, solutions of sulfites or bisulfites (1 in 20) acidified with acetic acid evolve the odor of sulfur dioxide and yield no turbidity. On the subsequent addition of 1 drop of sodium sulfide TS, the solutions immediately yield a white turbidity, which changes to a yellow precipitate.

Tartrate

(1) With silver nitrate solution (1 in 50), neutral solutions of tartrates (1 in 20) yield a white precipitate that dissolves in nitric acid. Also, the precipitate, when warmed with ammonia TS, dissolves, and metallic silver is deposited gradually.

(2) When 2 drops of diluted acetic acid (1 in 4), 1 drop of ferrous sulfate TS, 2 to 3 drops of hydrogen peroxide TS, and then an excess of sodium hydroxide solution (1 in 25) are added, solutions of tartrates (1 in 20) exhibit a red-purple to purple color.

(3) When 5 ml of sulfuric acid, previously mixed with 2 to 3 drops of resorcinol solution (1 in 50) and 2 to 3 drops of potassium bromide solution (1 in 10), is added to 2 to 3 drops of a solution of a tartrate (1 in 20), and then the mixture is heated for 5 to 10 minutes on a water bath, a dark blue color is produced. When the solution, previously cooled, is poured into an excess of water, a red color is produced.

Thiocyanate

(1) With an excess of silver nitrate solution (1 in 10), solutions of thiocyanates yield a white precipitate that does not dissolve in diluted nitric acid (1 in 10), but does dissolve in ammonia solution.

(2) With ferric chloride solution (1 in 10), solutions of thiocyanates produce a red color that does not fade with hydrochloric acid.

Zinc Salt

(1) With sodium sulfide TS, neutral to alkaline solutions of zinc salts yield a whitish precipitate that does not dissolve in diluted acetic acid (1 in 20). The precipitate dissolves on the subsequent addition of diluted hydrochloric acid (1 in 4).

(2) With freshly prepared potassium ferrocyanide solution (1 in 10), solutions of zinc salts yield a white precipitate that does not dissolve in diluted hydrochloric acid (1 in 4), but does dissolve in sodium hydroxide solution (1 in 25).

Quantitative Test for Generated Gas

The Quantitative Test for Generated Gas is designed to measure the quantity of gas generated from Baking Powder.

Apparatus Use the apparatus illustrated on the next page.

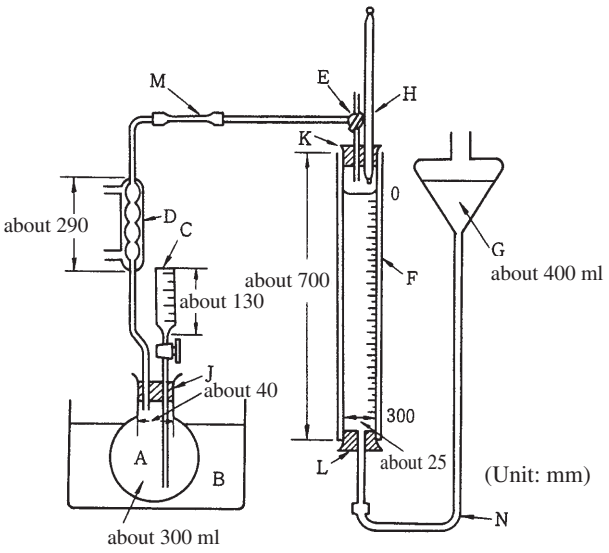
Preparation of Replacing Solution Weigh 100 g of sodium chloride, dissolve in 350 ml of water, add 1 g of sodium hydrogen carbonate, and add diluted hydrochloric acid (1 in 3) until the solution shows slight acidity to methyl orange TS.

Procedure Wrap 2.0 g of the sample (in the case of Duplex Baking Powder, use the mixture in the same ratio as specified for use) in Japanese paper (“washi,” which is traditional Japanese paper), and place into flask A for gas generation containing 100 ml of water. Connect the apparatus, open three-way stopcock E, and move leveling bottle G vertically to adjust the level of the replacing solution to the zero mark on the scale of gas buret F. Allow water to flow through condenser D, turn three-way stopcock E and, after the path is opened between condenser D and gas buret F, add 20 ml of diluted hydrochloric acid (1 in 3) dropwise from dropping funnel C, and then immediately close the cock of the dropping funnel. While occasionally shaking the flask gently, heat in a water bath at 75°C, and lower leveling bottle G according to the level of gas buret F. After 3 minutes, when the levels of the replacing solution in gas buret F and leveling

bottle G are equal, read the level on scale V (ml), and read the temperature (t°) of the generated gas with thermometer H. Calculate the volume of generated gas Vo (ml) in a normal state by the formula given below. Determine the blank value v (ml) in the same manner, and make any necessary correction.

$$\text{Vo (ml)} = (V - v) \times \frac{P - p}{101} \times \frac{273}{273 + t}$$

P = atmospheric pressure (kPa) at the time of measurement,
p = vapor pressure (kPa) of water at t°C.



A: Round-bottom flask for gas generation (about 300 ml capacity)
B: Water bath
C: Acid-dropping funnel
D: Condenser
E: Three-way stopcock
F: Gas buret with outer tube (about 300 ml capacity, with 1-ml divisions)
G: Leveling bottle (about 400 ml capacity)
H: Thermometer
J, K, and L: Rubber stoppers
M and N: Rubber tubes

Readily Carbonizable Substances Test

The Readily Carbonizable Substances Test is designed to determine the allowable limit of impurities in a sample which are readily colored by the addition of sulfuric acid.

Procedure Unless otherwise specified, proceed as directed below.

Before use, wash thoroughly a colorless, hard-glass test tube with 94.5–95.5% sulfuric acid. Unless otherwise specified, when the sample is a solid, place 5 ml of 94.5–95.5% sulfuric acid into the test tube, add the specified quantity of the powdered sample in small portions, and dissolve completely by stirring with a glass rod. When the sample is a liquid, measure the specified quantity of the sample, and place into the test tube. Add 5 ml of 94.5–95.5% sulfuric acid, and mix by shaking. Cool the tube if the temperature of the content in the tube rises. If the reaction may be affected by the temperature, maintain it at the standard temperature. Allow to stand for 15 minutes. Place the Matching Fluid, specified in the individual monograph, into another test tube of the same quality and form as used for the sample, and use this solution as the control solution. Compare the color with that of the control solution against a white background by examining from above and from the side. The color of the sample is not deeper than that of the control solution.

When the specification "to dissolve the sample in sulfuric acid while heating" is given, place the sample and sulfuric acid in the test tube, heat as directed in the individual monograph, and compare the color with that of the control solution.

Refractive Index

Refractive Index Determination is designed to measure the ratio of the velocity of light in air to its velocity in a sample. In isotropic substances, the refractive index is a constant unique to each substance at a definite wavelength, temperature, and pressure. This measurement is applicable to purity tests.

The refractive index (n_D^t) means the value obtained with respect to air and the sample when measured using the D line of the sodium spectrum at t°C. Unless otherwise specified, the refractive index is measured using a Abbe refractometer at a temperature in the range of ±0.2°C of that specified in the individual monograph.

Residue on Ignition

The Residue on Ignition Test is designed to measure the weight of the substance left when a sample is ignited with sulfuric acid.

In the Monog raphs, such a specification as "not more than 0.10%" for this test indicates that when determined by igniting 1 to 2 g of the sample, accurately weighed, with sulfuric acid at 450–550°C for 3 hours, the weight of the residue is not more than 0.10% of the sample. Also, such a specification as "not more than 0.02% (5 g, 850°C, 30 minutes)" indicates that when determined by igniting about 5 g of the sample, accurately weighed, with sulfuric acid at 850°C for 30 minutes, the weight of the residue is not more than 0.02% of the sample. When the stipulation "dried sample" is given in the Monographs, the sample to be used for the test should be previously dried under the conditions specified for the Loss on Drying Test in the individual monograph.

Procedure Ignite a platinum, quartz, or porcelain crucible under the conditions specified in the individual monograph for about 30 minutes, allow to cool in a desiccator, and weigh accurately.

If the sample consists of large crystals or lumps, quickly grind them to a size not exceeding about 2 mm in diameter. Unless otherwise specified, place 1 to 2 g of the ground sample in the crucible described above, and weigh accurately. Moisten the sample with a small amount of sulfuric acid, ignite slowly at as low a temperature as practicable until the sample is almost incinerated, and allow to cool. Add 1 ml of sulfuric acid, and heat slowly until white fumes no longer evolve. Transfer the crucible into an electric furnace, and unless otherwise specified, ignite it at 450–550°C for 3 hours. Cool the crucible in a desiccator, and then weigh accurately. When the amount of the residue so obtained exceeds the limit specified in the individual monograph, ignite to constant weight.

Specific Gravity

Specific Gravity means the ratio of the mass of a substance to that of an equal volume of a standard substance. In this test, the specific gravity ($d_t^{t'}$) means the ratio of the weight of the sample at t′°C to that of an equal volume of distilled water at t°C. When only the specific gravity is given, unless otherwise specified, it means the ratio of the weight (d_{20}^{20}) of the sample at 20°C to that of an equal volume of distilled water at 20°C. Unless otherwise specified, the measurement is performed using Method 1, Method 2, or Method 4. When the figure specified is accompanied by the word "about," Method 3 is also applicable.

Method 1. Measurement by Pycnometer

A pycnometer is a glass bottle with a capacity of usually 10 ml to 100 ml. It has a ground-glass stopper fitted with a thermometer and a side inlet-tube with a marked line and a ground-glass cap.

Weigh accurately a pycnometer, previously cleaned and dried, and record the weight (W). Remove the stopper and the cap, fill the pycnometer with the sample, keep at a temperature 1–3°C lower than the specified temperature (t′°C), and close the stopper, taking care not to leave any bubbles. Raise the temperature gradually until the thermometer shows the specified temperature. Remove the sample above the mark from the side tube, and close the cap. Wipe the outside surface thoroughly, weigh accurately, and record the weight (W_1). Perform the same procedure with distilled water using the same pycnometer, and record the weight (W_2) at the specified temperature (t°C). Calculate the specific gravity ($d_t^{t'}$) by the formula:

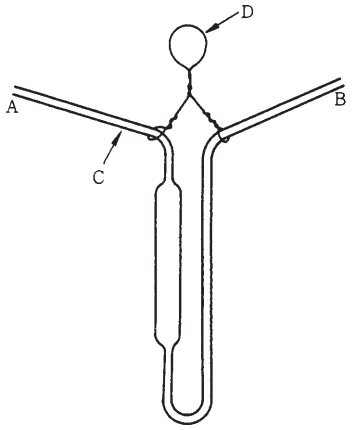
$$(d_t^{t'}) = \frac{W_1 - W}{W_2 - W}$$

Method 2. Measurement by Sprengel-Ostwald Pycnometer

The Sprengel-Ostwald pycnometer (shown in the Figure) usually has a capacity of 1 to 10 ml, and both ends are thick-walled fine tubes, one (A) of which has the marked line (C). A platinum wire (D) (or an aluminum wire or other appropriate wire) is attached to hang on the hook of a chemical balance when weighing.

Weigh accurately a cleaned and dried pycnometer, and record the weight (W). Immerse fine tube B without a marked line in the sample kept at a temperature 3–5°C lower than the specified temperature. Attach a rubber tube or a ground-glass tube to the end of A, and suction up the sample gently until it comes up above marked line C, taking care to prevent bubble formation. Immerse the pycnometer in a water bath kept at the specified temperature (t′°C) for 15 minutes, and by attaching a piece of filter paper to the end of B, adjust the end of the sample to marked line C. Remove the pycnometer from the water bath, wipe the water off its outside surface, weigh accurately, and record the weight (W_1). Perform the same determination with distilled water using the same pycnometer. Weigh accurately the pycnometer containing distilled water at the specified temperature (t°C), and record the weight (W_2). Calculate the specific gravity ($d_t^{t'}$) by the formula:

$$(d_t^{t'}) = \frac{W_1 - W}{W_2 - W}$$



Method 3. Measurement by Hydrometer

Use a hydrometer for the specified temperature with the required precision. Before using, clean the hydrometer with ethanol or diethyl ether.

Shake the sample well. After the bubbles disappear, place the hydrometer in the sample. When the hydrometer comes to a standstill at the specified temperature, read the specific gravity at the upper brim of the meniscus. If any hydrometer has specific instructions, follow those instructions.

Method 4. Measurement by Oscillating Transducer Density Meter

This method is designed to determine the specific gravity of a liquid or gaseous sample from the mass of reference substances by measuring the density of the sample from the intrinsic oscillation period (T(s)) of a cell filled with the sample. When a cell containing the sample to be examined is oscillated, it undergoes an oscillation with an intrinsic oscillation frequency, depending on the mass of the sample. If the volume of the oscillating part of the sample cell is constant, the relation of the square of the intrinsic oscillation period and density of the sample is linear.

To measure the sample density using this method, the respective intrinsic oscillation periods (T_{S1} and T_{S2}) for two reference substances (density: ρ_{S1}, ρ_{S2}) should be previously measured at the specified temperature (t′°C), and the cell constant $K_{t'}$ ($g \cdot cm^{-3}s^{-2}$) should be determined by the formula:

$$Kt' = \frac{\rho_{S1}^{t'} - \rho_{S2}^{t'}}{T_{S1}^{2} - T_{S2}^{2}}$$

Usually, water and dried air are used as reference substances. Here, the density of water ($\rho_{S1}^{t'}$) at the temperature (t′ °C) is obtained from the table above, and that of dried air ($\rho_{S2}^{t'}$) is calculated by the formula:

$$\rho_{S2}^{t'} = 0.0012932 \times \{273.15/(273.15 + t')\} \times (p/101.325),$$

where the pressure of dried air is at p kPa.

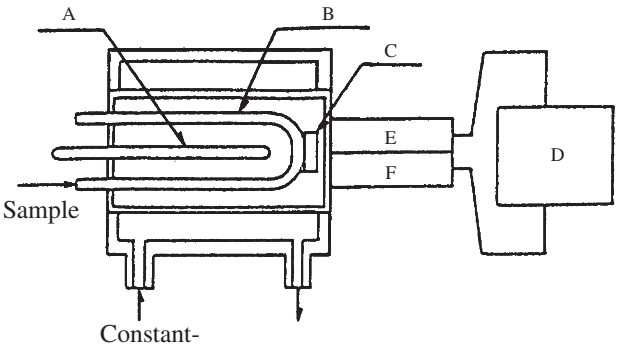
When the sample is introduced into a sample cell of a cell constant ($K_{t'}$) and the intrinsic oscillation period (T_T) of the sample is measured under the same operating conditions as used for the reference substances, the density of the sample ($\rho_T^{t'}$) can be determined by the following formula using the intrinsic oscillation period (T_{SI}) and the density of water ($\rho_{S1}^{t'}$) at the specified temperature (t′ °C).

$$\rho_T^{t'} = \rho_{S1}^{t'} + K_{t'}(T_T^{2} - T_{S1}^{2})$$

The specific gravity of the sample ($d_t^{t'}$) in relation to water of the temperature (t°C) can be determined by the formula below, using the density of water (ρ_{S1}^{t}) at the temperature (t°C) indicated in the table.

$$d_t^{t'} = \rho_T^{t'} / \rho_{S1}^{t}$$

Apparatus An oscillating transducer density/specific gravity meter is usually composed of a U-shaped glass sample cell, an oscillator to give an initial vibration to the sample cell, a detection system to measure the intrinsic vibration period, and a temperature controlling system. The sample cell is of an inner volume of about 1 ml and is fixed with its closed end. The structure of the sample cell chamber and its surrounding is illustrated in the right column.



A: Thermometer
B: Sample cell
C: Vibration plate
D: Amprifier
E: Detector
F: Vibrator

Procedure Adjust the sample cell, water, and the sample to the specified temperature (t′ °C). Wash the sample cell with water or an appropriate solvent, and then dry thoroughly with a stream of dried air. Stop the flow of dried air, confirm that the temperature is maintained constant, and then measure the intrinsic oscillation period (T_{S2}) given by the dried air. Separately, measure the atmospheric pressure (p kPa) at the place of determination. Next, introduce water into the sample cell and measure the intrinsic oscillation period (T_{SI}) of the water. Determine the cell constant ($K_{t'}$) using the intrinsic oscillation periods of water and dried air obtained by the above-mentioned formula.

Next, introduce the sample into the glass cell, confirm that the temperature is maintained constant, and measure the intrinsic oscillation period T_T of the sample. Obtain the density of the sample ($\rho_T^{t'}$), using the intrinsic oscillation periods of water and the sample, the density of water ($\rho_{S1}^{t'}$), and the cell constant ($K_{t'}$). If necessary, calculate the specific gravity of the sample ($d_t^{t'}$) in relation to water at the temperature (t°C), using the density of water (ρ_{S1}^{t}) given in the following table.

When introducing the sample or water into the cell, take care to avoid the formation of bubbles in the sample cell.

Temperature °C	Density g/cm³	Temperature °C	Density g/cm³	Temperature °C	Density g/cm³	Temperature °C	Density g/cm³
0	0.99984	10	0.99970	20	0.99820	30	0.99565
1	0.99990	11	0.99961	21	0.99799	31	0.99534
2	0.99994	12	0.99950	22	0.99777	32	0.99503
3	0.99996	13	0.99938	23	0.99754	33	0.99470
4	0.99997	14	0.99924	24	0.99730	34	0.99437
5	0.99996	15	0.99910	25	0.99704	35	0.99403
6	0.99994	16	0.99894	26	0.99678	36	0.99368
7	0.99990	17	0.99877	27	0.99651	37	0.99333
8	0.99985	18	0.99860	28	0.99623	38	0.99297
9	0.99978	19	0.99841	29	0.99594	39	0.99259

Specific Optical Rotation

Optical rotation is the property displayed by optically active substances or their solutions of rotating the plane of polarization of polarized light. The optical rotation is measured using a polarimeter.

The optical rotation is characterized as dextrorotatory or levorotatory, depending on whether the plane of the polarization is rotated to the right or to the left, respectively, as determined by viewing towards the light source. The optical rotation is expressed in angular degrees with the symbol (°), placed at the upper right of the number of degrees, and a plus sign (+) or a minus sign (–) preceding the number for dextrorotation or levorotation, respectively.

The angle of rotation α_x^t is the value obtained when measured with specific monochromatic light x (described in terms of the wavelength or the name) at temperature t°C. When the phrase "optical rotation" is only written, unless otherwise specified, it indicates the angle of rotation α_D^{20}, as measured at 20°C using a polarimeter tube of 100 mm in length and the D line of the sodium spectrum as the light source.

The specific rotation $[\alpha]_x^t$ is expressed by the formula:

$$[\alpha]_x^t = \frac{100\alpha}{lc}$$

- t = the temperature of measurement,
- x = the wavelength or the name of the specific monochromatic light of spectrum used (when D line is used, indicate as D),
- α = the angle of the rotation, in degrees, of the plane of the polarization,
- l = the thickness of the layer of the measured solution, i.e., the length of the polarimeter tube (mm),
- c = the number of grams of the sample in 1 ml of the solution.

In the Monographs, such a specification as "$[\alpha]_D^{20}$: +20.5 to +21.5° (1g, freshly boiled and cooled water, 10 ml, on the dried basis)" for this test indicates that the specific rotation of the substance is +20.5 to +21.5°, when determined on the dried basis for a solution that is prepared by weighing accurately about 1 g of the test substance and dissolving in newly boiled and cooled water to make exactly 10 ml.

Sulfate Limit Test

The Sulfate Limit Test is designed to determine the allowable limit of sulfate contained in a sample.

In the Monographs, such a specification as "not more than 0.024% as SO_4 (1.0 g, Control Solution 0.005 mol/L sulfuric acid 0.50 ml)" for this test indicates that when determined by weighing 1.0 g of the test substance as the sample and proceeding as directed in the following procedure, using 0.50 ml of 0.005 mol/L sulfuric acid in the preparation of the control solution, the sulfate content of the substance is not more than 0.024% as SO_4.

Preparation of Test Solution and Control Solution Unless otherwise specified, proceed as directed below.

Test Solution When only the quantity of the sample is specified, measure the specified quantity of the sample, transfer into a Nessler tube, and dissolve in about 30 ml of water. Neutralize the solution with diluted hydrochloric acid (1 in 4) if the solution is alkaline. Add 1 ml of diluted hydrochloric acid (1 in 4) and water to make 50 ml.

When the test solution is prepared using the sample solution prepared in the individual monograph, transfer the specified quantity of the sample solution into a Nessler tube, add 1 ml of diluted hydrochloric acid (1 in 4) and then water to make 50 ml.

Control Solution Measure the specified quantity of 0.005 mol/L sulfuric acid, and transfer into another Nessler tube. Add 1 ml of diluted hydrochloric acid (1 in 4) and water to make 50 ml. If the test solution is not clear, filter both the test and control solutions under the same conditions.

Procedure Unless otherwise specified, add 2 ml of barium chloride solution (3 in 25) to each of the test and control solutions, mix thoroughly, and allow to stand for 10 minutes. Examine both Nessler tubes from above and from the side against a black background, and compare the turbidity. The turbidity developed in the test solution is not thicker than that of the control solution.

Sulfite Determination

Sulfite Determination is designed to determine the quantity of sulfites from the quantity of iodine required to react with sulfites. In the test, sulfites are made to react with iodine, the excess iodine is back-titrated with sodium thiosulfate, and the amount of iodine required for the reaction is calculated.

Procedure Unless otherwise specified, proceed as directed below.

Measure accurately the specified quantity of the sample, transfer it into a flask with a ground-glass stopper containing exactly 50 ml of 0.05 mol/L iodine solution, and dissolve. Stopper, allow to stand for 5 minutes, and add 2 ml of diluted hydrochloric acid (2 in 3). Finally, titrate the excess iodine with 0.1 mol/L sodium thiosulfate solution (indicator: starch TS).

Thin-Layer Chromatography

Thin-Layer Chromatography is designed to separate the individual components of a mixture by developing in a mobile phase, using a thin-layer made of a suitable immobile phase. This method is generally applicable to identification tests and purity tests.

Preparation of Thin-Layer Plate Unless otherwise specified, prepare a thin-layer plate by the following method, and then store it protected from moisture.

Using suitable instruments, make a suspension by adding an adequate amount of water to the specified solid support. Apply this suspension on a 50 mm×200 mm or 200 mm× 200 mm glass plate that is smooth and of uniform-thickness to make a uniform layer of 0.2–0.3 mm in thickness. After air-drying, dry further under the specified conditions. A suitable plastic plate may be used instead of a glass plate. Also, commercial thin-layer plates prepared by applying solid supports specified in the individual monographs on glass plates, plastic plates, or aluminum sheets may be used.

Procedure Unless otherwise specified, proceed as directed below.

Designate a line about 20 mm distant from one end of the thin-layer plate as the starting line. Using a micropipette, apply the specified volumes of the test solution and control solution on the starting line at least 10 mm apart from each other and at least 10 mm distant from both edges of the plate so that the applied spots are about 3 mm in diameter. Air-dry the plates. Place the plate in a developing container with the starting line down, and tightly seal the container. The developing container should be filled beforehand with the specified developing solvent up to a depth of 10 mm, and should be saturated with the vapor of the solvent. When the solvent front has ascended from the starting line to the specified distance, take the plate out of the container, and air-dry. Examine and compare the location and color of each spot obtained from the test solution and the control solution by the specified method.

Turbidity Test

The Turbidity Test is designed to scientifically and objectively determine the solubility of a sample to the solvent specified in Clarity of Solution in Purity in the individual monograph. By examining the state of the solution, the characteristic properties of the substance and the existence of impurities in the substance can be easily identified.

In the Monographs, such a specification as "almost clear (1.0 g, water 20 ml)" for the "clarity of solution" indicates that a solution prepared by dissolving 1.0 g of the test substance in 20 ml of water is almost clear.

Preparation of Test Solution Unless otherwise specified, prepare the solution in a Nessler tube as specified in Clarity of Solution in the individual monograph, and use this solution as the test solution.

Preparation of Standard Solution

Turbidity Standard Stock Solution Measure exactly 14.1 ml of 0.1 mol/L hydrochloric acid, and add water to make exactly 50 ml. One ml of this solution contains 1 mg of chlorine (Cl).

Turbidity Standard Solution Measure exactly 1 ml of the Turbidity Standard Stock Solution, and add water to make exactly 100 ml. One ml of this solution contains 0.01 mg of chlorine (Cl).

Preparation of Reference Solutions

The turbidity is identified by the solutions prepared as directed below.

Clear. Measure 0.2 ml of the Turbidity Standard Solution, and add water to make 20 ml. Add 1 ml of diluted nitric acid (1 in 3), 0.2 ml of 2% (w/v) dextrin solution, and 1 ml of 2% (w/v) silver nitrate solution. Shake, and allow to stand for 15 minutes, protected from direct sunlight.

Almost clear. Measure 0.5 ml of the Turbidity Standard Solution, and add water to make 20 ml. Add 1 ml of diluted nitric acid (1 in 3), 0.2 ml of 2% (w/v) dextrin solution, and 1 ml of 2% (w/v) silver nitrate solution. Shake, and allow to stand for 15 minutes, protected from direct sunlight.

Very slightly turbid. Measure 1.2 ml of the Turbidity Standard Solution, and add water to make 20 ml. Add 1 ml of diluted nitric acid (1 in 3), 0.2 ml of 2% (w/v) dextrin solution, and 1 ml of 2% (w/v) silver nitrate solution. Shake, and allow to stand for 15 minutes, protected from direct sunlight.

Slightly turbid. Measure 6 ml of the Turbidity Standard Solution, and add water to make 20 ml. Add 1 ml of diluted nitric acid (1 in 3), 0.2 ml of 2% (w/v) dextrin solution, and 1 ml of 2% (w/v) silver nitrate solution. Shake, and allow to stand for 15 minutes, protected from direct sunlight.

Turbid. Measure 0.3 ml of the Turbidity Standard Stock Solution, and add water to make 20 ml. Add 1 ml of diluted nitric acid (1 in 3), 0.2 ml of 2% (w/v) dextrin solution, and 1 ml of 2% (w/v) silver nitrate solution. Shake, and allow to stand for 15 minutes, protected from direct sunlight.

Procedure Unless otherwise specified, proceed according to the follow the method: Place equal volumes of the test solution and the reference solution into separate Nessler tubes, and examine them from above and from the side, protected from sunlight; the turbidity of the test solution is not thicker than that of the reference solution corresponding to the specified turbidity. For solutions specified as "clear" or "almost clear," foreign matter, such as floating matter, should not be present practically.

Ultraviolet-Visible Spectrophotometry

Ultraviolet-Visible Spectrophotometry is designed to measure the degree of absorption of light in a definite and narrow wavelength range by a sample. The visible and ultraviolet absorption spectra of a solution of a substance depend on the chemical structure of the substance. Spectrophotometry is used to identify a substance by measuring the absorbances at various wavelengths. This method is applicable to identification tests, purity tests, and assays, in which the absorbance of a solution with a certain concentration is usually measured at the maximum absorption wavelength (λ_{max}) or the minimum absorption wavelength (λ_{min}).

When monochromatic light passes through a solution, the ratio of the transmitted light intensity (I) to the incident light intensity (I_0) is called transmittance (T), while the common logarithm of the reciprocal of transmittance is called absorbance (A).

$$T = \frac{I}{I_0} \qquad A = \log\frac{I_0}{I} = -\log T$$

The absorbance (A) is proportional to the concentration (c) of the solution and the length (l) of the layer of solution through which the light passes.

$$A = kcl \quad (k: \text{constant})$$

Calculated on the basis that l is 1 cm and c is a 1% (w/v) solution, the absorbance is called specific absorbance ($E^{1\%}_{1cm}$); calculated on the basis that l is 1 cm and c is 1 mol/L, the absorbance is called the molecular extinction coefficient (E). The molecular extinction coefficient at the wavelength of the maximum of absorption is expressed as E_{max}.

The absorbance is measured for solutions prepared by the solvent specified. The appropriate concentration of solutions is such that the measured absorbance falls between 0.2 and 0.7. If the absorbance is higher than the given range, the solution should be diluted with the solvent to a suitable concentration before measuring. Use the following formulae to obtain $E^{1\%}_{1cm}$ or E.

$$E^{1\%}_{1cm} = \frac{a}{c\ (\%) \times l} \qquad E = \frac{a}{c\ (\text{mol}) \times l}$$

l = length of solution layer (cm),
a = measured absorbance,
c (%) = concentration of solution (% w/v),
c (mol) = molarity of solution (mol/L).

In the Monographs, such a specification as "$E^{1\%}_{1cm}$ (265 nm): 445–485" for this test indicates that when determined at a wavelength of 265 nm by the specified spectrophotometric procedure, $E^{1\%}_{1cm}$ is 445–485.

Apparatus and Procedure A photoelectric spectrophotometer is used for the measurement of absorption. The photoelectric spectrophotometer consists of a monochrometer and a photoelectric photometer. The light source used is a tungsten lamp for measurements in the visible range and a heavy hydrogen discharge lamp for measurements in the ultraviolet range. A quartz or glass cell is used in the visible range and a quartz cell is used in the ultraviolet range. Unless otherwise specified, the path length of the cell is 1 cm.

Proceed as directed below for the measurement of absorbance.

Adjust the wavelength scale to the specified wavelength, put the reference solution into the light path, and adjust the instrument so that the absorbance of the reference solution is zero. Unless otherwise specified, use the solvent of the solution to be determined as the reference solution. Next, put the sample solution into the light path, and read the absorbance.

Calibration of Wavelength and Absorbance Scales The wavelength scale is usually calibrated using wavelengths of 239.95 nm, 253.65 nm, 302.15 nm, 313.16 nm, 334.15 nm, 365.48 nm, 404.66 nm, 435.83 nm, and 546.10 nm for a quartz-mercury or glass-mercury arc lamp, or 486.00 nm and 656.10 nm for a heavy hydrogen discharge lamp.

The absorbance scale is calibrated using a solution prepared as follows: Weigh accurately about 0.06 g of potassium dichromate (standard reagent), previously pulverized and dried between 100°C and 110°C for 3 to 4 hours, and dissolve in 0.005 mol/L sulfuric acid to make exactly 1,000 ml. The $E^{1\%}_{1cm}$ values of this solution are 122.9–126.2 (standard value, 124.5), 142.4–145.7 (standard value, 144.0), 47.0–50.3 (standard value, 48.6), and 104.9–108.2 (standard value, 106.6) at wavelengths of 235 nm (min), 257 nm (max), 313 nm (min), and 350 nm (max), respectively.

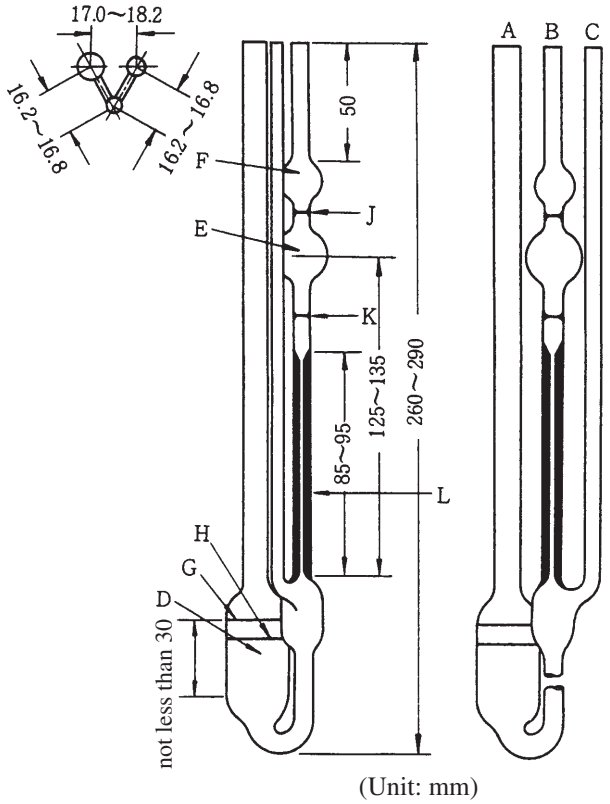
Viscosity

Viscosity Determination is designed to determine the kinematic viscosity and (absolute) viscosity of a sample, using a viscometer. The units are millimeters squared per second (mm^2/s) and milli-Pascal second (mPa·s), respectively.

Method 1 Viscosity Measurement by Capillary Tube Viscometer

The method is applied to the kinematic viscosity determination of Newtonian liquids.

Apparatus Use an Ubbelohde-type viscometer, illustrated below.



(Unit: mm)

A, B, C: Tube
D, E, F: Bulb
G, H, J, K: Mark
L: Capillary tube

The table below gives the approximate relations between the internal diameters of the capillary tubes and the kinematic viscosity ranges suitable for measurements.

Although the internal diameters of the capillary tubes need not be exactly the same as shown in the table on the next page, a viscometer should be selected so that the sample flow time ranges between 200 seconds and 1,000 seconds.

Internal diameter of capillary tubes (mm)	Range of kinematic viscosity (mm^2/s)
0.56–0.60	2–10
0.75–0.79	6–30
0.85–0.89	10–50
1.07–1.13	20–100
1.40–1.46	60–300
1.61–1.67	100–500
1.92–1.98	200–1,000
2.63–2.71	600–3,000
3.01–3.11	1,000–5,000
3.58–3.66	2,000–10,000
4.68–4.88	6,000–30,000
5.33–5.55	10,000–50,000
6.41–6.67	20,000–100,000

Procedure Transfer the sample into tube A, being careful to prevent the formation of bubbles in the sample solution, and adjust the meniscus of the sample so that the upper surface comes to between the two marks G and H on bulb D, when the viscometer is stood upright.

Place the viscometer in a thermostatic water bath maintained at the specified temperature (±0.1°C) so that bulb F of tube B is immersed completely in water.

Fix the viscometer vertically, and allow to stand for about 20 minutes until the sample reaches the specified temperature. Close tube C with a finger, transfer the sample to tube B by gentle suction until the meniscus of the sample rises to the middle of bulb F, open the inlet of tube C, and immediately close the inlet of tube B with the finger. When the sample has flowed down from the lower end of the capillary tube, open the inlet of tube B, and measure the time (t), in seconds, required for the meniscus of the sample to pass from mark J to mark K in tube B. Calculate the kinematic viscosity (ν) by the formula

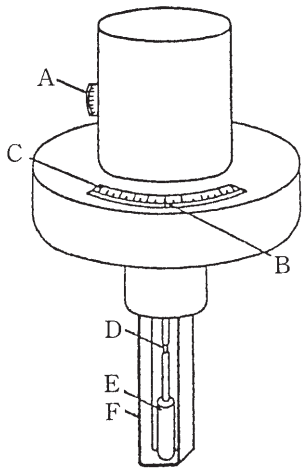
$$\nu = kt,$$

where k is the viscometer constant, which is determined previously using distilled water or a reference standard solution with known viscosity in the same manner as for the sample. The temperature of this measurement may differ from that of the measurement of the sample.

Method 2 Viscosity Measurement by Rotational Viscometer

This method is applied to both Newtonian and non-Newtonian liquids. The principle of the method is the detection and determination of the torque generated by viscosity resistance acting on the rotor surface when it rotates in a sample liquid at a constant angular velocity. The torque is detected in terms of the degree of the torsion of the spring in the viscometer, and the viscosity of the sample is calculated from the dial-reading corresponding to the degree of torsion.

Apparatus Use the Brookfield-type viscometer, illustrated in the right column. Because the type of rotor and the rotational frequency are not specified, select those that are appropriate for the sample used.



A: Rotational-frequency changing dial
B: Indicator
C: Scale
D: Immersion mark
E: Rotor
F: Guard

Procedure Attach rotor E and guard F (except that an adapter for low viscosity is used) specified in the individual monograph. Adjust rotational-frequency changing dial A to the specified frequency. Immerse rotor E slowly into the sample liquid, and adjust immersion mark D to the surface of the sample. Switch on the viscometer to rotate E. Indicator B starts to move from zero. Either when the readings indicated by B stabilize or after the specified amount of time has elapsed, as directed in the individual monograph, stop the rotor and take the reading on scale C. To obtain the viscosity of the sample, multiply the reading by the appropriate conversion factor, given in the following table, which is determined from the type of rotor used and the rotational frequency selected.

Conversion factor

Rotor \ Rotational frequency	60	30	12	6
Adapter	0.1	0.2	0.5	1.0
No. 1	1	2	5	10
No. 2	5	10	25	50
No. 3	20	40	100	200
No. 4	100	200	500	1,000

In the Monographs, such a specification as "1,500–2,500 mPa·s (No. 2, 12 rotations, 30 seconds)" for this test indicates that when a No. 2 rotor is rotated at 12 rotations/min, the viscosity observed 30 seconds later is 1,500–2,500 mPa·s. Also, such a specification as "30,000–40,000 mPa·s (No. 4, 12 rotations, stable)" indicates that when a No. 4 rotor is rotated at 12 rotations/min, the viscosity is 30,000–40,000 mPa·s when the readings have stabilized on the scale.

Water Determination (Karl Fischer Method)

Water Determination is designed to determine water, utilizing the quantitative reaction of water with iodine and sulfur dioxide in the presence of a lower alcohol, such as methanol, and an organic base, such as pyridine, as shown in the following formulae:

$$H_2O + I_2 + SO_2 + 3C_5H_5N \rightarrow 2(C_5H_5N^+H)I^- + C_5H_5N \cdot SO_3$$
$$C_5H_5N \cdot SO_3 + CH_3OH \rightarrow (C_5H_5N^+H)O^-SO_2 \cdot OCH_3$$

There are two determination methods: the volumetric titration method and the coulometric titration method.

In the volumetric titration method, the iodine required for reaction with water is dissolved beforehand in water determination TS, and the water content is determined by measuring the amount of iodine consumed as a result of reaction with water in a sample. In the coulometric titration method, first, iodine is produced by electrolysis of the reagent containing iodide ion. Next, the water content in a sample is determined by measuring the quantity of electricity which is required for the electrolysis, based on the quantitative reaction of the generated iodine with water.

In the Monographs, such a specification as "not more than 4.0% (0.5 g, Back Titration)" for this test indicates that when the test is conducted by weighing about 0.5 g of the sample accurately and performing back titration as directed in Volumetric Titration, the water content is not more than 4.0% of the weight of the sample.

Method 1. Volumetric Titration

Apparatus Generally, the apparatus consists of an automatic burette, a titration vessel, a stirrer, and a constant-voltage amperometric titration system or constant-current potentiometric titration system.

Because water determination TS is extremely hygroscopic, the apparatus should be protected from atmospheric moisture. A suitable desiccant such as silica gel or calcium chloride for water determination is usually used for protection against moisture.

Procedure There are two titration methods: amperometric titration at a constant voltage or potentiometric titration at a constant current. As a rule, the titration with water determination TS should be performed at the same temperature as that at which the TS has been standardized with protection from exposure to moisture.

Amperometric titration at a constant voltage: The apparatus is equipped with a variable resistor in the circuit, and the resistor is adjusted to apply a constant voltage (mV) between a pair of platinum electrodes immersed in the solution to be titrated. The current (μA) that changes with the dropwise addition of water determination TS is measured. As the titration progresses the current in the circuit changes abruptly but returns to the original state within several seconds. At the end of the titration, the change in current persists for a certain time (usually, 30 seconds or longer). The endpoint of titration is determined when this electric state has been attained.

Potentiometric titration at a constant current: The resistor is adjusted to pass a constant current between the two platinum electrodes, and the potential (mV) that changes with the dropwise addition of water determination TS is measured. With the progress of titration, the value indicated by the potentiometer in the circuit decreases suddenly from a polarization state of several hundred mV to the nonpolarization state, but it returns to the original state within several seconds. At the end of titration, the non-polarization state persists for a certain time (usually, 10–30 seconds or longer). The endpoint of titration is determined when this electric state has been attained.

In the case of back titration, when the amperometric titration method is used at a constant voltage, the microammeter needle is out of scale in the presence of an excessive quantity of water determination TS. It returns rapidly to the original position when the titration reaches the endpoint. Similarly, when the potentiometric titration method at a constant current is used, the millivoltmeter needle is at the original position in the presence of an excessive quantity of water determination TS. A definite voltage is applied when the titration reaches the endpoint.

Unless otherwise specified, the titration of water with water determination TS is performed by either of the methods below. Usually, the endpoint of the titration can be observed more clearly in the back titration method than in the direct titration method.

(1) Direct Titration Unless otherwise specified, proceed as directed below.

Put 25 ml of methanol for water determination in a dried titration vessel, and add water determination TS to the endpoint. Unless otherwise specified, weigh accurately a quantity of the sample estimated to contain 10 to 50 mg of water, transfer it quickly into the titration vessel, and dissolve by stirring. Titrate with water determination TS to the endpoint with vigorous stirring.

When the sample is insoluble in the solvent, powder the sample quickly, weigh a suitable amount of the sample accurately, transfer it quickly into the titration vessel, and stir the mixture for 30 minutes, protected from exposure to moisture. Titrate while stirring vigorously.

When the sample interferes with the Karl Fisher reaction, an evaporation technique may be used in which the water released and evaporated by heating the sample using an evaporation device is introduced into the titration vessel by means of a stream of nitrogen gas.

$$\text{Water } (H_2O)\ (\%) = \frac{\left(\begin{array}{c}\text{Volume (ml) of TS for water}\\ \text{determination consumed}\end{array}\right) \times f}{\text{Weight (mg) of the sample}} \times 100$$

f = the number of mg of water (H_2O) corresponding to 1 ml of water determination TS.

(2) Back Titration Unless otherwise specified, proceed as directed below.

Take 20 ml of methanol for water determination in a dried titration vessel, and titrate with water determination TS. Weigh accurately a suitable quantity of the sample estimated to contain 10–50 mg of water, transfer the sample quickly into the titration vessel, add a definite volume of excess water determination TS, stir for 30 minutes, while protecting from exposure to atmospheric moisture, and then titrate the solution with Water–Methanol Standard Solution with vigor-

ous stirring.

$$\text{Water } (H_2O)\ (\%) = \frac{\left[\left(\begin{array}{c}\text{Volume (ml) of}\\ \text{water deter -}\\ \text{mination TS}\\ \text{added}\end{array}\right) \times f\right] - \left[\left(\begin{array}{c}\text{Volume (ml) of}\\ \text{Water–Methanol}\\ \text{Standard Solution}\\ \text{consumed}\end{array}\right) \times f'\right]}{\text{Weight (g) of the sample}} \times 100$$

f = the number of mg of water (H_2O) corresponding to 1 ml of water determination TS,

f′ = the number of mg of water (H_2O) in 1 ml of Water–Methanol Standard Solution.

Method 2. Coulometric Titration

Apparatus Usually, the apparatus is comprised of an electrolytic cell for iodine production, a stirrer, a titration vessel, and a constant-current potentiometric titration system. The iodine production device is composed of an anode and a cathode, separated by a diaphragm. The anode is immersed in the anolyte solution for water determination and the cathode is immersed in the catholyte solution for water determination. Both electrodes are usually made of platinum-mesh.

Because both the anolyte and catholyte solutions for water determination are extremely hygroscopic, the apparatus should be protected from exposure to atmospheric moisture. For this purpose, an appropriate desiccant such as silica gel or calcium chloride for water determination is usually used.

Preparation of Anolyte and Catholyte Solutions for Water Determination

In the coulometric titration method, a pair of anolyte and catholyte solutions is used. The solutions should be prepared by any of the methods given below.

Preparation 1

Anolyte for Water Determination Dissolve 102 g of imidazole for water determination in 900 ml of methanol for water determination, cool the solution with ice, and pass dried sulfur dioxide gas through the solution while maintaining its temperature below 30°C. When the mass increase of the solution has reached 64 g, add and dissolve 12 g of iodine in the solution. Add water dropwise while stirring until the solution turns from brown to yellow. Finally, add methanol for water determination to make 1,000 ml.

Catholyte for Water Determination Dissolve 24 g of diethanolamine hydrochloride in 100 ml of methanol for water determination.

Preparation 2

Anolyte for Water Determination Dissolve 40 g of 1,3-di(4-pyridyl)propane and 30 g of diethanolamine in about 200 ml of methanol for water determination, and pass dried sulfur dioxide gas through the solution until the mass increase of the solution has reached 25 g. Add 50 ml of propylene carbonate, and dissolve 6 g of iodine in the solution. Add methanol for water determination to make 500 ml, and then add water dropwise until the solution turns from brown to yellow.

Catholyte for Water Determination Dissolve 30 g of choline chloride for water determination in 100 ml of methanol for water determination.

Preparation 3

Anolyte for Water Determination Dissolve 100 g of diethanolamine in 900 ml of methanol for water determination or a 3:1 mixture of methanol for water determination/chloroform for water determination, and pass dried sulfur dioxide gas through the solution while cooling. When the mass increase of the solution has reached 64 g, add and dissolve 20 g of iodine in the solution. Add water dropwise while stirring until the solution turns from brown to yellow.

Catholyte for Water Determination Dissolve 25 g of lithium chloride in 1,000 ml of a 4:1 mixture of methanol for water determination/nitromethane.

Procedure Place a suitable volume of an anolyte for water determination in a titration vessel, and immerse in this solution a pair of platinum electrodes or double platinum electrodes of the constant-current potentiometric titration system. Next, immerse the iodide production system filled with a catholyte for water determination in the anolyte.

Switch on the electrolytic system, and make the content of the titration vessel anhydrous. Next, weigh accurately an amount of the sample so that 1–5 mg of water is contained, transfer quickly into the vessel, and dissolve by stirring. Perform the titration to the endpoint with vigorous stirring. If the sample is insoluble in the anolyte, powder it quickly, and transfer an accurately weighed amount of the sample into the vessel. Titrate with vigorous stirring after stirring the mixture for 5–30 minutes while protecting from exposure to atmospheric moisture.

Determine the quantity of electricity (C) [electric current (A)×time (seconds)] required for the production of iodine during the titration, and calculate the content (%) of the water in the sample by the formula below.

When the sample interferes with the Karl Fisher reaction, an evaporating technique may be used in which the water released and evaporated by heating the sample using an evaporation device is introduced into the titration vessel by means of a stream of nitrogen gas.

$$\text{Water } (H_2O)\ (\%) = \frac{\text{Quantity of electricity (C) required for iodine production}}{10.72 \times \text{Weight (mg) of the sample}} \times 100$$

REAGENTS, SOLUTIONS, AND OTHER REFERENCE MATERIALS

C. REAGENTS, SOLUTIONS, AND OTHER REFERENCE MATERIALS

Unless otherwise specified, the reagents, test solutions (TS), volumetric solutions, standard solutions, reference standards, thermometers, filter papers, filters, and sieves to be used in the tests specified in JSFA-VIII shall meet the specifications given in the corresponding sections below. The Bertrand table and Reference Infrared Absorption Spectra are given in sections 10 and 11.

Reagents meeting the Japanese Industrial Standards are given a Japanese Industrial Standard Number (JIS number). Reagents classified as grades other than "special grade" or "first-grade" in the Japanese Industrial Standards are accompanied by the corresponding grade category in square brackets. The reagents for which the Japanese Industrial Standards have been withdrawn are accompanied by the old JIS numbers and the years when the last revision took place. When a reagent name in this publication is different from that in the Japanese Industrial Standards, the JIS name is given in square brackets following the reagent name in Section C.

Glass containers to store reagents, TS, volumetric solutions, and standard solutions shall be extremely low in solubility and in alkalinity and contain as little lead and arsenic as possible.

1. Reagents and Test Solutions (TS)

Absolute Ethanol See Ethanol, Absolute.

Absorbent Cotton Use absorbent cotton specified in the Japanese Pharmacopoeia.

Absorbing Solution for Arsine Dissolve 0.50 g of silver diethyldithiocarbamate in pyridine to make 100 ml. Store in a tightly stoppered, light-resistant bottle in a cold place.

Acetaldehyde CH_3CHO [K8030]

Acetate Buffer Weigh 82 g of anhydrous sodium acetate, and dissolve in 140 ml of water. Add 25 ml of acetic acid and water to make 250 ml. Adjust the pH to 5.51±0.03 with acetic acid or sodium acetate solution (2 in 15).

Acetate Buffer (pH 4.0) Weigh 2.95 g anhydrous sodium acetate, dissolve in 900 ml of water, add a few drops of acetic acid to adjust the pH to 4.0, and add water to make 1,000 ml.

Acetate Buffer (pH 4.5)

Solution 1 Add water to 6.0 g of acetic acid to make 1,000 ml.

Solution 2 Dissolve 8.2 g of anhydrous sodium acetate in water to make 1,000 ml.

Mix both solutions, and adjust the pH to 4.5 with either solution 1 or 2.

Acetate Buffer (pH 5.4)

Solution 1 To 5.78 ml of acetic acid, add water to make 1,000ml.

Solution 2 Weigh 8.5 g of anhydrous sodium acetate, and dissolve in water to make 1,000 ml.

Mix 176 volumes of Solution 1 and 824 volumes of Solution 2. Adjust the pH to 5.4 with either of the solutions 1 and 2.

Acetic Acid CH_3COOH [K8355]

Acetic Acid, Dilute Weigh 6 g of acetic acid, and add water to make 100 ml.

Acetic Acid for Nonaqueous Titration Measure 1,000 ml of acetic acid, add 5 g of chromium trioxide, and allow to stand overnight. Filter, and distill the filtrate. To the distillate obtained at 115ºC or above, add 20 g of acetic anhydride, and redistill. Use the fraction obtained at a constant boiling temperature of 117–118ºC.

Acetic Acid–Sodium Acetate Buffer (pH 4.5) for Iron Limit Test Dissolve 75.4 ml of acetic acid and 111 g of sodium acetate in water to make 1,000 ml.

Acetic Anhydride $(CH_3CO)_2O$ [K8886]

Acetic Anhydride–Pyridine TS Weigh 25 g of acetic anhydride, and add dehydrated pyridine to make 100 ml. Prepare fresh before use.

Acetone CH_3COCH_3 [K8034]

Acetonitrile CH_3CN [K8032]

Acetyl Chloride for Linalool Assay CH_3COCl Measure 128 ml of acetic acid, and transfer it to a 300-ml three-necked flask. Equip one of the two necks with a dropping funnel and the other with a reflux condenser with ground-glass joint each. Cool the flask in an ice water bath. Add dropwise 100 g of phosphorus trichloride slowly, keeping the temperature at 10ºC or below, and allow to stand for 30 minutes. Boil the mixture for 30 minutes, and leave it to stand to separate into two layers. Transfer the supernatant carefully into a distillation flask, and add 5 ml of acetic acid. Use the resultant solution for distillation. Proceed as directed under Method 2 in the Boiling Point and Distillation Range Tests. Set up the apparatus as directed. Use a three-branched adapter connected to two 100-ml flasks to collect the distillate and to a calcium chloride tube. All glass joints should be ground. Distill the solution, and discard the first distillate. To the fraction distilled at 45ºC or above, add 5 g of freshly molten anhydrous sodium acetate, and return it to the distillation flask. Distill again in the same manner, and collect the distillate at 50ºC or above. Use the collected distillate as the reagent. Prepare fresh before use.

2-Acetyl-4-tetrahydroxybutylimidazole $C_9H_{14}N_2O_5$ Grayish-white crystals or crystalline powder. Freely soluble in methanol or in ethanol, and sparingly soluble in water.

Melting point 234–236°C.

Purity Dissolve 10.0 mg of 2-Acetyl-4-tetrahydroxybutylimidazole in 100 ml of carbonyl-free methanol. Analyze this solution by liquid chromatography using the operating conditions given below. No peaks other than the peak of 2-acetyl-4-tetrahydroxybutylimidazole are observed.

Operating conditions

Detector: Ultraviolet spectrophotometer (determination wavelength: 280 nm).

Column: A stainless steel tube of 4.6 mm internal diameter and 15 cm length.

Packing material of column: 5-µm octadecylsilanized silica gel for liquid chromatography.

Mobile phase: A 45:60 mixture of 0.2% (w/v) phosphoric acid/methanol.

Flow rate: 0.6 ml/min.

Acetylene See Dissolved Acetylene.

***N*-Acetylglucosamine for Assay** $C_8H_{15}NO_6$ A white powder or crystalline powder.

Identification To 0.5 ml of a solution of *N*-Acetylglucosamine for Assay (1 in 100), add 0.1 ml of borate buffer (pH 9.1), and heat at 90–100°C for 3 minutes. After quick cooling, add 3.0 ml of *p*-dimethylaminobenzaldehyde TS, and warm at 37°C for 20 minutes. A red-purple color develops.

Purity (1) Specific rotation $[\alpha]_D^{20}$: +39° to +42° (2%, water, 6 hours).

(2) Related substances Prepare a test solution by dissolving 0.1 g of *N*-Acetylglucosamine for Assay in 10 ml of water. Prepare a control solution by adding water to 1.5 ml of the test solution, exactly measured, and making exactly 100 ml. Analyze 10 µl each of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Continue the chromatography for two times the retention time of the main peak, and measure the peak areas. Exclude the solvent peak from measurement. The total area of all peaks of the test solution, other than the main peak, is not greater than the area of the main peak of the control solution.

Operating conditions

Use the operating conditions given in the Assay for *N*-Acetylglucosamine in the Monographs.

Loss on drying Not more than 1.0% (105°C, 3 hours).

Acidic Ferrous Sulfate TS See Ferrous Sulfate, Acidic.

Acidic Stannous Chloride TS See Stannous Chloride TS, Acidic.

Acriflavine Hydrochloride A dark red-brown crystalline powder. A solution of Acriflavine Hydrochloride (1 in 100) is reddish brown. When 30 ml of water is added to 1 ml of this solution, the solution turns yellow, emitting fluorescence. On the subsequent addition of 1 ml of hydrochloric acid, the fluorescence disappears. When sodium hydrogen carbonate solution (1 in 20) is added to a solution of Acriflavine Hydrochloride (1 in 10), an effervescence occurs.

Acrylate Resin for Adsorption Porous resin made as adsorbent.

Active Carbon Use medicinal carbon in the Japanese Pharmacopoeia.

Adipic Acid $HOOC(CH_2)_4COOH$ "Adipic Acid"

Agar [K8263]

Albumin TS Take carefully egg white from a fresh chicken egg. Shake well with 100 ml of water, and filter. Prepare fresh before use.

Aldehyde-free Ethanol See Ethanol, Aldehyde-free.

Alizarin Red S $C_{14}H_5O_2(OH)_2SO_3Na \cdot H_2O$ [K8057]

Alizarin S See Alizarin Red S.

Alizarin Yellow GG $C_{13}H_8N_3NaO_5$ [K8056]

Alizarin Yellow GG TS Weigh 0.1 g of alizarin yellow GG, and dissolve in 100 ml of ethanol. Filter if necessary.

Alizarin Yellow GG–Thymolphthalein TS Mix 10 ml of alizarin yellow GG TS and 20 ml of thymolphthalein TS.

Alkaline Cupric Citrate TS See Cupric Citrate TS, Alkaline.

Alkaline Pyrogallol Solution See Pyrogallol Solution, Alkaline.

Alumina A white, almost odorless, and tasteless powder. Insoluble in water and in organic solvents.

Particle size Alumina passes through a 150-µm standard sieve and hardly pass through a 75-µm sieve.

pH Not more than 11.0. Weigh 50 g of Alumina, add 200 ml of water, and boil for 30 minutes. Cool, and filter. Measure the pH of the filtrate.

Adsorption 0.1–0.2. Put 30 g of Alumina in a glass tube (18 mm in internal diameter) stuffed with glass wool into the end, and pat the tube lightly to level the surface of the alumina layer. Cover the surface with a small, round filter paper, and pour benzene onto the filter paper surface to allow to pass down through the alumina. When the alumina is moistened completely and the solution surface of benzene reaches the top of alumina layer, pour immediately 20 ml of a solution of picric acid in benzene (1 in 20). When the solution surface reaches the top of alumina layer, pour 20 ml of benzene again. Measure the height of alumina layer (L) and the height of the layer that absorbed picric acid (*l*). The adsorption obtained by the following formula:

$$\text{Adsorption} = \frac{L}{l \times 30}$$

Aluminum Al [K8069]

Aluminum Chloride See Aluminum(III) Chloride Hexahydrate.

Aluminum(III) Chloride Hexahydrate $AlCl_3 \cdot 6H_2O$ [K8114]

Aluminum Potassium Sulfate See Potassium Aluminum

Sulfate Dodecahydrate.

Aminated Polyvinyl Alcohol Gel for Liquid Chromatography Use a product prepared for liquid chromatography.

4-Aminoantipyrine $C_{11}H_{13}N_3O$ [K8048]

4-Aminobenzensulfonic Acid $C_6H_7NO_3S$ A white to whitish powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength near 248 nm): Not less than 869. Weigh 0.0100 g of 4-Aminobenzensulfonic Acid, previously dried in a vacuum desiccator, and add ammonium acetate solution (3 in 2,000). Dissolve it to make exactly 100 ml. Refer to this solution as solution A. Exactly measure 10 ml of solution A, add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Measure the absorbance of this solution.

Purity Other aromatic compounds Exactly measure 10 ml of solution A, add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Analyze 20 µl of this solution by liquid chromatography using the operating conditions directed under Purity (6) for Food Yellow No. 4 in the Monographs. Only one peak is observed.

Amino-bonded Silica Gel for Liquid Chromatography Use a product produced for liquid chromatography.

2-Amino-2-hydroxymethyl-1,3-propanediol $H_2NC(CH_2OH)_3$ [K9704]

4-Amino-5-methoxy-2-methylbenzenesulfonic Acid $C_8H_{11}NO_4S$ A whitish powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength near 250 nm): Not less than 362. Weigh 0.0100 g of 4-Amino-5-methoxy-2-methylbenzenesulfonic Acid, previously dried for 24 hours in a vacuum desiccator, dissolve in ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Refer to this solution as solution A. Measure exactly 10 ml of solution A, and add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. This solution exhibits absorption maxima at wavelengths of 218 nm, 250 mm, and 291 nm.

Purity Other aromatic compounds Measure exactly 1.0 ml of solution A, and add ammonium acetate solution (7.7 in1,000) to make exactly 100 ml. Analyze 20 ml of this solution by liquid chromatography using the operating conditions specified in Purity (8) for Food Red No. 40 in the Monographs. Only one peak of 4-amino-5-methoxy-2-methylbenzenesulfonic acid is observed.

1-Amino-2-naphthol-4-sulfonic Acid $C_{10}H_5(NH_2)(OH)SO_3H$ [K8050]

1-Amino-2-naphthol-4-sulfonic Acid TS Weigh 0.2 g of 1-amino-2-naphthol-4-sulfonic acid, dissolve in 195 ml of sodium hydrogen sulfite solution (3 in 20) and 5 ml of anhydrous sodium sulfite solution (1 in 5), and filter if necessary. Stopper tightly, and store in a dark, cold place. Use within 10 days of preparation.

Ammonia–Ammonium Chloride Buffer (pH 10.7) Weigh 67.5 g of ammonium chloride, dissolve in 570 ml of ammonia solution, and add freshly boiled and cooled water to make 1,000 ml.

Ammonia Solution NH_4OH [K8085, Specific gravity: about 0.90]

Ammonia TS To 400 ml of ammonia solution, add water to make 1,000 ml.

Ammonium Acetate CH_3COONH_4 [K8359]

Ammonium Acetate Buffer Weigh 77 g of ammonium acetate, and dissolve in 10 ml of acetic acid and water to make 1,000 ml.

Ammonium Acetate Buffer (pH 3.0)

Solution 1 Dissolve 10 g of ammonium acetate to make 100 ml.

Solution 2 Add water to 31.0 g of acetic acid to make 100 ml.

Mix Solution 1 and Solution 2, and adjust to pH 3.0 with either solution.

Ammonium Amidosulfate $NH_4OSO_2NH_2$ [K8588]

Ammonium Carbonate [K8613]

Ammonium Carbonate TS Weigh 20 g of ammonium carbonate, add 20 ml of ammonia TS and water to dissolve, and make 100 ml.

Ammonium Cerium Nitrate See Ammonium Cerium(IV) Nitrate.

Ammonium Cerium(IV) Nitrate $(NH_4)_2Ce(NO_3)_6$ [Diammonium Cerium(IV) Nitrate, K8556]

Ammonium Cerium(IV) Sulfate See Ammonium Cerium(IV) Sulfate Dihydrate.

Ammonium Cerium(IV) Sulfate Dihydrate $Ce(NH_4)_4(SO_4)_4{\cdot}2H_2O$ [Tetraammonium Cerium(IV) Sulfate Dihydrate, K8977]

Ammonium Chloride NH_4Cl [K8116]

Ammonium Chloride Buffer Solution (pH10) Weigh 5.4 g of ammonium chloride, add 21 ml of ammonia solution and water to dissolve, and make exactly 100 ml.

Ammonium Iron(II) Sulfate Hexahydrate $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$ [K8979]

Ammonium Iron(III) Sulfate Dodecahydrate $Fe(NH_4)(SO_4)_2{\cdot}12H_2O$ [K8982]

Ammonium Iron(III) Sulfate TS To 10 g of iron(III) ammonium sulfate dodecahydrate, add 10 ml of diluted nitric acid (1 in 3) and 80 ml of water to dissolve.

Ammonium Metavanadate See Ammonium Vanadate(V).

Ammonium Molybdate See Hexaammonium Heptamolybdate Tetrahydrate.

Ammonium Molybdate TS Weigh 6.5 g of powdered molybdenum trioxide, dissolve in a mixture of 14 ml of water and 14.5 ml of ammonia solution, and cool. Add gradually the solution to a cooled mixture of 32 ml of nitric acid and 40 ml of water while stirring. Allow to stand for 48 hours, and filter through a glass-fiber filter under reduced pressure. This solution cannot withstand long storage. The solution is usable when it meets the following test: When 2 ml of disodium phosphate solution (1 in 8) is added to 5 ml of the prepared solution, an abundant yellow precipitate is formed immediately or after slight warming. Store protected from light. If a precipitate is formed during storage, use the supernatant.

Ammonium Molybdate–Sulfuric Acid TS Weigh 18.8 g of ammonium molybdate, and dissolve in 300 ml of water. Add 150 ml of sulfuric acid, then water to make 500 ml.

Ammonium Nickel Sulfate See Ammonium Nickel(II) Sulfate Hexahydrate.

Ammonium Nickel(II) Sulfate Hexahydrate $NiSO_4(NH_4)_2SO_4 \cdot 6H_2O$ [K8990]

Ammonium Nitrate NH_4NO_3 [K8545]

Ammonium Oxalate See Ammonium Oxalate Monohydrate.

Ammonium Oxalate Monohydrate $H_4NOOCCOONH_4 \cdot H_2O$ [K8521]

Ammonium Peroxodisulfate $(NH_4)_2S_2O_8$ [K8252]

Ammonium Persulfate See Ammonium Peroxodisulfate.

Ammonium Pyrrolidine Dithiocarbamate $C_5H_{12}N_2S_2$ (for atomic absorption spectrophotometry)

Ammonium Sulfamate See Ammonium Amidesulfate.

Ammonium Sulfate $(NH_4)_2SO_4$ [K8960]

Ammonium Sulfide TS $(NH_4)_2S$ [Ammonium Sulfide Solution (Colorless), K8943] Store in a small, completely filled, light-resistant bottle.

Ammonium Tartrate $H_4NOOCCH(OH)CH(OH)COONH_4$ [Ammonium (+)-Tartrate, K8534]

Ammonium Thiocyanate NH_4SCN [K9000]

Ammonium Thiocyanate–Cobalt Nitrate TS Weigh 17.4 g of ammonium thiocyanate and 2.8 g of cobalt nitrate, mix, and add water to make 100 ml.

Ammonium Vanadate(V) NH_4VO_3 [K8747]

Amyl Alcohol, Iso See 3-Methyl-1-butanol.

Amylase (Crystal) *Bacillus subtilis* liquefying α-amylase. A white, odorless crystalline powder.

Weigh accurately about 1 g of starch, dry at 105°C for 4 hours, and determine the weight loss. Weigh an amount of starch equivalent to 2.0 g on the dry basis, transfer into a Nessler tube, and add 5 ml of phosphate buffer (pH 7) and water to make 50 ml. Heat in a water bath for 10 minutes while shaking occasionally, and allow to stand at 40°C for 30 minutes. To this mixture, add 0.5 ml of a solution of Amylase (Crystal) (1 in 1,000), shake well, and allow to stand at 40°C for 30 minutes. Add immediately 1 ml of sodium hydroxide solution (1 in 25), shake well, and cool. Add 2 drops of phenolphthalein TS, and turn upside down twice. A uniformly pink color develops.

Amylase TS Weigh 0.2 g of amylase (crystal), add 100 ml of water, shake well, and filter. Prepare fresh before use.

Anhydrous Cupric Sulfate See Cupric Sulfate, Anhydrous.

Anhydrous Disodium Phosphate See Disodium Phosphate, Anhydrous.

Anhydrous Disodium Phosphate for pH Determination See Disodium Phosphate, Anhydrous, for pH Determination.

Anhydrous Potassium Carbonate See Potassium Carbonate, Anhydrous.

Anhydrous Sodium Acetate See Sodium Acetate, Anhydrous.

Anhydrous Sodium Carbonate See Sodium Carbonate, Anhydrous.

Anhydrous Sodium Sulfate See Sodium Sulfate, Anhydrous.

Anhydrous Sodium Sulfite See Sodium Sulfite, Anhydrous.

Aniline $C_6H_5NH_2$ [K8042]

Aniline Azo Schaeffer's Salt $C_{16}H_{11}N_2NaO_4S$ Monosodium 6-hydroxy-5-(phenylazo)-2-naphthalenesulfonate.
An orange-red powder.

Specific absorbance $E^{1\%}_{1cm}$ (maximum absorption wavelength near 483 nm): Not less than 595. Weigh 0.0100 g of Aniline Azo Schaeffer's Salt, previously dried for 24 hours in a vacuum desiccator, and add ammonium acetate solution (3 in 2,000). Dissolve and make up to exactly 100 ml. Refer to this solution as solution A. Exactly measure 10 ml of solution A, add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Measure the absorbance of this solution.

Purity Other colors Exactly measure 10 ml of solution A, add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Analyze 20 µl of this solution by liquid chromatography using the operating conditions directed under Purity (5) for Food Yellow No. 5 in the Monographs. Only one peak is observed.

Anion-exchange Resin, Strongly Basic A strongly basic quaternary ammonium salt of polystyrene. A yellow to yellow-brown powder. Passes through a 600-µm standard sieve but hardly passes through a 425-µm sieve.

Weigh about 50 g of Anion-exchange Resin Strongly Basic, immerse in water, allow to stand for 30 minutes, and pour the resin with water into a glass tube for chromatography (about 2.5 cm in internal diameter) to prepare a resin column. Pour 2,000 ml of sodium hydroxide solution (1 in 25) into the column, and allow to pass through at a rate of about 30 ml per minute. Then, wash the resin with water until the washings are neutral to phenolphthalein TS, and perform the following test: Measure 10 ml of the resin, pour it with water into a glass tube for chromatography (15 mm in internal diameter), and allow 70 ml of 0.1 mol/L hydrochloric acid to pass through the column at a rate of about 2 ml per minute. The pH of the effluent is 4.0–8.0.

Anion-exchange Resin, Weakly Basic Weakly basic polystyrene polyamine. A yellow to yellow-brown powder. Passes through a 600-μm standard sieve but hardly passes through a 425-μm sieve.

Weigh about 50 g of Weakly Basic Anion-exchange Resin, immerse in water, allow to stand for 30 minutes, and pour the resin with water into a glass tube for chromatography (about 25 mm in internal diameter) to prepare the resin column. Pour 500 ml of sodium hydroxide solution (1 in 25) into the column, and allow to pass through at a rate of about 8 ml per minute. Then, wash the resin with water until the washings are neutral to phenolphthalein TS, and perform the following test: Measure 10 ml of the resin, pour it with water into a glass tube for chromatography (15 mm in internal diameter), and allow 70 ml of 0.1 mol/L hydrochloric acid to pass through the column at a rate of about 2 ml per minute. The pH of the effluent is 4.0–8.0.

***p*-Anisaldehyde** See 4-Methoxybenzaldehyde.

0.5% *p*-Anisaldehyde–Ethyl Acetate TS See 0.5% 4-Methoxybenzaldehyde–Ethyl Acetate TS.

***p*-Anisaldehyde–Sulfuric Acid TS** See 4-Methoxybenzaldehyde–Sulfuric Acid TS.

***p*-Anisidine** $CH_3OC_6H_4NH_2$ White to light brown crystals or crystalline powder.

Purity Melting point 57–60°C.

***p*-Anisidine–Phthalic Acid TS** Dissolve 1.23 g of *p*-anisidine and 1.66 g of phthalic acid in methanol to make 100 ml. Store in a tightly-stoppered, light-resistant container in a cold place.

Anthrone $C_{14}H_{10}O$ [K8082]

Anthrone TS Weigh 0.05–0.2 g of anthrone, and dissolve in 100 ml of sulfuric acid. Prepare fresh before use.

Antimony(III) Chloride $SbCl_3$ [K8400]

Antimony Trichloride See Antimony(III) Chloride.

Antimony Trichloride TS Wash the surface of antimony trichloride with dehydrated chloroform until the washings become clear. Add dehydrated chloroform to the washed antimony trichloride to make a saturated solution. Store in a tightly-stoppered, light-resistant container in a cold place. Prepare fresh before use.

Aqua Regia Mix 3 parts hydrochloric acid and 1 part nitric acid by volume. Prepare fresh before use.

L-Arabitol $C_5H_{12}O_5$ White crystals or crystalline powder.

Clarity of solution Clear (1.0 g, water 20 ml).

Melting point 102–104°C.

Water Not more than 0.5% (1.0 g, Direct Titration).

Residue on ignition Not more than 0.10% (2 g).

L-Arabinose for Assay $C_5H_{10}O_5$ White crystals or powder.

Purity (1) Specific rotation $[\alpha]_D^{20}$: +103.0° to +105.5° (2 g, water 50 ml, on the dried basis). Allow the test solution to stand for 24 hours before measurement.

(2) Related substances Prepare a test solution by dissolving 1.0 g of L-Arabinose for Assay in 25 ml of water. Prepare a control solution by diluting 1 ml of the test solution, measured exactly, with water to make exactly 100 ml. Analyze 10 μl each of these solutions by liquid chromatography using the operating conditions given below. Continue the chromatography for two times the retention time of the main peak, and measure the peak areas. The total area of all peaks of the test solution, other than the main peak, is not greater than the area of the main peak of the control solution.

Operating conditions

Use the conditions specified in the Assay for L-Arabinose in the Monographs.

L-Arginine Hydrochloride
$H_2N(HN)CNH(CH_2)_3CH(NH_2)COOH \cdot HCl$ [L-Arginine Monohydrochloride, K9046: 1972] White fine crystals.

Identification (1) To a solution of L-Arginine Hydrochloride (1 in 10), add 5 ml of 30% (w/v) sodium hydroxide solution, and boil. Ammonia evolves.

(2) Cool 1 ml of a solution of L-Arginine Hydrochloride (1 in 100) in icy water, add 1 ml of 10% (w/v) sodium hydroxide, 1 ml of 0.02% (w/v) α-naphthol solution, and 0.3 ml of sodium hypochlorite (5% effective chlorine), and shake. A red-orange color develops.

Purity Specific rotation +22.3 to +23.0 (105°C, determine after drying for 3 hours).

Arsenic-free Hydrochloric Acid See Hydrochloric Acid, Arsenic-free.

Arsenic-free Zinc See Zinc, Arsenic-free.

Arsenic Trioxide See Diarsenic Trioxide.

Arsenic Trioxide (Standard Reagent) See Diarsenic Trioxide (Standard Reagent).

Arsenic Trioxide TS Weigh 1 g of diarsenic trioxide, and add 30 ml of sodium hydroxide solution (1 in 40), and dissolve while heating. Cool, and add acetic acid gradually to make 100 ml.

L-Ascorbic Acid $C_6H_8O_6$ "L-Ascorbic Acid"

Ascorbic Acid for Iron Limit Test $C_6H_8O_6$ [L-Ascorbic Acid, K 9502]

L-Ascorbic Acid 2-Glucoside for Assay $C_{12}H_{18}O_{11}$ White, oderless crystals or crystalline powder having an acid taste.

Content Not less than 99.9% of L-ascorbic acid 2-glucoside ($C_{12}H_{18}O_{11}$) on the dried basis.

Identification (1) To 5 ml of a solution of L-Ascorbic Acid 2-Glucoside for Assay (1 in 50), add one drop of potassium permanganate solution (1 in 300). The color of the solution disappears immediately. To 5 ml of a solution of L-Ascorbic Acid 2-Glucoside for Assay (1 in 50), add one to two drops of 2,6-dichlorophenolindophenol sodium salt TS. The color of solution disappears immediately

(2) To 5 ml of boiled Fehling's TS, add 2–3 drops of a solution of L-Ascorbic Acid 2-Glucoside for Assay (5 in 40), and heat for about 5 minutes. A red precipitate is formed.

(3) Determine the absorption spectrum of L-Ascorbic Acid 2-Glucoside for Assay as directed in the Potassium Bromide Disk Method under Infrared Spectrophotometry. It exhibits absorption bands at wavenumbers of about 3300 cm^{-1}, 1770 cm^{-1}, 1700 cm^{-1}, 1110 cm^{-1} and 1060 cm^{-1}.

Purity (1) Clarity of solution Clear (1.0g, Water 50 ml).

(2) Free ascorbic acid and free D-glucose Prepare a test solution. Dissolve 0.50 g of L-Ascorbic Acid 2-Glucoside for Assay in the mobile phase specified in the operating conditions to make exactly 25 ml.

Prepare a standard solution. Dissolve 0.50 g of L-ascorbic acid in the mobile phase to make exactly 25 ml. Take exactly 1.0 ml of this solution, and add the mobile phase to make exactly 100 ml. Use the prepared solution as the ascorbic acid standard stock solution. Each 1.0 ml of this solution contains 0.2 mg of ascorbic acid. Separately, dissolve 0.50 g of glucose in the mobile phase to make exactly 25 ml. Take exactly 1.0 ml of this solution, and add the mobile phase to make exactly 100 ml. Use the prepared solution as the D-glucose standard stock solution. Each 0.1 ml of this solution contains 0.2 mg of D-glucose. Place exactly 10 ml each of the ascorbic acid standard stock solution and D-glucose standard stock solution into a volumetric flask, and add the mobile phase to make exactly 100 ml of a solution. Use this as the standard solution of ascorbic acid and D-glucose.

Analyze 10 µl portions of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Measure the peak areas of ascorbic acid and D-glucose for each solution. Each of the peak areas of peaks of the test solution corresponding to the retention times of ascorbic acid and D-glucose is not greater than each of the peak areas of corresponding peaks of ascorbic and D-glucose for the standard solution.

Operating conditions

Detector: Differential refractometer.

Column: A stainless steel of 4–5 mm internal diameter and 15–30 cm length.

Column packing material: 5–10 µm amino-bonded silica gel for liquid chromatography.

Column temperature: 40ºC.

Mobile phase: A 3:2 mixture of acetonitrile/a solution of potassium dihydrogen phosphate in 0.5% (vol) phosphoric acid (5.44 in 1,000).

Flow rate: A constant rate about 0.7 ml/minute.

Loss on drying Not more than 1.0% (105ºC, 2 hours).

Assay Weigh accurately about 1 g of the sample, dissolve in 30 ml of water, and add two drops of phenolphthalein TS. Titrate with 0.2 mol/L sodium hydroxide solution to the first light red color that persists about for 30 seconds.

Each ml of 0.2 mol/L sodium hydroxide = 67.65 mg of $C_{12}H_{18}O_{11}$

Aspartame $C_{14}H_{18}N_2O_5$ "Aspartame"

L-α-Aspartyl-D-phenylalanine Methyl Ester $C_{14}H_{18}N_2O_5$ A white, crystalline powder. Soluble in water.

Melting point 142.0–145.0°C.

Purity Other amino acids or peptide compounds Use a solution of L-α-Aspartyl-D-phenylalanine Methyl Ester (1 in 1,000) as the test solution. Analyze 2 µl of the test solution by thin-layer chromatography using a 32:15:3:1 mixture of chloroform/methanol/water/acetic acid as the developing solvent. The control solution is not used. Use a thin-layer plate coated with silica gel for thin-layer chromatography and dried at 110°C for 1 hour. When the solvent front ascends to a point about 10 cm above the original line, stop the development, air-dry the plate, then dry at 80°C for 30 minutes. Spray with ninhydrin TS, dry at 80°C for 10 minutes, and examine in daylight. Only one spot is observed.

Barium Carbonate $BaCO_3$ A white powder.

Content Not less than 99.0%.

Purity (1) Sodium Not more than 0.01%. Prepare the test solution by dissolving 1.0 g of Barium Carbonate in diluted hydrochloric acid (1 in 10) to make 100 ml. Prepare the control solution: To 1.0 g of Barium Carbonate, add 1 ml of each of Sodium Standard Solution (0.1 mg/ml), Potassium Standard Solution (0.1 mg/ml), Calcium Standard Solution (0.1 mg/ml), and Strontium Standard Solution (5.0 mg/ml), then add diluted hydrochloric acid (1 in 10) to dissolve, and make 100 ml. Measure the absorbances of the test solution and the control solution, using the operating conditions given below. The absorbance of the test solution does not exceed the difference in absorbance between the control solution and the test solution.

Operating conditions

Light source: Sodium hollow cathode lamp.

Analytical line: 589.0 nm.

Supporting gas: Air.

Combustible gas: Acetylene.

(2) Potassium Not more than 0.01%. Measure the absorbances of the test solution and the control solution prepared in (1), using the operating conditions given below. The absorbance of the test solution does not exceed the difference in absorbance between the control solution and the test solution.

Operating conditions

Light source: Potassium hollow cathode lamp.

Analytical line: 766.5 nm.

Supporting gas: Air.

Combustible gas: Acetylene.

(3) Calcium Not more than 0.01%. Measure the absorbances of the test solution and the control solution prepared in (1), using the operating conditions given below. The absorbance of the test solution does not exceed the difference in absorbance between the control solution and the test solution.

Operating conditions

Light source: Calcium hollow cathode lamp.

Analytical line: 422.7 nm.

Supporting gas: Air.

Combustible gas: Acetylene.

(4) Strontium Not more than 0.5%. Measure the absorbances of the test solution and the control solution prepared in (1), using the operating conditions given below. The absorbance of the test solution does not exceed the difference in absorbance between the control solution and the test solution.

Operating conditions

Light source: Strontium hollow cathode lamp.

Analytical line: 460.7 nm.

Supporting gas: Air.

Combustible gas: Acetylene.

(5) Barium hydroxide Not more than 0.02%. To 5 g of Barium Carbonate, add 50 ml of water not containing carbon dioxide. Shake for 5 minutes, and filter through a filter paper for quantitative analysis (5C). Titrate this solution with 0.05 mol/L hydrochloric acid (indicator: bromothymol blue TS).

Each ml of 0.05 mol/L hydrochloric acid = 4.284 mg of $Ba(OH)_2$

Assay Weigh accurately about 1 g of Barium Carbonate, and add 50 ml of water and 40 ml of 1 mol/L hydrochloric acid. Boil and cool the mixture. Titrate with 1 mol/L sodium hydroxide (indicator: bromothymol blue TS). Separately perform a blank test to make any necessary correction.

Each ml of 1 mol/L hydrochloric acid = 98.67 mg of $BaCO_3$

Barium Chloride See Barium Chloride Dihydrate.

Barium Chloride Dihydrate $BaCl_2 \cdot 2H_2O$ [K8155]

Barium Hydroxide See Barium Hydroxide Octahydrate.

Barium Hydroxide Octahydrate $Ba(OH)_2 \cdot 8H_2O$ [K8577]

Barium Oxide BaO [desiccant, K8428: 1961] A white or grayish white, hygroscopic powder.

Identification (1) A solution of Barium Oxide is alkaline.

(2) Dissolve in water, made acidic with hydrochloric acid, and add sulfuric acid. A white precipitate is produced.

(3) In the Flame Coloration Test, Barium Oxide gives a green flame.

Basic Bismuth Nitrate A white fine crystalline powder. Causes a moistened blue litmus paper to turn red.

Residue on ignition 79.0–82.0.

Basic Lead Acetate TS See Lead Acetate TS, Basic.

Benzene C_6H_6 [K8858]

Benzidine $C_{12}H_{12}N_2$ A white to slightly pinkish crystalline powder. Gradually turns dark upon exposure to light in air.

Identification Dissolve 0.1 g of Benzidine in 10 ml of acetic acid, and add potassium bichromate. A dark green precipitate is produced.

Purity Melting point 127–129°C.

Benzoic Acid C_6H_5COOH [K8073]

***N*-Benzoyl-L-arginine Ethyl Ester Hydrochloride** $C_{15}H_{22}N_4O_3 \cdot HCl$ A white crystalline powder.

Melting point 128–133°C.

Purity Dissolve 0.10 g of *N*-Benzoyl-L-arginine Ethyl Ester Hydrochloride in water to make exactly 10 ml. Use this solution as the test solution. Analyze 10 µl of the test solution by thin-layer chromatography using a 4:1:1 mixture of 1-butanol/acetic acid/water as the developing solvent. No control solution is used. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and dried at 110°C for 1 hour. Stop the development when the solvent front ascends to a point about 10 cm above the original line, air-dry, and allow to stand in iodine vapor for 30 seconds. Only one spot is observed.

Benzyl Alcohol $C_6H_5CH_2OH$ [K8854]

5-Benzyl-3,6-dioxo-2-piperazineacetic Acid $C_{13}H_{14}N_2O_4$ A white to gray crystalline powder. Slightly soluble in acidic water, freely soluble in neutral to alkaline water, and soluble in dimethylsulfoxide.

Melting point 242–246°C.

Purity Other amino or imino compounds Analyze 10 µl of a solution of 5-Benzyl-3,6-dioxo-2-piperazineacetic Acid (1 in 1,000) by thin-layer chromatography, using a 32:15:3:1 mixture of chloroform/methanol/water/acetic acid as the developing solvent. No control solution is used. Use a thin-layer plate coated with silica gel for thin-layer chromatography and dried at 110°C for 1 hour. When the solvent front ascends to a point about 10 cm above the original line, stop the development, and air-dry the plate for 30 minutes. Prepare a beaker filled with chlorine gas: Place about 3 g of bleaching powder in a beaker, add 1 ml of hydrochloric acid cautiously to generate chlorine gas, and leave it tightly sealed for 30 seconds. Transfer the air-dried plate to the beaker filled with chlorine gas, seal the beaker tightly, and allow to stand for 20 minutes. Take the plate out of the beaker, allow to stand for 10 minutes, spray with ethanol, and air-dry. Spray it with potassium iodide–starch TS, and examine in daylight. Only one spot is observed.

Bertrand's TS A Weigh 40 g of fine cupric sulfate crystals, and dissolve in water to make 1,000 ml. Store in an almost-filled, glass-stoppered bottle.

Bertrand's TS B Weigh 200 g of potassium sodium tartrate and 150 g of sodium hydroxide, and dissolve in water to make 1,000 ml. Store in a rubber-stoppered container.

Bertrand's TS C Weigh 50 g of iron(III) sulfate, dissolve in about 500 ml of water, add 200 ml of sulfuric acid gradually, and shake. Cool, add dropwise Bertrand's TS D until a slightly red-brown color develops, and add water to make 1,000 ml.

Bertrand's TS D Weigh 5 g of potassium permanganate, and dissolve in water to make 1,000 ml.

Standardization Weigh exactly 0.25 g of ammonium oxalate, and dissolve in 100 ml of water. Add 2 ml of sulfuric acid, warm to 60–70°C. Titrate this solution with Bertrand's TS D. Refer to the volume of Bertrand's TS D consumed as a (ml). One ml of Bertrand's TS D is equivalent to 0.2238/a g of Cu.

Betaine for Assay See Betaine Monohydrate.

Betaine Monohydrate $C_5H_{11}NO_2 \cdot H_2O$ White, hygroscopic,

deliquescent crystals having a slight odor and a sweet, slightly bitter taste.

Identification Determine the absorption spectrum of Betaine Monohydrate, previously dried, as directed in the Paste Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity Related substances Prepare a test solution by dissolving about 1 g of Betaine Monohydrate, previously dried, in water to make exactly 100 ml. Prepare a control solution by diluting 1 ml of the test solution, measured exactly, with water to make exactly 100 ml. Analyze 10 µl each of the test solution and the control solution by liquid chromatography using the operating conditions given below. Continue the chromatography for about two times the retention time of the main peak and measure the peak areas. Exclude the solvent peak from the measurement. The total area of all peaks of the test solution, other than the main peak, is not greater than the area of the main peak of the control solution.

Operating conditions
Detector: Differential refractometer.
Column: A stainless steel tube of 4 mm internal diameter and 25 cm length.
Column packing material: Strongly acidic cation exchange resin for liquid chromatography.
Column temperature: 70°C.
Mobile phase: Water
Flow rate: Adjust so that the retention time of betaine is about 9 minuets.

Loss on drying 12.0–14.6% (105°C, reduced pressure, 3 hours).

Biphenyl $C_6H_5C_6H_5$ Use a high-quality product produced for gas chromatography.

2,2′- Bipyridyl $(C_5H_4N)_2$ [K8486]

Bis(3-methyl-1-phenyl-5-pyrazolone) $C_{20}H_{18}N_4O_2$ [K9545]

Bismuth Nitrate See Bismuth Nitrate Pentahydrate.

Bismuth Nitrate Pentahydrate $Bi(NO_3)_3{\cdot}5H_2O$ [K8566]

Bismuth Nitrate TS Weigh 5 g of bismuth nitrate, and add 25 ml of water and 25 ml of acetic acid to dissolve. Dilute with water to make exactly 250 ml.

Bis(1-phenyl-3-methyl-5-pyrazolone) See Bis(3-methyl-1-phenyl-5-pyrazolone).

***N,O*-Bis(trimethylsilyl)acetamide**
$CH_3C[NSi(CH_3)_3]OSi(CH_3)_3$ A colorless liquid.
Refractive index n_D^{20}: 1.414–1.418.
Specific gravity 0.825–0.835.
Boiling point 71.0–73.0℃ (4.7 kPa).

Bleaching Powder [K8388:1961] A white or whitish powder having a chlorine odor.

Content Equivalent to not less than 30% effective chlorine.

Assay Proceed as directed in the Assay for High-Test Hypochlorite.

Store in a cool and dark place.

Blue Litmus Paper See Litmus Paper, Blue.

Borate Buffer (pH 9.1) Dissolve 4.95 g of boric acid in 50 ml of water, and adjust to pH 9.1 with sodium hydroxide solution (7 in 100), and add water to make exactly 100 ml (0.8 mol/L).

Boric Acid H_3BO_3 [K8863]

Boric Acid–Sodium Hydroxide Buffer Mix 12.36 g of boric acid and 4.00 g of sodium hydroxide, and dissolve the mixture in water to make 1,000 ml.

Boron Trifluoride BF_3 A colorless gas having a pungent odor.

Boiling point –100.3℃.
Melting point –127.1℃.

Boron Trifluoride–Methanol TS Dissolve 14 g of boron trifluoride in methanol to make 100 ml.

Bouillon, General Dissolve 5 g of meat extract and 10 g of peptone in 1,000 ml of water, while warming gently. After sterilizing, adjust the pH to 6.4–7.0, and cool. Replenish the evaporated water, and filter. Autoclave the filtrate at 121℃ for 30 minutes.

Brilliant Green $C_{27}H_{34}N_2O_4S$ Fine yellow crystals with luster. Soluble in water and in ethanol. Exhibits an absorption maximum at a wavelength of 623 nm.

Bromine Br_2 [K8529]

Bromine TS A saturated solution of bromine.

Set up a glass bottle with a stopper to which vaseline is applied. Take 2–3 ml of bromine into the bottle, and add 100 ml of cold water. Stopper tightly, and shake. Use the water phase. Store in a cold place, protected from light.

Bromine–Hydrochloric Acid TS Measure 1 ml of bromine–potassium bromide TS, and add 100 ml of arsenic-free hydrochloric acid.

Bromine–Potassium Bromide TS Weigh 30 g of bromine and 30 g of potassium bromide, and mix them. Dissolve the mixture in water, and make 100 ml.

Bromocresol Green $C_{21}H_{14}Br_4O_5S$ [K8840]

Bromocresol Green TS Weigh 0.050 g of bromocresol green, and dissolve in 100 ml of ethanol. Filter if necessary.

Bromocresol Green–Methyl Red Mixture TS Mix equal volumes of bromocresol green TS and methyl red TS.

Bromophenol Blue $C_{19}H_{10}Br_4O_5S$ [K8844]

Bromophenol Blue TS Weigh 0.1 g of bromophenol blue, and dissolve in 100 ml of 50% (vol) ethanol. Filter if necessary.

Bromophenol Blue TS for Citric Acid To bromophenol blue TS, add an equal volume of ethanol, and adjust the pH

to 7.0 with 0.01 mol/L sodium hydroxide.

Bromophenol Blue–Sodium Hydroxide TS Weigh 0.1 g of bromophenol blue, dissolve in 3 ml of 0.05 mol/L sodium hydroxide while shaking well, and add water to make 25 ml.

Bromothymol Blue $C_{27}H_{28}Br_2O_5S$ [K8842]

Bromothymol Blue TS Weigh 0.1 g of bromothymol blue, and dissolve in 100 ml of 50% (vol) ethanol. Filter it if necessary.

Bromothymol Blue–Sodium Hydroxide TS To 0.2 g of bromothymol blue, previously powdered, add 5 ml of sodium hydroxide solution (4.3 in 1,000), and add a small amount of water. Dissolve it while stirring in a water bath at 50ºC, and add water to make 100 ml.

Brucine See Brucine *n*-Hydrate.

Brucine *n*-Hydrate $C_{23}H_{26}N_2O_4 \cdot nH_2O$ [K8832]

Buffer for Bacillus Natto Gum (pH 3.3) Dissolve 6.19 g of trisodium citrate, 5.66 g of sodium chloride, 19.80 g of citric acid, 130.0 ml of ethanol, 5.0 ml of 2,2′-thiodiethanol, 4.0 ml of a solution of polyoxyethylene(23) lauryl ether (1 in 4), and 0.1 ml of octanoic acid by adding water to make exactly 1,000 ml.

Butanol See 1-Butanol.

***tert*-Butanol** See *t*-Butyl Alcohol.

1-Butanol $CH_3(CH_2)_2CH_2OH$ [K8810]

2-Butanone $CH_3COC_2H_5$ [K8900]

***t*-Butyl Alcohol** $(CH_3)_3COH$ [K8813]

Butyl Alcohol, Iso See 2-Methyl-1-propanol.

Butylated Hydroxytoluene $C_{15}H_{24}O$ "Butylated Hydroxytoluene"

Caffeine $C_8H_{10}N_4O_2 \cdot H_2O$ Use Caffeine specified in the Japanese Pharmacopoeia.

Calcium Acetate See Calcium Acetate Monohydrate.

Calcium Acetate Monohydrate $Ca(CH_3COO)_2 \cdot H_2O$ [K8364]

Calcium Carbonate $CaCO_3$ [K8617]

Calcium Chloride See Calcium Chloride Dihydrate.

Calcium Chloride Dihydrate $CaCl_2 \cdot 2H_2O$ [K8122]

Calcium Chloride for Water Determination $CaCl_2$ [Calcium Chloride (for water determination), K8125]

Calcium Hydroxide $Ca(OH)_2$ [K8575]

Calcium Hydroxide for pH Determination $Ca(OH)_2$ [Calcium Hydroxide, K8575]

Use a saturated solution that is obtained at 23–27ºC and has pH 12.45 at 25ºC.

Calcium Hydroxide TS Weigh 10 g of calcium oxide, add 40 ml of freshly boiled and cooled water, and allow to stand for a while. Add 1,000 ml of freshly boiled and cooled water, stopper tightly, shake, and allow to stand. Discard the supernatant by decantation, and to the residue, add 1,000 ml of freshly boiled and cooled water. Stopper tightly, and allow to stand for 1 hour with occasional shaking vigorously. Collect the supernatant by decantation or filtration before use.

Calcium Oxide CaO [Quick Lime, K8410]

Camphor $C_{10}H_{16}O$ Use *dl*-camphor in the Japanese Pharmacopoeia.

Carbon Dioxide CO_2 "Carbon Dioxide"

Carbon Disulfide CS_2 [K8732]

Carbon Monoxide CO A colorless gas. Carbon Monoxide is produced by reacting formic acid with sulfuric acid and passing the produced gas through sodium hydroxide TS layer. Carbon Monoxide contained in a hermetic, pressure-resistant metal container may be used.

Carbon Tetrachloride CCl_4 [K8459]

Carbonyl-free Methanol See Methanol, Carbonyl-free.

Carob Bean Gum "Carob Bean Gum"

Casein, Milk See Casein (Milk).

Casein (Milk) [K8234]

Casein Peptone See Peptone, Casein.

Casein TS (pH 2.0) Weigh accurately about 1 g of milk casein, dry at 105ºC for 2 hours, and determine the loss on drying. Weigh exactly an amount equivalent to 1.2 g of milk casein on the dry basis, and add 12 ml of lactic acid TS and 150 ml of water. Warm it in a water bath to dissolve, and cool with running water. Add 1 mol/L hydrochloric acid to adjust to pH 2.0. Add water to make exactly 200 ml. Prepare fresh before use.

Casein TS (pH 7.0) Weigh accurately about 1 g of milk casein, dry at 105ºC for 2 hours, and determine the loss on drying. Weigh exactly an amount equivalent to 0.6 g of milk casein on the dry basis, and add 80 ml of 0.05 mol/L disodium phosphate and 80 ml of water. Warm it in a water bath for 20 minutes to dissolve. Cool with running water, and add 1 mol/L hydrochloric acid to adjust the pH to 7.0. Add water to make exactly 100 ml. Prepare fresh before use.

Casein TS (pH 8.0) Weigh accurately about 1 g of milk casein, dry at 105ºC for 2 hours, and determine the loss on drying. Weigh exactly an amount equivalent to 1.2 g of milk

casein on the dry basis, and add 160 ml of 0.05 mol/L disodium phosphate. Warm it in a water bath to dissolve. Cool with running water, and add 0.1 mol/L sodium hydroxide to adjust to pH 8.0. Add water to make exactly 200 ml. Prepare fresh before use.

Catechol $C_6H_4(OH)_2$ [1,2-Benzenediol, K8240]

Cation-exchange Resin, Strongly Acidic The sodium salt of strongly acidic polystyrene sulfonic acid. A light yellow to yellow-brown powder. Passes through a 600-μm standard sieve, and hardly passes a 420-μm sieve.

Weigh about 50 g of Strongly Acidic Cation-exchange Resin, immerse in water for 30 minutes. Pour the resin with water into a glass tube for chromatography (about 25 mm internal diameter) to prepare a resin column. Pour 250 ml of diluted hydrochloric acid (1 in 4) into the column, and allow it to pass through at a rate of about 4 ml per minute. Then pour water to wash the column until the color of the washings becomes green to blue with bromocresol green TS, and perform the following test:

Measure 10 ml of the resin, pour it with water into a glass tube for chromatography (15 mm internal diameter), and allow 80 ml of 0.1 mol/L sodium hydroxide to pass through at a rate of about 2 ml per minute. The pH of the effluent is 5.0–6.5.

Cation-exchange Resin, Strongly Acidic (Fine) A hydrogen ion type of strongly acidic polystyrene sulfonic acid. A light yellow to yellow-brown powder. Passes through a 150-μm standard sieve and hardly passes a 75-μm sieve.

Weigh about 50 g of Strongly Acidic Cation-exchange Resin (Fine), immerse in water for about 1 hour, and decant 2 or 3 times until the supernatant becomes clear. Pour the resin with water into a glass tube for chromatography (about 25 mm internal diameter) to prepare resin column. Pour 250 ml of diluted hydrochloric acid (1 in 4) into the column, and allow to pass through at a rate of about 4 ml per minute. Then pour water to wash the column until the color of the washings becomes green to blue with bromocresol green TS, and perform the following test:

Measure 10 ml of the resin, pour it with water into a glass tube for chromatography (15 mm in internal diameter), and allow 80 ml of 0.1 mol/L sodium hydroxide to pass through at a rate of about 2 ml per minute. The pH of the effluent is 4.0–6.5.

Cation-exchange Resin, Weakly Acidic (Fine) A hydrogen ion type of weakly acidic methacrylic carboxylic acid. A white powder. Passes through a 150-μm standard sieve and hardly passes through a 75-μm sieve.

Weigh about 50 g of Weakly Acidic Cation-exchange Resin (Fine), immerse in water, allow to stand for about 1 hour, and decant 2 or 3 times until the supernatant becomes clear. Pour the resin with water into glass tube for chromatography (about 25 mm internal diameter) to prepare a resin column. Pour 250 ml of diluted hydrochloric acid (1 in 4) into the column, and allow to pass through at a rate of about 4 ml per minute. Then, pour water to wash the column until the color of the washings changes to green to blue with bromocresol green TS, and perform the following test:

Measure 10 ml of the resin, pour it with water into a glass tube for chromatography (15 mm internal diameter), and allow 80 ml of 0.1 mol/L sodium hydroxide to pass through at a rate of about 2 ml per minute. The pH of the effluent is 4.0–6.5.

Centrifugal Ultrafiltration Unit A polypropyrene tube (about 3 cm diameter and 11–12 cm length) lined with a regenerated cellulose film with 3,000 molecular weight cut off, or other units comparable to this in resolution capability.

Ceric Ammonium Nitrate See Cerium(IV) Ammonium Nitrate.

Ceric Ammonium Sulfate See Cerium(IV) Ammonium Sulfate.

Cerium(IV) Ammonium Nitrate $Ce(NH_4)_2(NO_3)_6$ [Diammonium Cerium(IV) Nitrate, K8556]

Cerium(IV) Ammonium Sulfate See Cerium(IV) Ammonium Sulfate Dihydrate.

Cerium(IV) Ammonium Sulfate Dihydrate $Ce(NH_4)_4(SO_4)_4{\cdot}2H_2O$ [Tetraammonium Cerium(IV) Sulfate Dihydrate, K8977]

Chloral Hydrate $CCl_3CHO{\cdot}H_2O$ [K8869: 1961] Transparent, colorless or white crystals having a pungent aroma.

Content 99.5–101.0%. Weigh accurately about 5 g of Chloral Hydrate, add exactly 50 ml of 1 mol/L sodium hydroxide, and allow to stand for 2 minutes. Titrate with 0.5 mol/L sulfuric acid (indicator: phenolphthalein TS).

Each ml of 1 mol/L sodium hydroxide = 165.4 mg of $CCl_3CHO{\cdot}H_2O$

Chloramine T See Sodium *p*-Toluenesulfonchloramide Trihydrate.

Chloramine T TS Weigh 1.25 g of chloramine T, and dissolve in water to make 100 ml. Prepare fresh before use.

Chloramphenicol $C_{11}H_{12}C_{12}N_2O_5$ Use chloramphenicol specified in the Japanese Pharmacopoeia.

Chloroform $CHCl_3$ [K8322]

Chloroform, Dehydrated $CHCl_3$ Measure 20 ml of chloroform, add 20 ml of water, shake gently and well for 3 minutes, and separate the chloroform layer from the mixture. Repeat twice the above procedure with 20 ml of water each time. Filter the chloroform layer through a dry filter paper. To the filtrate, add 5 g of anhydrous potassium carbonate freshly ignited, stopper tightly, and allow to stand overnight protected from light. Filter through a dry filter paper, and distill the filtrate, protecting from light.

Chloroform, Ethanol-free $CHCl_3$ Measure 20 ml of chloroform, add 20 ml of water, shake well and gently for 3 minutes, and separate the chloroform layer from the mixture. Repeat twice the above procedure with 20 ml of water each time. Filter the chloroform layer through a dry filter paper. To the filtrate, add 5 g of anhydrous sodium sulfate, and shake well for 5 minutes. Allow to stand for 2 hours, and filter through a dry filter paper.

Chloroform for Water Determination To 1,000 ml of chloroform, add 30 g of synthetic zeolite for desiccation, and stopper tightly. With occasional shaking, allow to stand for about 8 hours. After additional about 16 hours of standing, confirm that the chloroform layer is clear and separate it. Store protected from moisture. The water content should not be more than 0.1 mg in 1 ml of the sample.

Choline Chloride $[(CH_3)_3NCH_2CH_2OH]Cl$ [K8130:1981] White crystals or crystalline powder having a faint characteristic odor.

Content 98–101%. Weigh accurately about 0.2 g of Choline Chloride, previously dried 110ºC for 3 hours, titrate with 0.05 mol/L sulfuric acid.

Each ml of 0.05 mol/L sulfuric acid = 0.01396 g of $(CH_3)_2NCH_2CH_2OH]Cl$

Choline Chloride for Water Determination
$[(CH_3)_3NCH_2CH_2OH]Cl$ A white crystalline powder.

Melting point 303–305ºC (decomposition).

Water Not more than 1 mg/g sample.

Chromium Oxide Cr_2O_3 [Dichromium Trioxide (Chromium Oxide), Class I designated chemical, K1401]

Chromium(VI) Oxide CrO_3 [K8434:1980] Dark red-purple, deliquescent crystals or lumps.

Identification To a solution of Chromium(VI) Oxide, add lead acetate. A yellow precipitate is produced.

Purity (1) Clarity Clear (1.0 g, water 10 ml).

(2) Alkali earth metals Not more than 1.0%. Transfer 1.0 g of Chromium(VI) Oxide to an Erlenmeyer flask, and add 17 ml of water, 5 ml of hydrochloric acid (1 in 3), and 5 ml of ethanol. Heat for 1 hour under a reflux condenser. Evaporate the ethanol, and add 70 ml of hot water and 7 ml of ammonia solution (2 in 5). Heat on a water bath until it no longer smells ammonia, and continue to heat to dryness. To the residue, add 30 ml of hot water, filter, and take the filtrate into an evaporating dish, previously weighed. Wash the filter paper three times with three 10-ml portions of hot water, and combine washings with the filtrate. Evaporate the water in the evaporating dish on the water bath. To the residue, add 0.5 ml of sulfuric acid, and evaporate on a heating panel. When ignited, the residue is not more than 1 mg.

Chromium Trioxide See Chromium(VI) Oxide.

Chromotropic Acid See Disodium Chromotropate Dihydrate.

Chromotropic Acid TS Weigh 0.5 g of disodium chromotropate dihydrate, add dilute sulfuric acid (10 in 15) to 50 ml, and shake. Centrifuge the mixture, and use the supernatant as Chromotropic Acid TS. Prepare fresh before use.

Citrate Buffer

Solution 1 Weigh 21 g of citric acid, and dissolve in water to make 1,000 ml.

Solution 2 Weigh 28.4 g of disodium phosphate, and dissolve in water to make 1,000 ml.

Mix 11 volumes of Solution 1 and 389 volumes of Solution 2.

Citrate Buffer (pH 2.2) Weigh 1.4 g of sodium citrate, 13 g of citric acid, and 10.9 g of sodium chloride, and mix them. Dissolve in water to make 1,000 ml.

Citrate Buffer (pH 3.0)

Solution 1 Weigh 21 g of citric acid, and dissolve in water to make 1,000 ml.

Solution 2 Weigh 71.6 g of disodium phosphate, and dissolve in water to make 1,000 ml.

Mix 159 volumes of Solution 1 and 41 volumes of Solution 2.

Citrate Buffer (pH 5.0)

Solution 1 Weigh 21 g of citric acid, and dissolve in water to make 1,000 ml.

Solution 2 Weigh 71.6 g of disodium phosphate, and dissolve in water to make 1,000 ml.

Mix 97 volumes Solution 1 and 103 volumes Solution 2.

Citrate Buffer (pH 5.28) Weigh 34.3 g of sodium citrate, and dissolve in 400 ml of water. Add 7.5 ml of hydrochloric acid, 5 ml of benzyl alcohol, and water to make 1,000 ml. Adjust the pH to 5.28 ± 0.03 with diluted hydrochloric acid (1 in 4) or sodium hydroxide solution (1 in 25).

Citrate Buffer (pH 6.0)

Solution 1 Dissolve 21 g of citric acid in water to make 1,000ml.

Solution 2 Dissolve 71.6 g of disodium hydrogen monophosphate in water to make 1,000 ml.

Mix 72 volumes of Solution 1 and 128 volumes of Solution 2. If necessary, adjust the pH to 6.0 with either of Solution 1 or Solution 2.

Citrate Buffer (pH 7.0).

Solution 1 Dissolve 21 g of citric acid in water to make 1,000 ml.

Solution 2 Dissolve 71.6 g of disodium hydrogen phosphate in water to make 1,000 ml.

Mix 35 volumes of Solution 1 and 165 volumes of Solution 2, and adjust the pH to 7.0 with either solution.

Citric Acid See Citric Acid Monohydrate.

Citric Acid Monohydrate $H_3C_6H_5O_7{\cdot}H_2O$ [K8283]

Citrinin $C_{13}H_{14}O_3$ Yellow, odorless crystals. Very soluble in water.

Identification Proceed as directed in the Potassium Bromide Disk Method under Infrared Spectrophotometry. It exhibits absorption bands at about 1634 cm^{-1}, 1492 cm^{-1}, 1266 cm^{-1}, 1018 cm^{-1} and 818 cm^{-1}.

Purity Related substance Prepare a test solution by dissolving about 0.01 g Citrinin, weighed accurately, in methanol to make 100 ml. Prepare a control solution by measuring exactly 1 ml of the test solution and diluting with methanol to exactly 100 ml. Analyze 5 µl each of the test solution and the control solution by liquid chromatography using the operating conditions given below. Measure the peak areas. The total area of all peaks of the test solution, other than the main peak and methanol peak, is not greater than the area of the main peak of the control solution.

Operating conditions
Detector: Spectrophotofluorometer (excitation wavelength 330 nm, fluorescence wavelength 500 nm).
Column: A stainless steel tube of 3.9–4.6 mm internal diameter and 25–30 cm length.
Column packing materials: 5-µm octadecylsilanized silica gel for liquid chromatography.
Column temperature: 30ºC.
Mobile phase: A 100:100:0.1 mixture of acetonitrile/water/trifluoroacetic acid.
Flow rate: 1.0 ml/min.

Cobalt(II) Chloride See Cobalt(II) Chloride Hexahydrate.

Cobalt(II) Chloride Hexahydrate $CoCl_2 \cdot 6H_2O$ [K8129]

Cobalt Chloride TS Weigh 2.0 g of cobalt(II) chloride, add 1 ml of hydrochloric acid and water to dissolve, and make exactly 100 ml.

Cobalt Nitrate See Cobalt(II) Nitrate Hexahydrate.

Cobalt(II) Nitrate Hexahydrate $Co(NO_3)_2 \cdot 6H_2O$ [K8552]

Cobaltous Chloride See Cobalt(II) Chloride.

Color Fixing TS for D-Glucose Determination Dissolve 0.50 g of phenol, 130 units of mutarotase, 9,000 units of glucose oxidase, 650 units of peroxidase, and 0.1 g of 4-aminoantipyrine in phosphate buffer (pH 7.1), and make exactly 1,000 ml. Store at 2–10°C, and use within 1 month of preparation.

Copper(II) Acetate See Copper(II) Acetate Monohydrate.

Copper(II) Acetate Monohydrate $Cu(CH_3COO)_2 \cdot H_2O$ [Copper(II) Acetate Monohydrate, K8370]

Copper(II) Acetate TS, Strong Weigh 13.3 g of Copper(II) Acetate, and add 5 ml of acetic acid and 195 ml of water to dissolve.

Copper(II) Chloride See Copper(II) Chloride Dihydrate.

Copper(II) Chloride Dihydrate $CuCl_2 \cdot 2H_2O$ [K8145]

Copper Disodium Ethylenediaminetetraacetate See Copper Disodium Ethylenediaminetetraacetate Tetrahydrate.

Copper Disodium Ethylenediaminetetraacetate Tetrahydrate $C_{10}H_{12}CuN_2Na_2O_8 \cdot 4H_2O$ A blue powder.

Content Not less than 98.0%.

pH 7.0–9.0

Clarity Dissolve 0.10 g of the sample in 10 ml of newly distillated and cooled water. The solution is clear and blue.

Assay Accurately weigh about 0.45 g of Copper Disodium Ethylenediaminetetraacetate Tetrahydrate, dissolve in water, and make exactly 1,000 ml. Measure exactly 10 ml of this solution, add water and dilute nitric acid to adjust the pH to about 1.5. Add 5 ml of methanol solution of *o*-phenanthroline (1 in 20), and titrate with 0.01 mol/L bismuth nitrate (indicator: xylenol orange TS) until the yellow color of the solution changes to red.

Each ml of 0.01 mol/L bismuth nitrate = 4.698 mg of $C_{10}H_{12}CuN_2Na_2O_8 \cdot 4H_2O$

Copper Fragment Cu [K8660] Use fragmentary copper.

Copper(II) Sulfate Pentahydrate $CuSO_4 \cdot 5H_2O$ [K8983]

***p*-Cresidine** See 2-Methoxy-5-methylaniline.

Cresidine Azo Schaeffer's Salt $C_{18}H_{15}N_2NaO_5S$ Monosodium 6-hydroxy-5-(2-methoxy-5-methylphenylazo)-2-naphthalenesulfonate. A red powder.

Specific absorbance $E^{1\%}_{1cm}$ (maximum absorption wavelength near 500 nm): Not less than 597. Weigh 0.0100 g of Cresidine Azo Schaeffer's Salt, previously dried for 24 hours in a vacuum desiccator, and dissolve in ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Refer to this solution as solution A. Measure exactly 10 ml of solution A, and add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. This solution exhibits an absorption maximum at a wavelength of 498–502 nm.

Purity Other coloring matters Measure exactly 1.0 ml of solution A, and add ammonium acetate solution (7.7 in 1,000) to make exactly 100 ml. Analyze 20 µl of this solution by liquid chromatography using the operating conditions specified in Purity (6) for Food Red No. 40 in the Monographs. Only one peak of cresidine azo Schaffer's salt is observed.

Cresidine Sulfonic Acid Azo β-Naphthol $C_{18}H_{15}N_2NaO_5S$ Monosodium 4-(2-hydroxy-1-naphthylazo)-5-methoxy-2-methylbenzenesulfonate. A reddish brown powder.

Specific absorbance $E^{1\%}_{1cm}$ (maximum absorption wavelength near 500 nm): Not less than 644. Weigh 0.0100 g of Cresidine Sulfonic Acid Azo β-Naphthol, previously dried for 24 hours in a vacuum desiccator, and dissolve in ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Refer to this solution as solution A. Measure exactly 10 ml of solution A, and add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. This solution exhibits an absorption maximum at a wavelength of 499–503 nm.

Purity Other coloring matters Measure exactly 1.0 ml of solution A, and add ammonium acetate solution (7.7 in 1,000) to make 100 ml. Analyze 20 µl of this solution by liquid chromatography using the operating conditions specified in Purity (6) for Food Red No. 40 in the Monographs. Only one peak of cresidine sulfonic acid azo β-naphthol is observed.

Cresidine Sulfonic Acid Azo G Salt $C_{18}H_{13}N_2Na_3O_{11}S_3$ Trisodium 7-hydroxy-8-(2-methoxy-5-methyl-4-sulfophenylazo)-1,3-naphthalenedisulfonate. An orange-red powder.

Specific absorbance $E^{1\%}_{1cm}$ (maximum absorption wavelength near 500 nm): Not less than 461. Weigh 0.0100 g of Cresidine Sulfonic Acid Azo G Salt, previously dried for 24 hours in a vacuum desiccator, and dissolve in ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Refer to this solution as solution A. Measure exactly 10 ml of solution A, and add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. This solution exhibits an absorption maximum at a wavelength of 498–502 nm.

Purity Other Coloring Matters Measure exactly 1.0 ml of solution A, and add ammonium acetate solution (7.7 in 1,000) to make 100 ml. Analyze 20 µl of this solution by liquid chromatography using the operating conditions as speci-

fied in Purity (6) for Food Red No. 40 in the Monographs. Only one peak of cresidine sulfonic acid azo G salt is observed.

Cresidine Sulfonic Acid Azo R Salt $C_{18}H_{13}N_2Na_3O_{11}S_3$ Trisodium 3-hydroxy-4-(2-methoxy-5-methyl-4-sulfophenylazo)-2,7-naphthalenedisulfonate. A reddish brown powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength near 500 nm): Not less than 494. Weigh 0.0100 g of Cresidine Sulfonic Acid Azo R Salt, previously dried for 24 hours in a vacuum desiccator, and dissolve in ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Refer to this solution as solution A. Measure exactly 10 ml of solution A, and add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. This solution exhibits an absorption maximum at a wavelength of 513–517 nm.

Purity Other Coloring Matters Measure exactly 1.0 ml of solution A, and add ammonium acetate solution (7.7 in 1,000) to make 100 ml. Analyze 20 µl of this solution by liquid chromatography using the operating conditions specified in Purity (6) for Food Red No. 40 in the Monographs. Only one peak of cresidine sulfonic acid azo R salt is observed.

***m*-Cresol** $CH_3C_6H_4OH$ [K8305]

***o*-Cresol** $CH_3C_6H_4OH$ [K8304]

***p*-Cresol** $CH_3C_6H_4OH$ [K8306]

Cresol Red $C_{21}H_{18}O_5S$ [K8308]

Cresol Red–Thymol Blue TS Weigh 0.1 g of cresol red and 0.3 g of thymol blue, mix them, and dissolve in 100 ml of ethanol. Add water to make 400 ml. Filter if necessary.

Crystal Violet $C_{25}H_{30}ClN_3 \cdot 9H_2O$ [K8294]

Crystal Violet–Acetic Acid TS Weigh 0.050 g of crystal violet, and dissolve in 100 ml of acetic acid.

Cu–PAN A grayish-orange-yellow, grayish-red-brown, or light grayish-purple powder. To prepare Cu-PAN, mix 1 g of 1-(2-pyridylazo)-2-naphthol (free acid) and 11.1 g of copper disodium ethylenediaminetetraacetate tetrahydrate.

Absorbance Weigh 0.50 g of Cu–PAN and dissolve in diluted dioxane (1 in 2) to make exactly 50 ml. Measure exactly 1 ml of this solution, add methanol to make exactly 100 ml. Measure the absorbance of this solution as directed in Ultraviolet-Visible Spectrophotometry using water as reference. It is not less than 0.48 at a wavelength of 470 nm.

Purity Clarity and color of solution Dissolve 0.5 g of Cu –PAN in 50 ml of diluted dioxane (1 in 2). The solution is clear and yellow-brown.

Cu–PAN TS Dissolve 1 g of Cu–PAN in 100 ml of diluted dioxane (1 in 2).

Cupferron $C_6H_9N_3O_2$ [K8289]

Cupferron TS Weigh 6 g of cupferron, and dissolve in water to make 100 ml. Prepare fresh before use.

Cupric Acetate See Copper(II) Acetate Monohydrate.

Cupric Citrate TS, Alkaline Weigh 173 g of sodium citrate and 117 g of sodium carbonate, add 100 ml of water, and dissolve while heating. Filter if necessary. To a solution prepared by dissolving 17.3 g of cupric sulfate in 700 ml of water, add the obtained solution gradually while stirring. Cool, and add water to make 1,000 ml.

Cupric Sulfate See Copper(II) Sulfate Pentahydrate.

Cupric Sulfate, Anhydrous $CuSO_4$ [Copper(II) Sulfate, K8984]

Cupric Sulfate–Ammonia TS Weigh 0.4 g of cupric sulfate, and dissolve in 50 ml of a 3:2 mixture of citric acid solution (1 in 5)/ammonia TS.

Cyanidin 3-Glucoside Chloride $C_{21}H_{21}ClO_{11}$

Identification (1) Weigh 1 mg of Cyanidin 3-Glucoside Chloride, and add citrate buffer (pH 3.0) to make 5 ml. A red to dark red-orange color develops.

(2) To the solution prepared in (1), add sodium hydroxide solution (1 in 25) to make the solution alkaline. The solution turns dark-green.

(3) A solution of Cyanidin 3-Glucoside Chloride in citrate buffer (pH 3.0) exhibits an absorption maximum at a wavelength of 505–525 nm.

(4) Measure the absorption spectrum of Cyanidin 3-Glucoside Chloride as directed in the Potassium Bromide Disk Method under Infrared Spectrophotometry. It exhibits its absorption bands at wavenumbers of about 3378 cm^{-1}, 1640 cm^{-1}, 1332cm^{-1}, 1070 cm^{-1}, and 630 cm^{-1}.

Purity Related substances Use the solution prepared in Identification (1) as the test solution. Prepare Control Solution A by adding citrate buffer (pH 3.0) to 1 ml of the test solution, exactly measured, to make exactly 100 ml. Analyze the test solution and Control Solution A by liquid chromatography using the operating conditions given below. Continue the chromatography for three times the retention time of the main peak, and measure the peak areas. Exclude the solvent peaks from measurement. The total area of all peaks of the test solution, other than the main peak, is not greater than the area of the main peak of Control Solution A.

Operating conditions Use the conditions given in Identification (4) for Purple Corn Color in the Monographs.
Detection sensitivity: Adjust the sensitivity so that the automatic integration method can measure the peak area of the main peak from 10 µl of Control Solution B. Also, adjust so that the peak height of the main peak from 10 µl of Control Solution A is about 20% of the full scale. Prepare Control Solution B as follows: Measure exactly 1 ml of Control Solution A, and add citrate buffer (pH 3.0) to make exactly 20 ml.

Cyanogen Bromide TS for Thiamine Assay Measure 100 ml of ice-cold water, and add 2 ml of bromine, and shake vigorously. Add dropwise ice-cooled potassium thiocyanate solution (1 in 10) until it discolors. Prepare in a draft chamber, and use within a month of preparation. As cyanogen bromine vapor is highly poisonous, take the greatest care not to inhale it while handling.

α-Cyclodextrin for Assay $C_{36}H_{60}O_{30}$ White, odorless crystals or crystalline powder having a slightly sweet taste.

Identification To 0.2 g of α-Cyclodextrin for Assay, add 2 ml of iodine TS, warm in a water bath to dissolve, and allow to stand at room temperature. A blue-purple precipitate is formed.

Purity (1) Specific rotation $[\alpha]_D^{20}$: +147 to +152°. Weigh accurately about 1 g of α-Cyclodextrin for Assay, dried previously, and add water to make exactly 100 ml. Measure the angular rotation of the obtained solution.

(2) Related substances Prepare a test solution by dissolving about 1.5 g of α-Cyclodextrin for Assay in water to make exactly 100 ml. Prepare a control solution by adding water to 1 ml of the test solution, measured exactly, to make exactly 100 ml. Analyze 20–100 μl each of the test solution and the control solution by liquid chromatography using the conditions below. Continue the chromatography for two times the retention time of the main peak, and measure the peak areas. The total area of all peaks of the test solution, other than the main peak, is not greater than the area of the main peak of the control solution.

Operating conditions

Use the conditions specified in the Assay for α-Cyclodextrin in the Monographs.

Loss on drying Not more than 14.0% (1.0 g, 105ºC, 0.67 kPa, 4 hours).

β-Cyclodextrin for Assay $C_{42}H_{70}O_{35}$ White crystals or a crystalline powder. Odorless and has a slightly sweet taste.

Identification Add 2 ml of iodine TS to 0.2 g of β-Cyclodextrin for Assay, and warm in a water bath to dissolve. Allow to stand at room temperature and yellow-brown precipitate is formed.

Purity (1) Specific rotation $[\alpha]_D^{20}$: +160 to +164º. Weigh accurately about 1 g of β-Cyclodextrin for Assay, previously dried, add water to make exactly 100 ml. Measure the angular rotation of the prepared solution.

(2) Related substances Prepare a test solution by dissolving about 1.5 g of β-Cyclodextrin for Assay in water to make 100 ml. Prepare a control solution by taking exactly 1 ml of the test solution and adding water to make 100 ml. Analyze 20–100 μl each of the test solution and the control solution by liquid chromatography using the operating conditions given below. Continue the chromatography for two times the retention time of the main peak. Measure the peak areas. The total area of all peaks of the test solution, other than the main peak, is not greater than the area of the main peak of the control solution.

Operating conditions

Use the conditions specified in the Assay for β-Cyclodextrin in the Monographs.

Loss on drying Not more than 14.0% (1.0 g, 105ºC, 0.67 kPa, 4 hours).

γ-Cyclodextrin for Assay $C_{48}H_{80}O_{40}$ White, odorless crystals or crystalline powder having a slightly sweet taste.

Identification: To 0.2 g of γ-Cyclodextrin for Assay, add 2 ml of iodine TS, warm in a water bath to dissolve, and allow to stand at room temperature. A blue-purple precipitate is formed.

Purity (1) Specific rotation $[\alpha]_D^{20}$: +172 to +178°. Weigh accurately about 1 g of γ-Cyclodextrin for Assay, previously dried, and add water to make exactly 100 ml. Measure the angular rotation of the prepared solution.

(2) Related substances Prepare a test solution by dissolving about 1.5 g of γ-Cyclodextrin for Assay in water to make 100 ml. Prepare a control solution by adding water to 1 ml of the test solution, measured exactly, to make exactly 100 ml. Analyze 20–100 μl each of the test solution and the control solution by liquid chromatography using the conditions below. Continue the chromatography for two times the retention time of main peak, and measure the peak areas. The total area of all peaks of the test solution, other than the main peak, is not greater than the area of the main peak for the control solution.

Operating conditions

Use the conditions specified in the Assay for γ-Cyclodextrin in the Monographs.

Loss on drying: Not more than 14.0% (1.0 g, 105ºC, 0.67 kPa, 4 hours).

Cyclohexane C_6H_{12} [K8464]

Cysteine Hydrochloride See L-Cysteine Hydrochloride Monohydrate.

L-Cysteine Hydrochloride Monohydrate $C_3H_7NO_2S \cdot HCl \cdot H_2O$ [K8470]

L-Cysteine Monohydrochloride See L-Cysteine Hydrochloride Monohydrate.

Cysteine–Sulfuric Acid TS Weigh 0.30 g of L-cysteine monohydrochloride, add 10 ml of water to dissolve. To 0.5 ml of this solution, add 25 ml of 86% (vol) sulfuric acid, and mix. Prepare fresh before use.

DEAE-Cellulose Anion Exchanger ($-O-C_2H_4-N(C_2H_5)_2$ Type), Weakly Basic Use a weakly basic anion-exchanger prepared by introducing diethylaminoethyl groups into porous cellulose.

Dehydrated Chloroform See Chloroform, Dehydrated.

Dehydrated Pyridine See Pyridine, Dehydrated.

Devarda's Alloy [K8653]

Dextrin See Dextrin Hydrate.

Dextrin Hydrate $(C_6H_{10}O_5)_n \cdot nH_2O$ [K8646]

4,4′-Diaminodiphenylamine Sulfate $C_{12}H_{13}N_3 \cdot H_2SO_4$ [K8476:1962] A colorless to grayish blue, crystalline powder.

Melting point 157–160ºC. Dissolve 1 g of 4,4′-Diaminodiphenylamine Sulfate in 10 ml of dilute sulfuric acid while warming, add excess ammonia solution, and heat. When cooled, crystals are produced. Measure the melting point of the crystals.

4,4′-Diaminodiphenylamine TS Triturate well 4,4′-diaminodiphenylamine sulfate with a small quantity of ethanol, and add ethanol again. Heat on a water bath under a reflux condenser to make a saturated solution.

Diammonium Phosphate $(NH_4)_2HPO_4$ [Diammonium Hydrogen Phosphate, K9016]

Diammonium Phosphate Buffer Weigh 150 g of diammonium phosphate, dissolve in 700 ml of water, adjust the pH to 5.5 with diluted hydrochloric acid (1 in 2), and add water to make 1,000 ml.

Diarsenic Trioxide As_2O_3 [K8044]

Diarsenic Trioxide (Standard Reagent) As_2O_3 [Standard Material for Volumetric Analysis, Arsenic(III) Oxide, K8005]

Diatomaceous Earth for Chromatography Use white to grayish white diatomaceous earth of high quality.

Diatomaceous Earth for Gas Chromatography Use diatomaceous earth of high quality purified for gas chromatography.

2,6-Dibromo-*N*-chloro-*p*-benzoquinone Monoimine $C_6H_2Br_2ClNO$ [K8491]

2,6-Dibromoquinonechloroimide See 2,6-Dibromo-*N*-chloro-*p*-benzoquinone Monoimine.

Dibutyl Ether $[CH_3(CH_2)_3]_2O$ A clear liquid.
Refractive index n_D^{20}:1.398–1.400.
Specific gravity 0.764–0.770.
Boiling point 141–143°.

2,6-Dichlorophenolindophenol Sodium Salt
See 2,6-Dichlorophenolindophenol Sodium Salt Dihydrate.

2,6-Dichlorophenolindophenol Sodium Salt Dihydrate $C_{12}H_6Cl_2NNaO_2{\cdot}2H_2O$
[2,6-Dichloroindophenol Sodium Salt Dihydrate, K8469]

2,6-Dichlorophenolindophenol Sodium Salt TS
Weigh 0.1 g of 2,6-dichlorophenolindophenol sodium salt, and add 100 ml of water. Warm and filter it. Store in a brown bottle, and use within 3 days.

2,6-Dichloroquinonechloroimide $C_6H_2Cl_3NO$
Melting point 65–67°C.
Clarity of ethanolic solution Clear (0.10 g, ethanol 10 ml).
Residue on ignition Not more than 0.2%.

Diethanolamine $C_4H_{11}NO_2$ A colorless, viscous liquid.
Melting point 27–30ºC.
Water Not more than 1 mg in 1 g of Diethanolamine.

Diethanolamine Hydrochloride $C_4H_{11}NO_2{\cdot}HCl$ A light yellow liquid.
Refractive index n_D^{20}: 1.515–1.519.
Specific gravity 1.259–1.263.
Water Not more than 1 mg/g sample.

Diethylene Glycol Succinate Polyester Use diethylene glycol succinate polyester of high quality prepared for gas chromatography.

Diethyl Ether $C_2H_5OC_2H_5$ [K8103]

Diethyl Ether for Vitamin A Determination Distill diethyl ether, and discard 10% of the initial distillate and 10% of the distillation residue. Determine the absorbance of the distillate, using redistilled water as the reference. The absorbance is not more than 0.01 at 300–350 nm.
Peroxide Measure 5 ml of Diethyl Ether for Vitamin A Determination, and add 5 ml of iron(II) sulfate TS and 5 ml of ammonium thiocyanate solution (2 in 25). No red color develops.

Digitonin $C_{56}H_{92}O_{29}$ [K8452]

1,3-Dihydroxynaphthalene $C_{10}H_6(OH)_2$ Red-brown crystals or a gray to grayish brown powder. Freely soluble in water, in ethanol, and in diethyl ether.
Melting point 122–124ºC (decomposition).
Sensitivity To 2 drops of tartaric acid solution (1 in 1,000), add 1 ml of a solution of 1,3-Dihydroxynaphthalene in diluted sulfuric acid (1 in 10,000), and heat at 90ºC for 1 hour. A blue-green to green-blue color develops.

Dilute Acetic Acid See Acetic Acid, Dilute.

Dilute Hydrochloric Acid See Hydrochloric Acid, Dilute.

Dilute Iron(III) Chloride TS See Iron(III) Chloride TS, Dilute.

Dilute Methylene Blue TS See Methylene Blue TS, Dilute.

Dilute Nitric Acid See Nitric Acid, Dilute.

Dilute Phenol Red TS See Phenol Red TS, Dilute.

Dilute Sodium Hydroxide TS See Sodium Hydroxide TS, Dilute.

Dilute Sulfuric Acid See Sulfuric Acid, Dilute.

1,2-Dimethoxyethane $C_4H_{10}O_2$ A colorless, transparent liquid having a diethyl ether-like odor. Very soluble in water, in ethanol, and in hydrocarbon solvents.
Content Not less than 99.0% of 1,2-dimethoxyethane ($C_4H_{10}O_2$).
Boiling point 82–83ºC.
Assay Analyze 1,2-Dimethoxyethane by gas chromatography using the following conditions, and calculate the area percentage of the main peak.
Operating conditions
Detector: Flame ionization detector.
Column: A glass or stainless steel tube of 3–4 mm internal diameter and 2 m length.
Column packing material
Liquid phase: 10% Polyethylene glycol 20M of the amount of support.
Support: 177- to 250-μm diatomaceous earth for gas chromatography.
Column temperature: A constant temperature of 70–80ºC.
Carrier gas: Helium.
Flow rate: A constant rate of 50 ml/min.

1,2-Dimethoxyethane Containing 5% Methanol TS To 5 ml of methanol, add 1,2-dimethoxyethane to make 100 ml.

The solution is stable at least for 3 months in a refrigerator.

Dimethylamine Hydrochloride $(CH_3)_2NH{\cdot}H_2O{\cdot}HCl$
White, deliquescent crystals. Very soluble in water and melts at 170–172ºC.

***p*-Dimethylaminobenzaldehyde** See 4-Dimethylaminobenzaldehyde.

4-Dimethylaminobenzaldehyde $(CH_3)_2NC_6H_4CHO$ [K8496]

***p*-Dimethylaminobenzaldehyde TS** Weigh 125 mg of *p*-dimethylaminobenzaldehyde, dissolve in 100 ml of cooled diluted sulfuric acid (13 in 20), and add 0.05 ml of iron(III) chloride solution (1 in 10). Use within 7 days of preparation.

***p*-Dimethylaminobenzylidenerhodanine** $C_{12}H_{12}N_2OS_2$ [K8495]

***p*-Dimethylaminobenzylidenerhodanine TS** Weigh 0.02 g of *p*-dimethylaminobenzylidenerhodanine, and dissolve in acetone to make 100 ml.

***p*-Dimethylaminocinnamaldehyde** See 4-Dimethylaminocinnamaldehyde.

4-Dimethylaminocinnamaldehyde $C_{11}H_{13}NO$ Orange crystals or crystalline powder having a characteristic odor.
Melting point 140–142ºC.
Purity Clarity Dissolve 0.2 g of 4-Dimethylaminocinnamaldehyde in 20 ml of ethanol. The solution is clear.
Loss on drying Not more than 0.5% (105ºC, 2 hours).
Residue on ignition Not more than 0.10% (1 g).
Nitrogen content 7.8–8.1% (dry before the test at 105ºC for 2 hours, Nitrogen Determination).

***p*-Dimethylaminocinnamaldehyde TS** Before use, add 1 ml of acetic acid to 10 ml of an ethanol solution of 4-dimethylaminocinnamaldehyde (1 in 2,000).

Dimethylaniline $C_6H_5N(CH_3)_2$ [*N*,*N*-Dimethylaniline, K8493:1980] A liquid having a characteristic odor. Freshly obtained distillate is colorless but it gradually turns red to red-brown.
Congealing point Not lower than 1.9ºC.
Refractive index n_D^{20}: 1.556–1.560.
Specific gravity 0.955–0.960.

Dimethylformamide See *N*,*N*-Dimethylformamide.

***N*,*N*-Dimethylformamide** $HCON(CH_3)_2$ [K8500]

Dimethylglyoxime $(CH_3)_2C_2(NOH)_2$ [K8498]

Dimethyl Sulfoxide $(CH_3)_2SO$ [K9702]

Dimethyl Sulfoxide for Ultraviolet Absorption Spectrum Measurement $(CH_3)_2SO$ Colorless, transparent, and strongly hygroscopic crystals or liquid having a characteristic odor.
Congealing point 18.3ºC.
Water content Not more than 0.1%.
Absorbance Saturate the sample with nitrogen, and determine the absorbance immediately, using distilled water as the reference. Absorbances are not more than 0.20 at 270 nm, not more than 0.09 at 275 nm, not more than 0.06 at 280 nm, not more than 0.015 at 300 nm. No characteristic absorption is observed at 260–350 nm.

Dimethyl Sulfoxide TS Place 300 ml of dimethyl sulfoxide for ultraviolet absorption spectrum measurement in a 1-L separating funnel, and add 75 ml of phosphoric acid. Shake the mixture, and allow to stand for 10 minutes. Add 150 ml of isooctane for ultraviolet absorption spectrum measurement, shake, and allow to stand for 10 minutes. Separate the lower layer, and store in a tightly stoppered glass bottle.

3,5-Dinitrobenzoyl Chloride $(NO_2)_2C_6H_3COCl$
[K8477:1961] A slightly yellowish crystalline powder.
Melting point 67–69ºC.
Residue on ignition Not more than 0.10%.

2,4-Dinitrochlorobenzene $C_6H_3(NO_2)_2Cl$ [1-Chloro-2,4-dinitrobenzene, K8478]

2,4-Dinitrophenylhydrazine $C_6H_6N_4O_4$ [K8480]

2,4-Dinitrophenylhydrazine Hydrochloride TS Transfer 10 ml of hydrochloric acid into a 100 ml Erlenmeyer flask, add 5 g of 2,4-dinitrophenylhydrazine, and shake gently until free base (red color) converts to hydrochloride (yellow color). Add 100 ml of ethanol, and heat on a water bath to dissolve. Cool, crystallize at room temperature, and filter. Wash with diethyl ether, and dry at room temperature, and store in a desiccator. Use it as the reagent 2,4-dinitrophenylhydrazine hydrochloride. During storage, it gradually converts from hydrochloride to free base, but the free base is removed by rinsing with 1,2-dimethoxyethane. Prepare 2,4-Dinitrophenylhydrazine Hydrochloride TS by dissolving 0.5 g of 2,4-dinitrophenylhydrazine hydrochloride in 15 ml of 1,2-dimethoxyethane containing 5% methanol. Store in a refrigerator.

Dioxane See 1,4-Dioxane.

1,4-Dioxane $C_4H_8O_2$ [K8461]

Diphenyl See Biphenyl.

Diphenylamine $(C_6H_5)_2NH$ [K8487]

Diphenylamine TS To 1 g of diphenylamine, add 100 ml of sulfuric acid to dissolve. The solution is colorless.

Diphenyl Ether $C_{13}H_{10}O$
Description Colorless crystals having a characteristic odor.
Purity (1) Boiling point 254–259°C.
(2) Melting point 25–28°C.
(3) Related substances Prepare a test solution by dissolving 1.0 g of Diphenyl Ether in 100 ml of ethyl acetate. Prepare a control solution by adding ethyl acetate to 1 ml of the test solution, exactly measured, to make exactly 100 ml. Analyze 0.5 µl each of the test solution and the control solution by gas chromatography using the operating conditions given below, and measure the peak areas. Continue the chromatography for two times the retention time of the

main peak. Exclude the solvent peaks from measurement. The total area of all peaks of the test solution, other than the main peak, is not greater than the area of the main peak of the control solution.

Operating conditions

Detector: Flame ionization detector.

Column: A silicate glass capillary tube (0.53 mm internal diameter and 12 m length) coated with a 1.0-µm thick layer of dimethylpolysiloxane.

Column temperature: Raise the temperature from 100°C to 300°C at a rate of 10°C/minute.

Inlet temperature: 300°C.

Injection method: Split (10:1).

Carrier gas: Helium.

Flow rate: Adjust so that the peak of diphenyl ether appears about 3 minutes after injection.

Dipotassium Phosphate K_2HPO_4 [Dipotassium Hydrogen Phosphate, K9017]

α,α′-Dipyridyl See 2,2′-Bipyridyl.

1,3-Di-(4-pyridyl)propane $C_{13}H_{14}N_2$ A light yellow powder.

Melting point 61–62°C.

Water Not more than 1 mg/g.

Disodium Chromotropate See Disodium Chromotropate Dihydrate.

Disodium Chromotropate Dihydrate $C_{10}H_6Na_2O_8S_2 \cdot 2H_2O$ [K8316]

Disodium 4,4′-(Diazoamino)dibenzenesulfonate $C_{12}H_9N_3Na_2O_6S_2$ A white to whitish powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength near 358 nm): Not less than 677. Weigh 0.0100 g of Disodium 4,4′-(Diazoamino)dibenzenesulfonate, previously dried for 24 hours in a vacuum desiccator , and dissolve in sodium hydroxide solution (4 in 1,000) to make exactly 100 ml. Refer to this solution as solution A. Measure exactly 10 ml of solution A, and add ammonium acetate solution (4 in 1,000) to make exactly 100 ml. This solution exhibits absorption maxima at wavelengths of 240 nm and 358 nm.

Purity Other aromatic compounds Measure exactly 10 ml of solution A, and add sodium hydroxide solution (4 in 1,000) to make exactly 100 ml. Analyze 20 µl of this solution by liquid chromatography using the operating conditions specified in Purity (6) for Food Yellow No. 4 in the Monographs. Only one peak is observed.

Disodium Ethylenediaminetetraacetate See Disodium Ethylenediaminetetraacetate Dihydrate.

Disodium Ethylenediaminetetraacetate Dihydrate $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ [K8107]

Disodium Ethylenediaminetetraacetate TS Dissolve 37.2 g of disodium ethylenediaminetetraacetate in water, and make 1,000 ml.

Disodium 5′-Guanylate $C_{10}H_{12}N_5Na_2O_8P \cdot 4–7H_2O$ "Disodium 5′-Guanylate"

Disodium 3-Hydroxy-2,7-naphthalenedisulfonate $C_{10}H_6Na_2O_7S_2$ A white to whitish powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength near 281 nm): Not less than 126. Weigh 0.0100 g of Disodium 3-Hydroxy-2,7-naphthalenedisulfonate, previously dried for 24 hours in a vacuum desiccator, and dissolve in ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Refer to this solution as solution A. Exactly measure 10 ml of solution A, add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. This solution exhibits absorption maxima at wavelengths of 236 nm, 273 nm, 281 nm, and 340 nm.

Purity Other aromatic compounds Exactly measure 10 ml of solution A, add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Analyze 20 µl of this solution by liquid chromatography, using the operating conditions directed under Purity (6) for Food Red No. 2 in the Monographs. Only one peak is observed.

Disodium 7-Hydroxy-1,3-naphthalenedisulfonate $C_{10}H_6Na_2O_7S_2$ A white to whitish powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength near 288 nm): Not less than 150. Weigh 0.0100 g of Disodium 7-Hydroxy-1,3-naphthalenedisulfonate, previously dried for 24 hours in a vacuum desiccator, dissolve in ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Refer to this solution as solution A. Exactly measure 10 ml of solution A, add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. This solution exhibits absorption maxima at wavelengths of 237 nm, 288 nm, and 336 nm.

Purity Other aromatic compounds Exactly measure 10 ml of solution A, add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Analyze 20 µl of this solution by liquid chromatography using the operating conditions directed in Purity (6) for Food Red No. 2 in the Monographs. Only one peak is observed.

Disodium 5′-Inosinate $C_{10}H_{11}N_4Na_2O_8P \cdot 6–8H_2O$ "Disodium 5′-Inosinate"

Disodium Molybdate(VI) Dihydrate $Na_2MoO_4 \cdot 2H_2O$ [K8906]

Disodium 1-Nitroso-2-naphthol-3,6-disulfonate $C_{10}H_5NNa_2O_8S_2$ [K 8714]

Disodium 6,6′-Oxybis(2-naphthalenesulfonate) $C_{20}H_{12}Na_2O_7S_2$ A whitish powder.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength near 240 nm): Not less than 2,020. Weigh 0.0100 g of Disodium 6,6′-Oxybis(2-naphthalenesulfonate), previously dried for 24 hours in a vacuum desiccator, and dissolve in ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Refer to this solution as solution A. Measure exactly 10 ml of solution A, and add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. This solution exhibits absorption maxima at wavelengths of 220 nm and 240 nm.

Purity Other aromatic compounds Measure exactly 1.0 ml of solution A, and add ammonium acetate solution (7.7 in 1,000) to make exactly 100 ml. Analyze 20 µl of this solution by liquid chromatography using the operating conditions specified in Purity (8) for Food Red No. 40 in the Monographs. Only one peak of disodium 6,6'-oxybis(2-

naphthalenesulfonate) is observed.

Disodium Phosphate $Na_2HPO_4 \cdot 12H_2O$ [Disodium Hydrogen Phosphate Dodecahydrate, K9019]

Disodium Phosphate, Anhydrous Na_2HPO_4 [Disodium Hydrogen Phosphate, K9020]

Disodium Phosphate, Anhydrous, for pH Determination Na_2HPO_4 [Disodium Hydrogen Phosphate for pH Standard Solution, K9020]

Dissolved Acetylene C_2H_2 [K1902]

Distilled Water Use purified water specified in the Japanese Pharmacopoeia.

Dithizone $C_{13}H_{12}N_4S$ [K8490]

Dithizone TS for Zinc Weigh 0.01 g of dithizone, and add 100 ml of chloroform to dissolve. Store in a glass-stoppered, colored bottle.

Dragendorff Reagent

Solution 1 Weigh 0.85 g of basic bismuth nitrate, and add 10 ml of acetic acid and 40 ml of water to dissolve.

Solution 2 Weigh 8 g of potassium iodide, and add 20 ml of water to dissolve.

Prepare the reagent by mixing 5 ml of Solution 1, 5 ml of Solution 2, 20 ml of acetic acid, and 100 ml of water before use.

Egg White Use fresh egg white.

Egg White TS Weigh 10 g of egg white, add 40 ml of water, and shake.

Eosine See Eosine Y.

Eosine Y $C_{20}H_6Br_4Na_2O_5$ [K8651: 1988] Red to reddish white lumps or powder.
A solution of Eosin Y exhibits an absorption maximum at 517 nm.

Loss on drying Not more than 16% (105ºC, 4 hours).

Eriochrome Black T $C_{20}H_{12}N_3NaO_7S$ [K8736]

Eriochrome Black T–Sodium Chloride Indicator Mix 0.1 g of eriochrome black T and 10 g of sodium chloride, and triturate thoroughly to be homogeneous.

Eriochrome Black T TS Weigh 0.5 g of eriochrome black T and 4.5 g of hydroxylamine hydrochloride, and dissolve in 100 ml of ethanol. Store in a light-resistant container.

Erythritol See *meso*-Erythritol.

***meso*-Erythritol** $C_4H_{10}O_4$ White crystals or crystalline powder.

Clarity of solution Clear (1.0 g, water 20 ml).

Melting point 118–120°C.

Water Not more than 0.5% (1.0 g, Direct Titration).

Residue on ignition Not more than 0.10% (2 g).

Ethanol C_2H_5OH See Ethanol (95).

Ethanol (99.5) C_2H_5OH [K8101]

Ethanol (95) C_2H_5OH [K8102]

Ethanol, Absolute See Ethanol (99.5).

Ethanol, Aldehyde-free C_2H_5OH To 1,000 ml of ethanol, add 5 ml of sulfuric acid and 20 ml of water, and distill. To 1,000 ml of the distillate, add 10 g of silver nitrate and 1 g of potassium hydroxide, and boil under a reflux condenser for 3 hours. Distill it again.

Ethanol, Neutralized Measure a suitable quantity of ethanol, add several drops of phenolphthalein TS, and add sodium hydroxide solution (1 in 1,250) until a light pink color develops. Prepare fresh before use.

Ethanol-free Chloroform See Chloroform, Ethanol-free.

Ethanolic Potassium Hydroxide TS See Potassium Hydroxide TS, Ethanolic.

Ethanolic 10% Potassium Hydroxide TS See 10% Potassium Hydroxide TS, Ethanolic.

Ethyl Acetate $CH_3COOC_2H_5$ [K8361]

Ethyl Formate $HCOOC_2H_5$ A colorless, transparent liquid having a characteristic odor.

Content Not less than 97.0% of ethyl formate ($HCOOC_2H_5$).

Refractive index n_D^{20}: 1.3595–1.3601.

Specific gravity d_4^{20}: 0.915–0.924.

Boiling point 53–54ºC.

Assay Weigh accurately about 5.0 g of Ethyl Formate, and proceed as directed under Ester Value and Acid Value in the Flavoring Substances Tests. Calculate the content by the following formula:

$$\text{Content (\%) of ethyl formate } (HCOO_2H_5) = \frac{\text{Saponification Value} - \text{Acid Value}}{561.1} \times 74.08$$

Ethyl Methyl Ketone See 2-Butanone.

Ethylene Glycol $HOCH_2CH_2OH$ [K8105]

Ethylene Glycol for Water Determination Distill ethylene glycol, and collect the fraction at 195–198°C. The water content in 1 ml of it is not more than 1.0 mg.

Ethylene Glycol Monomethyl Ether See 2-Metoxyethanol.

***N*-Ethylmaleimide** $C_4H_2O_2NC_2H_5$ White crystals. Very soluble in ethanol and in diethyl ether. A solution of *N*-Ethylmaleimide (1 in 10,000) exhibits an absorption maximum at 298–302 nm.

Melting point 44.0–46.0°C.

Fehling's TS

Copper Solution Weigh 34.66 g of fine cupric sulfate crystals, and dissolve in water to make 500 ml. Store in a almost-filled, grass-stoppered bottle.

Alkaline Tartrate Solution Weigh 173 g of potassium sodium tartrate and 50 g of sodium hydroxide, and mix. Dissolve the mixture in water to make 500 ml. Store in a rubber-stoppered container.

Mix equal volumes of both solutions before use.

Ferric Ammonium Sulfate See Iron(III) Ammonium Sulfate.

Ferric Ammonium Sulfate TS Weigh 14 g of iron(III) ammonium sulfate, add 100 ml of water, and dissolve by shaking well. Filter, and add 10 ml of sulfuric acid. Store in a brown bottle.

Ferric Ammonium Sulfate–Sulfuric Acid TS
Weigh 15 g of iron(III) ammonium sulfate, and dissolve in 90 ml of water. Filter, and add 10 ml of diluted sulfuric acid (1 in 35).

Ferric Chloride See Iron(III) Chloride Hexahydrate.

Ferric Chloride–Hydrochloric Acid TS
See Iron(III) Chloride–Hydrochloric Acid TS.

Ferric Sulfate See Iron(III) Sulfate.

Ferric Sulfate TS Weigh 50 g of iron(III) sulfate, add about 500 ml of water, and shake well. Add 200 ml of sulfuric acid, dissolve by shaking well, and add water to make 1,000 ml.

Ferrous Ammonium Sulfate See Ammonium Iron(II) Sulfate Hexahydrate.

Ferrous Sulfate See Iron(II) Sulfate Heptahydrate.

Ferrous Sulfate TS Weigh 8 g of iron(II) sulfate, and dissolve in 100 ml of freshly boiled and cooled water. Prepare fresh before use.

Ferrous Sulfate TS, Acidic To 100 ml of water, add 7.5 ml of sulfuric acid. To the resultant solution, add about 80 g of ferrous sulfate, and dissolve while heating. To 20 ml of water, add 7.5 ml of nitric acid, and warm. To the diluted nitric acid, add the ferrous sulfate solution. Concentrate the mixture until it turns black to red, evolving red vapor. Add a few drops of nitric acid until it is free of ferrous iron, and warm again. After cooling, add water to the concentrated solution to make 110 ml. Prepare fresh before use.

Ferrous Sulfide See Iron(II) Sulfide.

Fluorescent Silica Gel for Thin-Layer Chromatography See Silica Gel for Thin-Layer Chromatography (Containing Fluorescent Indicator).

Folin's TS Weigh 20 g of sodium tungstate and 5 g of sodium molybdate, transfer into a 300-ml flask, and add about 140 ml of water, 10 ml of diluted phosphoric acid (17 in 20), and 20 ml of hydrochloric acid. Equip the flask with a reflux condenser with a ground-glass joint, and boil gradually for 10 hours. Add 30 g of lithium sulfate and 10 ml of water, and add a very small amount of bromine until the dark green color of the solution changes to yellow. Boil gradually for 15 minutes without a condenser to expel the excess bromine. Cool, add water to make 200 ml, and filter through a glass filter. Store in a tightly stoppered bottle.

Formaldehyde Solution HCHO [K8872]

Formalin See Formaldehyde Solution.

Formalin–Sulfuric Acid TS Measure 0.2 ml of formalin, and mix with 10 ml sulfuric acid. Prepare fresh before use.

Formic Acid HCOOH [K8264]

Formic Acid Buffer (pH 2.5) Measure 4 ml of formic acid, and add 90 ml of water. Adjust the pH to 2.5 with ammonia solution, and add water to make 1,000 ml.

Fructose $C_6H_{12}O_6$ Use fructose specified in the Japanese Pharmacopoeia.

Fumonisin B_1 $C_{34}H_{59}NO_{15}$ A white to yellowish white powder.

Identification Determine the absorption spectrum of Fumonisin B_1 as directed in the Potassium Bromide Disk Method under Infrared Spectrophotometry. It exhibits absorption bands at wavenumbers of about 3450 cm^{-1}, 2934 cm^{-1}, 1730 cm^{-1}, and 1632 cm^{-1}.

Purity Prepare a test solution by dissolving 0.010 g of Fumonisin B_1 in 10 ml of a 1:1 mixture of water/acetonitrile. Analyze 10 µl of the test solution by thin-layer chromatography using a 7:3 mixture of methanol/water as the developing solvent. Control solution is not used. Use a thin-layer plate coated with octadecylsilanized silica gel as the solid support. Stop the development when the solvent front ascends to a point about 10 cm above the original line, and air-dry the plate. Spray with a solution prepared by dissolving 1 g of vanillin in 100 ml of a 4:1 mixture of sulfuric acid/ethanol. Only one spot is observed in natural light.

Galactitol $C_6H_{14}O_6$ White crystals or crystalline powder.

Clarity of solution Clear (1.0 g, water 30 ml).

Melting point 188–189ºC.

Water Not more than 0.5% (1.0 g, Direct Titration).

Residue on ignition Not more than 0.10% (2 g).

Gallic Acid $C_7H_6O_5 \cdot H_2O$ [K8898:1961] White to pale yellowish white crystals or powder.

Identification To 5 ml of a solution of Gallic Acid (1 in 50), add 1 drop of ferric chloride solution (1 in 500). A bluish-black precipitate is formed.

Purity Tannic acid To 1.0 g of Gallic Acid, add 20 ml of water, shake well, and filter. When 5–6 drops of a warm solution of 1% gelatin is added to the filtrate, no turbidity appears.

Loss on drying Not more than 10% (105ºC, 3 hours)

Gelatin Use gelatin specified in the Japanese Pharmacopoeia.

Gelatin TS Dissolve gently 1 g of gelatin in water while heating, and filter if necessary. Prepare fresh before use.

Gelatin Peptone See Peptone, Gelatin.

General Bouillon See Bouillon, General.

Geniposide $C_{17}H_{24}O_{10}$ White, odorless crystals or crystalline powder.

Identification Weigh accurately about 5 mg of Geniposide, dissolve in methanol to make exactly 10 ml. Measure exactly 1 ml of this solution, and add methanol to make 10 ml. This solution exhibits an absorption maximum at a wavelength of about 238 nm.

Purity (1) Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength near 240 nm): 249–269. Measure the absorbance of the following solution at the maximum absorption wavelength at about 240 nm: Dissolve about 0.01 g of Geniposide, weighed accurately, in methanol (1 in 2) to make exactly 500 ml.

(2) Related substances Prepare a test solution by dissolving 0.01 g of Geniposide, weighed accurately, in a 17:3 mixture of water/acetonitrile, and making exactly 100 ml. Prepare a control solution by diluting 2 ml of the test solution, measured exactly, with a 17:3 mixture of water/acetonitrile to exactly 100 ml. Analyze 20 µl each of the test solution and the control solution by liquid chromatography using the operating conditions given below. Continue the chromatography for about two times the retention time of main peak, and measure the peak areas. Exclude the solvent peaks from measurement. The total area of all peaks of the test solution, other than the main peak, is not greater than the area of the main peak of the control solution.

Operating conditions

Detector: Ultraviolet spectrophotometer (measurement wavelength: 238 nm).

Column: A stainless steel tube of 4–5 mm internal diameter and 15–30 cm length.

Column packing material: 5-µm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 40°C.

Mobil phase: A 17:3 mixture of water/acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of geniposide is about 15 minutes.

Girard Reagent P $[C_5H_5NCH_2CONHNH_2]Cl$ A white to light yellow-orange powder having a slight characteristic odor. Freely soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol.

Content Not less than 95.0% of 1-(2-hydrazino-2-oxoethyl)pyridinium chloride ($C_7H_{10}N_3OCl$).

Melting point 200–203ºC.

Assay Weigh accurately about 0.3 g of Girard Reagent P, previously dried at 105ºC to constant weight, and add 50 ml of water to dissolve. Add 3 ml of diluted nitric acid (1 in 3), and titrate with 0.1 mol/L silver nitrate. Use a potentiometer to confirm the endpoint. Perform a blank test in the same manner, and make any necessary correction.

Each ml of 0.1 mol/L silver nitrate = 18.76 mg of $C_7H_{10}N_3OCl$

Glucoamylase A white to brown powder or a light yellow to dark brown liquid. Is odorless or has a characteristic odor. It is obtained from *Aspergillus niger*. One unit is the amount of enzyme required to produce 1 mg of D-glucose from starch as a substrate in 60 minutes at 40°C and pH 4.5.

Glucose $C_6H_{12}O_6$ Use glucose specified in the Japanese Pharmacopoeia.

Glucose Oxidase A white powder. It is obtained from *Penicillium* molds. One unit is the amount of enzyme required to produce 1 µmol of D-glucono-1,5-lactone from D-glucose as a substrate in 1 minute at 25°C and pH 7.0.

L-Glutamic Acid for Assay $C_5H_9NO_4$ L-Glutamic Acid [K9047]

Glycerol $CH_2(OH)CH(OH)CH_2OH$ [K8295]

Glycyrrhizic Acid for Thin-Layer Chromatography $C_{42}H_{62}O_{16}{\cdot}nH_2O$

Description A white crystalline powder having a characteristic sweet taste. Soluble in boiling water and in ethanol, and practically insoluble in diethyl ether.

Melting point 213–218°C (decomposed).

Purity Related substance Prepare a test solution by dissolving 0.010 g of Glycyrrhizic Acid for Thin-Layer Chromatography in 5 ml of a 1:1 mixture of water/ethanol. Prepare a control solution by diluting 1 ml of the test solution, measured exactly, with a 1:1 mixture of water/ethanol to exactly 100 ml. Analyze 10 µl each of the test solution and control solution by thin-layer chromatography as directed under Identification for Crude Licorice Extract. Spots, other than the main spot with about R_f 0.3, from the test solution are not darker in color than the spot from the reference solution.

Graphite Carbon Cartridge (500 mg) Use a polyethylene column with the internal diameter of 10–15 mm packed with 0.5 g graphite carbon or its equivalent in separation capability.

Helium He Use helium containing not less than 99.995% (vol) of He.

Heptane C_7H_{16} [K9701]

Hexaammonium Heptamolybdate Tetrahydrate $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ [K8905]

Hexachlorobenzene C_6Cl_6 Contains not less than 98% of hexachlorobenzene (C_6Cl_6).

Melting point 226°C

Hexadecane for Ultraviolet Absorption Spectrum Measurement $CH_3(CH_2)_{14}CH_3$ Prepare a test solution by adding isooctane for ultraviolet absorption spectrum measurement to 1 ml of Hexadecane for Ultraviolet Absorption Spectrum Measurement and making exactly 25 ml. Measure the absorbance of the test solution in a 5-cm path length cell using isooctane for ultraviolet absorption spectrum measurement as the reference solution. It is not more than 0.00 cm^{-1} at 280–400 nm. If necessary, purify the test solution by filtration through a column packed with active silica gel or by distillation.

Hexamethyldisilazane $(CCH_3)_3SiNHSi(CH_3)_3$ [1,1,1,3,3,3-Hexamethyldisilazane, K9556]

Hexane C_6H_{14} [K8848]

***n*-Hexane** See Hexane.

Hexane for Ultraviolet Absorption Spectrum Measurement C_6H_{14} When determined, using distilled water as the reference, the absorbance is not more than 0.10 at a wavelength of 220 nm and not more than 0.02 at 260 nm. No characteristic absorbance is observed at 260–350 nm.

1-Hexanol $CH_3(CH_2)_5OH$ A colorless, clear liquid.
Specific gravity d_4^{20}: 0.818–0.819.
Boiling point 157ºC.

Hydrazine (Hydrate) See Hydrazine Monohydrate.

Hydrazine Monohydrate $NH_2NH_2{\cdot}H_2O$ [K8871:1980]
A colorless, hygroscopic liquid having a characteristic odor.
Content Not less than 98% of hydrazine monohydrate ($H_2NNH_2{\cdot}H_2O$).
Identification Hydrazine Monohydrate reduces Fehling's TS.
Assay Weigh accurately about 1 g of Hydrazine Monohydrate, and dissolve in water to make exactly 200 ml. Transfer exactly 10 ml of this solution into a 300-ml stoppered Erlenmeyer flask, add 20 ml of water and 30 ml of hydrochloric acid, and cool. Titrate with 0.05 mol/L potassium iodate. Add 5 ml of chloroform just before the endpoint, and stir constantly. The endpoint is when pink color of chloroform disappears.
Each ml of 0.05 mol/L potassium iodate = 2.503 mg of $H_2NNH_2{\cdot}H_2O$

Hydrazine Sulfate See Hydrazinium Sulfate.

Hydrazinium Sulfate $N_2H_6SO_4$ [K8992]

4-Hydrazinobenzenesulfonic Acid $C_6H_8N_2O_3S$ A white to whitish powder.
Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength near 253 nm): Not less than 749. Weigh 0.0100 g of 4-Hydrazinobenzenesulfonic Acid, previously dried for 24 hours in a vacuum desiccator, add ammonium acetate solution (3 in 2,000), and dissolve to make exactly 100 ml. Refer to this solution as solution A. Exactly measure 10 ml of solution A, add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Measure the absorbance of this solution.
Purity Other aromatic compounds Exactly measure 10 ml of solution A, add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Analyze 20 µl of this solution by liquid chromatography using the operating conditions directed under Purity (6) for Food Yellow No. 4 in the Monographs. Only one peak is observed.

Hydrindantin $C_{18}H_{10}O_6$ A white powder. Practically insoluble in water, and freely soluble in dioxane.
Purity Ninhydrin positive substances Weigh 7 mg of Hydrindantin, dissolve in 10 ml of ninhydrin-ethylene glycol monomethyl ether TS, and heat for 3 minutes. No color develops.
Sensitivity To 10 ml of a solution of Hydrindantin in ethylene glycol monomethyl ether (1 in 10,000), add 1 ml of ammonia TS. A red color develops.
Loss on drying Not more than 2.0% (105ºC, 3 hours).

Hydriodic Acid HI [K8917]

Hydrochloric Acid HCl [K8180]

Hydrochloric Acid, Arsenic-free HCl (Hydrochloric Acid for Arsenic Analysis)

Hydrochloric Acid, Dilute Measure 23.6 ml of hydrochloric acid, and add water to make 100 ml. (10%)

Hydrochloric Acid, Purified HCl Measure 1,000 ml of diluted hydrochloric acid (1 in 2), add 0.3 g of potassium permanganate, and distill. Discard 250 ml of the initial distillate, and collect the subsequent 500 ml of distillate.

Hydrochloric Acid–Ammonium Acetate Buffer (pH 3.5) Weigh 25 g of ammonium acetate, dissolve in 45 ml of 6 mol/L hydrochloric acid, and add water to make 100 ml.

Hydrofluoric Acid HF [K8819]

Hydrogen H_2 Use hydrogen containing not less than 99.99% (vol) of H_2.

Hydrogen Peroxide H_2O_2 [K8230]

Hydrogen Peroxide TS Use oxydol specified in the Japanese Pharmacopoeia.

Hydrogen Sulfide H_2S A colorless gas having a characteristic odor. Heavier than air, and soluble in water. It is prepared by reacting ferrous sulfide with diluted sulfuric acid (1 in 20) or diluted hydrochloric acid (1 in 4).

Hydrogen Sulfide TS Use a saturated solution of hydrogen sulfide. Store in a small, almost filled, light-resistant bottle, and if possible in a cold place. It has a strong odor of hydrogen sulfide.

2-Hydroxy-1-(2-hydroxy-4-sulfo-1-naphthylazo)-3-naphthoic Acid $C_{21}H_{14}N_2O_7S$ [K8776]

5-Hydroxy-1-(4-sulfophenyl)-3-pyrazolecarboxylic Acid $C_{10}H_8N_2O_6S$ A white to whitish powder.
Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength near 261 nm): Not less than 494. Weigh 0.0100 g of 5-Hydrxy-1-(4-sulfophenyl)-3-pyrazolecarboxylic Acid, previously dried for 24 hours in a vacuum desiccator, dissolve in ammonium acetate solution (3 in 2,000), and make exactly 100 ml. Refer to this solution as solution A. Measure exactly 10 ml of solution A, and add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Measure the absorbance of this solution.
Purity Other Aromatic Compounds Measure exactly 10 ml of the solution A, add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Analyze 20 µl of this solution by liquid chromatography using the operating condi-

tions directed under Purity (6) for Food Yellow No.4 in the Monographs. Only one peak is observed.

Hydroxylamine Hydrochloride See Hydroxylammonium Chloride.

Hydroxylamine TS Weigh 20 g of hydroxylamine hydrochloride, dissolve in 40 ml of water, and add 400 ml of ethanol, 300 ml of 0.5 mol/L ethanolic potassium hydroxide, and 2.5 ml of bromophenol blue–sodium hydroxide TS. Allow to stand for 30 minutes, and filter. Prepare fresh before use.

Hydroxylammonium Chloride $HONH_3Cl$ [K8201]

Hypophosphorous Acid H_3PO_2 [Phosphinic Acid, K 8440]

Imidazole for Water Determination $C_3H_4N_2$ A white crystalline powder. Very soluble in water and in methanol.

Melting point 89–92°.

Absorbance $E^{1\%}_{1cm}$ (313 nm): Not more than 0.031 (8 g, water, 100 ml).

Water Not more than 1 mg/ml.

Indigo Carmine $C_{16}H_8N_2Na_2O_8S_2$ [K8092]

Indigo Carmine TS Weigh an amount of the sample equivalent to 0.18 g of indigo carmine ($C_{16}H_8N_2Na_2O_8S_2$), and dissolve in water to make 100 ml. Use within 2 months of preparation.

***myo*-Inositol for Assay**

Description White, odorless crystals or crystalline powder having a sweet taste.

Identification Determine the absorption spectrum of *myo*-Inositol for Assay, previously dried at 105°C for 4 hours, as directed in the Potassium Bromide Disk Method under Infrared Spectrophotometry. It exhibits absorption bands at about 3380 cm^{-1}, 3220 cm^{-1}, 1446 cm^{-1}, 1147 cm^{-1}, 1114 cm^{-1}, and 1049 cm^{-1}.

Purity Related substances Prepare a test solution by dissolving 0.2 g of *myo*-Inositol for Assay in 20 ml of water. Prepare a control solution by diluting 1 ml of the test solution, exactly measured, with water to exactly 100 ml. Analyze 10 µl each of the test solution and the control solution by liquid chromatography using the operating conditions below. Continue the chromatography for two times the retention time of the main peak and measure the peak areas of both solutions. Exclude the solvent main peak from the measurement. The total area of all peaks of the test solution, other than the main peak, is not greater than the area of the main peak of the control solution.

Operating conditions

Use the conditions specified in the Assay for *myo*-Inositol in the Monographs.

Iodine I_2 [K8920]

Iodine TS Weigh 14 g of iodine, dissolve in 100 ml of potassium iodide solution (2 in 5), and add 1 ml of diluted hydrochloric acid (1 in 4) and water to make 1,000 ml. Store protected from light.

Iodine–Carbon Tetrachloride TS Weigh 12.5 g of iodine, add 1,000 ml of carbon tetrachloride, and allow to stand overnight to dissolve.

Iodine–Potassium Iodide TS Weigh 0.5 g of iodine and 1.5 g of potassium iodide, and dissolve in 25 ml of water.

Iodine Trichloride ICl_3 [K8403]

Iron(II) Ammonium Sulfate See Ammonium Iron(II) Sulfate Hexahydrate.

Iron(III) Ammonium Sulfate See Ammonium Iron(III) Sulfate Dodecahydrate.

Iron(III) Chloride See Iron(III) Chloride Hexahydrate.

Iron(III) Chloride Hexahydrate $FeCl_3 \cdot 6H_2O$ [K8142]

Iron(III) Chloride TS Dissolve 9 g of iron(III) chloride in water to make 100 ml.

Iron(III) Chloride TS, Dilute To 2 ml of Iron(III) Chloride, add water to make 100 ml. Prepare fresh before use.

Iron(III) Chloride–Hydrochloric Acid TS Weigh 5 g of iron(III) chloride, add 5 ml of hydrochloric acid and water to dissolve, and make 100 ml.

Iron Fragment Fe Use fragmentary iron containing not less than 97.7% of Fe. It is attracted by a magnet.

Iron(II) Sulfate See Iron(II) Sulfate Heptahydrate.

Iron(II) Sulfate Heptahydrate $FeSO_4 \cdot 7H_2O$ [K8978]

Iron(III) Sulfate See Iron(III) Sulfate *n*-Hydrate.

Iron(III) Sulfate *n*-Hydrate $Fe_2(SO_4)_3 \cdot nH_2O$ [K8981]

Iron(II) Sulfide FeS [For generation of hydrogen sulfide, K8948]

Isoamyl Acetate See 3-Methylbutyl Acetate.

Isoamyl Alcohol See 3-Methyl-1-butanol.

Isobutyl Alcohol See 2-Methyl-1-propanol.

Isooctane See 2,2,4-Trimethylpentane.

Isooctane for Ultraviolet Absorption Spectrum Measurement See 2,2,4-Trimethylpentane for Ultraviolet Absorption Spectrum Measurement.

Isooctane TS Place 300 ml of dimethyl sulfoxide for ultraviolet absorption spectrum measurement in a 1-L separating funnel, add 75 ml of phosphoric acid, shake, and allow to stand for 10 minutes. Add 150 ml of isooctane for ultraviolet absorption spectrum measurement, shake, and allow to stand for 10 minutes. Separate the upper layer, and store in a tightly stoppered glass bottle.

Isopropyl Alcohol See 2-Propanol.

Isopropyl Alcohol for Vitamin A Determination See 2-Propanol for Vitamin A Determination.

Isopropyl Iodide for Assay C_3H_7I A clear, colorless liquid. Upon exposure to light, turns brown releasing iodine. Miscible with ethanol, with diethyl ether, and with petroleum benzine. Not miscible with water. Use the distillate obtained at 89.0–89.5°C for the following tests.

Content Not less than 98.0% of isopropyl iodide (C_3H_7I).

Specific gravity d_4^{20}: 1.700–1.710.

Purity Analyze 1 µl of Isopropyl Iodide for Assay by gas chromatography using the conditions directed in the Assay for Hydroxypropyl Methylcellulose in the Monographs. Using the automatic integration method, determine the peak area of each peak recorded in the chromatogram, and obtain the content of isopropyl iodide by the Peak Area Percentage Method. The content is not less than 99.8%. Adjust the detection sensitivity, so that the peak height of isopropyl iodide obtained from 1 µl of Isopropyl Iodide for Assay is about 80% of the full scale.

Assay Place 10 ml of ethanol in a 100-ml brown volumetric flask, and weigh accurately the flask with the ethanol. To the flask, add 1 ml of Isopropyl Iodide for Assay, and weigh the flask accurately. To the mixture, add ethanol to make exactly 100 ml. Measure exactly 20 ml of this solution into another volumetric flask, add exactly 50 ml of 0.1 mol/L silver nitrate solution and 2 ml of nitric acid, and stopper. Allow to stand in a dark place for 2 hours with occasional shaking. Then leave to stand in a dark place for night, and shake occasionally for additional 2 hours. Add water to make exactly 100 ml, and filter through a dry filter paper. Remove the initial 20 ml of the filtrate, and collect exactly the following 50 ml. Titrate the excess silver nitrate with 0.1 mol/L ammonium thiocyanate solution. Use 2 ml of ferric ammonium sulfate solution as an indicator. Separately perform a blank test.

Each ml of 0.1 mol/L silver nitrate solution = 17.00 mg of C_3H_7I

Lactic Acid $CH_3CH(OH)COOH$ [K8726]

Lactic Acid TS Weigh 12.0 g of lactic acid, and dissolve in water to make 100 ml.

Lactose See Lactose Monohydrate.

Lactose Broth Prepare by adding lactose monohydrate to general bouillon at a rate of 0.5%. To 1,000 ml of the medium, add about 12 ml of bromothymol blue–sodium hydroxide TS. Then dispense about 10-ml portions into several fermentation tubes. Sterilize them at 100°C for 15 to 30 minutes once a day on three successive days by using a steam boiler, or autoclave them once at 121°C for about 20 minutes, and cool immediately by immersing in cold water.

Lactose Monohydrate $C_{12}H_{22}O_{11}\cdot H_2O$ Use lactose specified in the Japanese Pharmacopoeia.

Lead Acetate See Lead(II) Acetate Trihydrate.

Lead(II) Acetate Trihydrate $Pb(CH_3COO)_2\cdot 3H_2O$ [K8374]

Lead Acetate TS Weigh 11.8 g of lead acetate, dissolve in water to make 100 ml, and add 2 drops of diluted acetic acid (1 in 4). Store in a tightly-stoppered container.

Lead Acetate TS, Basic Weigh 3 g of lead acetate and 1 g of lead monoxide, add 0.5 ml of water, and triturate them. Transfer the resultant yellowish mixture into a beaker, cover with a watch glass, and heat on a water bath. When the contents have become uniformly white to reddish white, add 9.5 ml of boiling water in small portions, cover again with the watch glass, and allow to stand. Collect the supernatant by decantation, and add water to adjust the specific gravity d_{25}^{25} to between 1.23 and 1.24. Store in a tight-stoppered container.

Lead Monoxide See Lead(II) Oxide.

Lead Nitrate See Lead(II) Nitrate.

Lead(II) Nitrate $Pb(NO_3)_2$ [K8563]

Lead(II) Oxide PbO [K8090]

Light Green SF Yellow $C_{37}H_{34}N_2Na_2O_9S_3$ Disodium 4-(bis{4-[*N*-ethyl-*N*-(3-sulfonatophenylmethyl)amino]phenyl}methyliumyl)benzenesulfonate. Darkish green granules or powder.

Identification Add 1 ml of sodium hydroxide solution (1 in 10) to 5 ml of a solution of Light Green SF Yellow (1 in 1,000). The solution turns light green.

Specific absorbance $E_{1cm}^{1\%}$: Not less than 606 (maximum absorption wavelength near 633 nm). Weigh 0.0100 g of Light Green SF Yellow, dissolve in ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Measure exactly 10 ml of this solution, and add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. This solution exhibits an absorption maximum at a wavelength of 631–635 nm.

Liquid Paraffin See Paraffin, Liquid.

Lithium Acetate See Lithium Acetate Dihydrate.

Lithium Acetate Buffer Weigh 40.8 g of lithium acetate, and dissolve in water to make 100 ml. Adjust the pH to 9 with sodium hydroxide solution (1 in 25).

Lithium Acetate Dihydrate $CH_3COOLi\cdot 2H_2O$ Colorless to white crystals. Freely soluble in water.

Melting point 70°C.

Clarity and color of solution Colorless and almost clear (0.5 g, water 10 ml).

Lithium Chloride LiCl [Lithium Chloride, K8162: 1992] White, deliquescent crystals or small lumps.

Content Not less than 99.0% of lithium chloride (LiCl) on the dried basis.

Identification To 5 ml of a solution of Lithium Chloride (1 in 100), add 1 ml of silver nitrate solution (1 in 50). A white precipitate is formed. The precipitate dissolves with the addition of 10 ml of diluted ammonia solution (2 in 5).

Loss on drying Not more than 2.0% (130°C, 42 hours).

Assay Prepare a test solution. Weigh accurately about 0.8 g of Lithium Chloride, previously dried, and add water. Dissolve and make up to exactly 100 ml. To 20 ml of this solution, measured exactly, add 50 ml of water. Use the obtained

solution as the test solution. To the test solution, gradually add exactly 50 ml of 0.1 mol/L silver nitrate while stirring. Then add 9 ml of diluted nitric acid (1 in 3) and 3 ml of nitrobenzene. Titrate the excess silver nitrate with 0.1 mol/L ammonium thiocyanate. Use ammonium iron(III) sulfate TS as the indicator. Separately, perform a blank test.

Each ml of 0.1 mol/L ammonium thiocyanate = 4.239 mg of LiCl

Lithium Lactate $LiC_3H_5O_3$ Odorless, white powder or crystals.

pH 6.0–7.5 (1.0 g, water 20 ml).

Residue on ignition 56.5– 58.0% (use after drying at 105°C for 4 hours)

Lithium Sulfate See Lithium Sulfate Monohydrate.

Lithium Sulfate Monohydrate $Li_2SO_4 \cdot H_2O$ [K8994]

Litmus Paper, Blue [Litmus Paper, Blue Litmus Paper, K9071]

Litmus Paper, Red [Litmus Paper, Red Litmus Paper, K9071]

L-Lysine Hydrochloride See L-Lysine Monohydrochloride.

L-Lysine Monohydrochloride
$H_2N(CH_2)_4CH(NH_2)COOH \cdot HCl$ [L(+)-Lysine Monohydrochloride, K9053:1993]
White crystals or crystalline powder.

Content Not less than 99.0% of L-lysine monohydrochloride ($H_2N(CH_2)_4CH(NH_2)COOH \cdot HCl$) when dried.

Identification (1) L-Lysine Monohydrochloride responds to all tests for Chloride Salts as described in the Qualitative Tests.

(2) Determine the absorption spectrum as directed in the Potassium Bromide Disk Method under Infrared Spectrophotometry. The spectrum exhibits absorption bands at wavenumbers of about 2100 cm^{-1}, 1630 cm^{-1}, 1500 cm^{-1}, 1420 cm^{-1}, and 1330 cm^{-1}.

Specific rotation $[\alpha]_D^{20}$: +20.5 to +21.5° (after drying, 4 g, diluted hydrochloric acid (1 in 2) 50 ml).

Loss on drying Not more than 0.5% (105°C, 3 hours).

Assay Weigh accurately about 0.1 g of L-Lysine Monohydrochloride, previously dried, dissolve in 3 ml of formic acid. Add 20 ml of 0.1 mol/L perchloric acid, measured accurately, heat for 30 minutes on a water bath, and cool. Add acetic acid for nonaqueous titration to make 60 ml, and titrate the excess perchloric acid with 0.1 mol/L sodium acetate. Use a potentiometer to confirm the endpoint. When 1 ml of crystal violet–acetic acid TS is used as the indicator, the end point is when the yellow color of the solution changes to blue-green through yellow-green. Separately, perform a blank test.

Each ml of 0.1 mol/L perchloric acid = 9.133 mg of $C_6H_{14}N_2O_2 \cdot HCl$

Magnesia TS Weigh 5.5 g of magnesium chloride and 7 g of ammonium chloride, mix them, and dissolve in 65 ml of water. Add 35 ml of ammonia TS, allow to stand for several days in a tightly stoppered bottle, and filter. If the solution is not clear, filter before use.

Magnesium Acetate See Magnesium Acetate Tetrahydrate.

Magnesium Acetate Tetrahydrate $Mg(CH_3COO)_2 \cdot 4H_2O$ [Magnesium Acetate (Tetrahydrate), K8380:1978] Colorless to white, deliquescent crystals or powder.

Content 99.0–101.0%.

Identification Magnesium Acetate Tetrahydrate responds to all tests for Acetate Salt and for Magnesium Salt.

Assay Weigh accurately about 0.5 g of Magnesium Acetate Tetrahydrate, and add 100 ml of water to dissolve. Add 2 ml of ammonia–ammonium chloride buffer (pH 10.7), and titrate with 0.01 mol/L EDTA solution. Use two drops of eriochrome black T TS as the indicator. The endpoint is when the solution turns from red to blue.

Each ml of 0.01 mol/L EDTA solution = 21.47 mg of $Mg(CH_3COO)_2 \cdot 4H_2O$

Magnesium Carbonate Use magnesium carbonate specified in the Japanese Pharmacopoeia.

Magnesium Chloride See Magnesium Chloride Hexahydrate.

Magnesium Chloride Hexahydrate $MgCl_2 \cdot 6H_2O$ [K8159]

Magnesium Dust See Magnesium Powder.

Magnesium Nitrate See Magnesium Nitrate Hexahydrate.

Magnesium Nitrate Hexahydrate $Mg(NO_3)_2 \cdot 6H_2O$ [K8567]

Magnesium Oxide MgO [K8432]

Magnesium Powder Mg [K8876]

Magnesium Sulfate See Magnesium Sulfate Heptahydrate.

Magnesium Sulfate Heptahydrate $MgSO_4 \cdot 7H_2O$ [K8995]

Magnesium Sulfate TS Weigh 11 g of magnesium sulfate, and dissolve in 50 ml of water to make 100 ml (0.5 mol/L).

Maltol for Assay Weigh 1 g of "Maltol" and 1 g of active carbon in a beaker, add 10 ml of water, dissolve by heating at 95°C, and filter while hot. Cool the filtrate to 10°C, and collect the formed crystals by filtration. Repeat the procedure for the recrystallized product, and dry the resulting twice-recrystallized product under reduced pressure of not more than 1.3 kPa at 40°C for 8 hours.

Manganese Sulfate See Manganese(II) Sulfate Pentahydrate.

Manganese(II) Sulfate Pentahydrate $MnSO_4 \cdot 5H_2O$ [K8997]

Manganese Sulfate TS Weigh 90 g of manganese sulfate, add about 200 ml of water, about 175 ml of phosphoric acid, and about 350 ml of diluted sulfuric acid (1 in 2) to dissolve, and add water to make 1,000 ml.

D-Mannitol $C_6H_{14}O_6$ [K8882]

D-Mannitol for Assay Weigh 40 g of "D-Mannitol," transfer to a 300-ml flask, and add 100 ml of water. Dissolve it by warming in a water bath, and cool to 40°C. Transfer the solution into a 300-ml beaker, add 0.02 g of "D-Mannitol," mix, and allow to stand for 24 hours. Filter the formed crystals by suction, and wash with 10 ml of cold water. Dry the resultant recrystallized product under reduced pressure at 105°C for 4 hours.

Meat Extract Use beef extract or its equivalent.

Meat Peptone See Peptone, Meat.

2-Mercaptoethanol $HSCH_2CH_2OH$ A colorless, clear liquid.

Specific gravity d_4^{20}: 1.112–1.117.

Menaquinone-4 for Assay $C_{31}H_{40}O_2$ A yellow powder or crystalline powder.

Melting point 36.0–38.0°C.

Purity (1) Clarity Yellow, clear (0.10 g, hexane 1 ml).

(2) Related substances The following operation should be protected from direct sunlight, and the equipment used should be light-resistant.

Prepare a test solution as follows. Dissolve about 0.1 g of Menaquinone-4 for Assay, accurately weighed, in 50 ml of 2-propanol, and add absolute ethanol to make exactly 100 ml. Measure exactly 10 ml of this solution, and add absolute ethanol to make 100 ml. Measure exactly 2 ml of the obtained solution, and add 4 ml of 2-propanol. Use the prepared solution as the test solution. Prepare a control solution by diluting 2 ml of the test solution, measured exactly, to exactly 100 ml with a 2:1 mixture of 2-propanol/ethanol. Analyze 20 µl each of the test solution and the control solution by liquid chromatography using the operating conditions below. Continue the chromatography for two times the retention time of the main peak, and measure the peak areas. The total area of all peaks of the test solution, other than the main peak, is not greater than the main peak area of the control solution.

Operating conditions

Use the conditions specified in the Assay for Menaquinone (Extract) in the Monographs.

Mercuric Acetate See Mercury(II) Acetate.

Mercuric Acetate TS for Nonaqueous Titration Weigh 6 g of mercury(II) acetate, and dissolve in acetic acid for nonaqueous titration to make 100 ml.

Mercuric Bromide See Mercury(II) Bromide.

Mercuric Bromide Test Paper Weigh 5 g of mercury(II) bromide, add 100 ml of ethanol, and dissolve while heating gently. Cut a filter paper for chromatography into a 3× 10 cm strip, immerse it in the solution, and allow to stand in a dark place for about 1 hour while shaking occasionally. Remove the filter paper from the solution, dry naturally in a dark place while keeping it horizontal, and cut into a circle with about 18 mm in diameter. Store in a brown, tightly-stoppered bottle in a dark place. Avoid direct contact with fingers.

Mercuric Chloride See Mercury(II) Chloride.

Mercuric Nitrate TS Weigh 40 g of yellow mercuric oxide, and add 32 ml of nitric acid and 15 ml of water to dissolve. Store in a tightly stoppered, light-resistant bottle. (4 mol/L)

Mercuric Oxide, Yellow See Mercury(II) Oxide, Yellow.

Mercuric Potassium Iodide TS Weigh 1.358 g of mercury(II) chloride, and dissolve in 60 ml of water. Add 10 ml of potassium iodide solution (1 in 2) and water to make 100 ml.

Mercuric Sulfate TS Weigh 5 g of yellow mercuric oxide, add 40 ml of water, and slowly add 20 ml of sulfuric acid while stirring. Add 40 ml of water, and dissolve by stirring well.

Mercury(II) Acetate $Hg(CH_3COO)_2$ [K8369]

Mercury(II) Bromide $HgBr_2$ [K8513]

Mercury(II) Chloride $HgCl_2$ [K8139]

Mercury(II) Oxide, Yellow HgO [Mercury(II) Oxide (Yellow), K8418]

Metaphosphoric Acid HPO_3 [K8890]

Methanol CH_3OH [K8891]

Methanol, Carbonyl-free Add 5 g of Girard regent P and 0.2 ml of hydrochloric acid to 500 ml of methanol, and reflux for 2 hours. Distill using a short Vigreux column. Store in a tightly stoppered, glass bottle.

Methanol for Water Determination CH_3OH Use methanol containing not more than 0.05% (w/v) of water. Otherwise, use methanol prepared in the following manner: To 1,000 ml of methanol, add 5 g of magnesium dust, and heat under a reflux condenser connected to a water absorption tube (filled with calcium chloride for water determination). Add 0.1 g of mercuric chloride to accelerate the reaction if necessary. When the gas no longer evolves, distill protected from moisture. Store protected from moisture.

Methanolic 35% Potassium Hydroxide TS See 35% Potassium Hydroxide TS, Methanolic.

Methanolic 5% Sodium Hydroxide TS See 5% Sodium Hydroxide TS, Methanolic.

4-Methoxybenzaldehyde $C_8H_8O_2$ A colorless to light-yellow, clear liquids. Miscible with ethanol and with diethyl ether, but practically insoluble in water.

Content Not less than 97.0%.

Specific gravity d_4^{20}: 1.123–1.129.

Assay Weigh accurately about 0.8 g of 4-Metyoxybenzaldehyde, add exactly 7.5 ml of hydroxylamine TS, shake well, and allow to stand for 30 minutes. Titrate with 0.5 mol/L hydrochloric acid (indicator: 3 drops of bromophenol blue TS) until the color of the solution changes from blue to yellow-green through green. Perform a blank test in the

same manner.

Each ml of 0.5 mol/L hydrochloric acid = 68.08 mg of $C_8H_8O_2$

0.5% 4-Methoxybenzaldehyde–Ethyl Acetate TS Mix 0.5 ml of 4-methoxybenzaldehyde and 99.5 ml of ethyl acetate.

4-Methoxybenzaldehyde–Sulfuric Acid TS To 9 ml of ethanol, add 0.5 ml of 4-methoxybenzaldehyde and 0.5 ml of sulfuric acid, and mix well.

2-Methoxyethanol $CH_3OCH_2CH_2OH$ [K8895]

2-Methoxy-5-methylaniline $C_8H_{11}NO$ A white to gray crystalline powder. Sparingly soluble in water, and soluble in methanol and in ethanol.

Identification (1) Dissolve Methoxy-5-methylaniline in a 1:1 mixture of methanol/0.01 mol/L ammonium acetate. It exhibits an absorption maximum at about 290 nm.

(2) Determine the absorption spectrum as directed in the Potassium Bromide Disk Method under Infrared Spectrophotometry. It exhibits absorption bands at wavenumbers of about 3410 cm^{-1}, 2950 cm^{-1}, 1630 cm^{-1}, 1520 cm^{-1}, 1230 cm^{-1}, 1030 cm^{-1}, and 780 cm^{-1}.

Melting point 47–54°C.

1-Methoxy-2-propanol $C_5H_{12}O_2$ A colorless, clear liquid.

Specific gravity 0.920–0.925

Refractive index 1.402–1.405.

Water Not more than 0.5% (0.1 g, coulometric titration).

Methyl Benzoate $C_6H_5COOCH_3$ A colorless, clear liquid.

Refractive index n_D^{20}: 1.515–1.520.

Specific gravity 1.087–1.095.

Purity Dissolve 0.1 ml of Methyl Benzoate in the mobile phase directed under the Assay for Thiamine Hydrochloride in the Monographs, and make 50 ml. Analyze 10 µl of this solution by liquid chromatography using the operating conditions directed under the Assay for Thiamine Hydrochloride. Continue the chromatography for twice the retention time of the main peak, and measure the peak area of each peak. Calculate the content of methyl benzoate. The content is not less than 99.0%.

3-Methyl-1-butanol $(CH_3)_2CHCH_2CH_2OH$ [K8051]

3-Methylbutyl Acetate $CH_3COOC_5H_{11}$ [K8358]

Methyl Ethyl Ketone See 2-Butanone.

2-Methylimidazole $C_4H_6N_2$ White to light-yellow, hygroscopic crystals or crystalline powder having a slight, characteristic odor. Soluble in water, in ethanol, in ethyl acetate, and in acetone.

Content Not less than 98% of 2-methylimidazole ($C_4H_6N_2$).

Boiling point 267–268°C.

Melting point 142–145°C.

Assay Weigh accurately about 0.2 g of 2-Methylimidazole, and dissolve in 50 ml of acetic acid for nonaqueous titration. Titrate with 0.1 mol/L perchloric acid. Use a potentiometer to determine the endpoint. Perform a blank test in the same manner, and make necessary correction.

Each ml of 0.1 mol/L perchloric acid = 8.211 mg of $C_4H_6N_2$

4-Methylimidazole $C_4H_6N_2$ Light-yellow, hygroscopic crystals or crystalline powder having a slight, characteristic odor. Soluble in water, in ethanol, in acetone, and in chloroform.

Content Not less than 97% of 4-methylimidazole ($C_4H_6N_2$).

Boiling point 262–264°C.

Melting point 46–48°C.

Assay Weigh accurately about 0.2 g of 4-Methylimidazole, and dissolve in 50 ml of acetic acid for nonaqueous titration. Titrate with 0.1 mol/L perchloric acid. Use a potentiometer to determine the endpoint. Perform a blank test in the same manner, and make necessary correction.

Each ml of 0.1 mol/L perchloric acid = 8.211 mg of $C_4H_6N_2$

Methyl Iodide for Assay CH_3I A clear, colorless liquid. Upon exposure to light, turns brown releasing iodine. Miscible with ethanol and with diethyl ether, and slightly soluble in water.

For the tests, use the fraction obtained by distillation at 42.2–42.6°C.

Content Not less than 98.0% of methyl iodide (CH_3I).

Specific gravity d_{25}^{25}: 2.27–2.28.

Purity Analyze 1 µl of Methyl Iodide for Assay by gas chromatography using the conditions directed in the Assay for Hydroxypropyl Methylcellulose. Measure the peak area of each peak recorded in the chromatogram, and obtain the content of methyl iodide by the Peak Area Percentage Method. The content is not less than 99.8%. Adjust the detection sensitivity, so that the peak height of methyl iodide obtained from 1 µl of Methyl Iodide for Assay is about 80% of the full scale.

Assay Perform the test in the same manner as for Isopropyl Iodide for Assay.

Each ml of 0.1 mol/L silver nitrate solution = 14.19 mg of CH_3I

Methyl Isobutyl Ketone See 4-Methyl-2-pentanone.

Methyl Orange $C_{14}H_{14}N_3NaO_3S$ [K8893]

Methyl Orange TS Weigh 0.1 g of methyl orange, and dissolve in 100 ml of water. Filter if necessary.

Methyl Orange–Indigo Carmine TS Weigh 0.1 g of methyl orange and 0.25 g of indigo carmine, mix, and add water to make 100 ml. Store protected from light, and use it within 15 days after preparation.

Methyl Orange–Xylene Cyanol FF TS Weigh 1 g of methyl orange and 1.4 g of xylene cyanol FF, mix, and dissolve in 500 ml of 50% (vol) ethanol.

4-Methyl-2-pentanone $CH_3COCH_2CH(CH_3)_2$ [K8903]

3-Methyl-1-phenyl-5-pyrazolone $C_{10}H_{10}N_2O$ [K9548]

2-Methyl-1-propanol $(CH_3)_2CHCH_2OH$ [K8811]

Methyl Red $C_{15}H_{15}N_3O_2$ [K8896]

Methyl Red TS Weigh 0.1 g of methyl red, and dissolve in 100 ml of ethanol. Filter if necessary.

Methyl Red–Methylene Blue Mixture TS Mix equal volumes of methyl red TS and methylene blue TS.

Methyl Salicylate $HOC_6H_4COOCH_3$ [K8398:1981]
A colorless to pale-yellow, oily substance having a characteristic odor.

Specific gravity 1.1821–1.192.

Methyl Silicone Polymer Use high-quality methyl silicone polymer prepared for gas chromatography.

Methyl yellow $C_{14}H_{15}N_3$ [K8494]

Methyl yellow TS Dissolve 0.10 g of methyl yellow in 200 ml of ethanol.

Methylene Blue $C_{16}H_{18}N_3S{\cdot}Cl{\cdot}3H_2O$ [K8897]

Methylene Blue TS Weigh 0.1 g of methylene blue, and dissolve in 100 ml of ethanol. Filter if necessary.

Methylene Blue TS, Dilute Measure 1 ml of methylene blue TS, and add water to make 100 ml.

Microcrystalline Cellulose for Thin-Layer Chromatography Use microcrystalline cellulose prepared for thin-layer chromatography.

Milk Casein See Casein, Milk.

Mogroside V ($C_{60}H_{102}O_{29}$) A white to light yellow powder having a sweet taste.

Identification Measure the absorption spectrum of Mogroside V, previously dried at 105°C for 2 hours, as directed in the Potassium Bromide Disk Method under Infrared Spectrophotometry. It exhibits absorption bands at wavenumbers of about 3430 cm^{-1}, 2930 cm^{-1}, 1634 cm^{-1}, 1383 cm^{-1}, 1170 cm^{-1}, 1075 cm^{-1}, and 1038 cm^{-1}.

Purity Related substance Prepare a test solution by dissolving 5 mg of Mogroside V in 1 ml of a 74:26 mixture of acetonitrile/water. Prepare a control solution by diluting 0.5 ml of the test solution, measured exactly, with a 74:26 mixture of acetonitrile/water to make exactly 10 ml. Analyze 5 µl each of the test solution and the control solution by liquid chromatography using the operating conditions given below. Continue the chromatography for two times the retention time of the main peak, and measure the peak areas. Exclude the solvent peaks from the measurement. The total area of all peaks of the test solution, other than the main peak, is not greater than the area of the main peak of the control solution.

Operating conditions
Follow the conditions specified in the Assay for Luohanguo Extract in the Monographs.

Molybdenum(III) Oxide MoO_3 [Molybdenum Trioxide, K8436:1979]

Molybdenum Trioxide See Molybdenum(III) Oxide.

Monoglucosyl Hesperidin for Assay A light yellow to yellow-brown crystalline powder having a slight characteristic odor.

Identification (1) Dissolve 5 mg of Monoglucosyl Hesperidin for Assay in 10 ml of water, and add 1–2 drops of dilute iron(III) chloride TS. A brown color develops.

(2) Dissolve 0.01 g of Monoglucosyl Hesperidin for Assay in 500 ml of water. The solution exhibits an absorption maximum at a wavelength of 280–286 nm.

Loss on drying Not more than 6.0% (Not more than 2.7 kPa, 120°C, 2 hours).

Purity Related substances Prepare a test solution by dissolving about 0.1 g of Monoglucosyl Hesperidin for Assay, weighed accurately, in an 80:20:0.01 mixture of water/acetonitrile/acetic acid to make exactly 200 ml. Prepare a control solution by diluting 1 ml of the test solution, measured exactly, to exactly 50 ml with an 80:20:0.01 mixture of water/acetonitrile/acetic acid. Analyze 10 µl each of the test solution and the control solution by liquid chromatography using the operating conditions given below. Continue the chromatography for two times the retention time of the main peak, and measure the peak areas. The total area of all peaks of the test solution, other than the main peak, is not greater than the area of the main peak of the control solution.

Operating conditions Use the conditions specified in the Assay for Enzymatically Modified Hesperidin in the Monographs.

Monopotassium Phosphate KH_2PO_4 [Potassium Dihydrogen Phosphate, K 9007]

Monopotassium Phosphate for pH Determination KH_2PO_4 [Potassium Dihydrogen Phosphate, For pH Standard Solution, K9007]

Monosodium 4-Amino-1-naphthalenesulfonate $C_{10}H_8NNaO_3S{\cdot}4H_2O$ A white to whitish powder.

Specific absorbance $E^{1\%}_{1cm}$ (maximum absorption wavelength near 319 nm): Not less than 338. Dry Monosodium 4-Amino-1-naphthalenesulfonate for 24 hours in a vacuum desiccator, weigh 0.0100 g of it, add ammonium acetate solution (3 in 2,000), and dissolve to make exactly 100 ml. Refer to it as solution A. Measure exactly 10 ml of solution A, add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. This solution exhibits absorption maxima at the wavelengths of 237 nm and 319 nm.

Purity Other aromatic compounds Measure exactly 10 ml of solution A, add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Analyze 20 µl of this solution by liquid chromatography using the operating conditions directed under Purity (6) for Food Red No. 2 in the Monographs. Only one peak is observed.

Monosodium L-Aspartate $C_4H_6NNaO_4{\cdot}H_2O$
"Monosodium L-Aspartate"

Monosodium L-Glutamate See Monosodium L-Glutamate Monohydrate.

Monosodium L-Glutamate Monohydrate $C_5H_8NNaO_4{\cdot}H_2O$ "Monosodium L-Glutamate"

Monosodium 6-Hydroxy-2-naphthalenesulfonate
$C_{10}H_7NaO_4S$ A whitish powder.

Specific absorbance $E^{1\%}_{1cm}$ (maximum absorption wavelength near 280 nm): Not less than 200. Weigh 0.0100 g of Monosodium 6-Hydroxy-2-naphthalenesulfonate, dried previously for 24 hours in a vacuum desiccator, and dissolve in ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Refer to this solution as solution A. Measure exactly 10 ml of solution A, and add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. This solution exhibits absorption maxima at wavelengths of 280 nm and 330 nm.

Purity Other aromatic compounds Measure exactly 1.0 ml of solution A, and add ammonium acetate solution (7.7 in 1,000) to make exactly 100 ml. Analyze 20 µl of this solution by liquid chromatography using the operating conditions specified in Purity (8) for Food Red No. 40 in the Monographs. Only one peak of monosodium 6-hydroxy-2-naphthalenesulfonate is observed.

Monosodium Phosphate $NaH_2PO_4{\cdot}2H_2O$ [Sodium Dihydrogen Phosphate Dihydrate, K9009]

Morpholine C_4H_9NO A basic, colorless liquid having an ammonia-like odor. Soluble in water.

Refractive index n_D^{20}: 1.452–1.457.

Specific gravity 0.998–1.005.

Mutarotase A white, 50% glycerol suspension. It is obtained from the kidney of swine. One unit is equivalent to the amount of enzyme required to produce 1 µmol of β-D-glucose from α-D-glucose as a substrate in 1 minute at 25°C and pH 7.2.

Myricitrin for Assay $C_{21}H_{20}O_{12}{\cdot}nH_2O$ A light grayish yellow to light yellow, almost odorless powder.

Identification Measure the absorption spectrum of Myricitrin for Assay as directed in the Potassium Bromide Disk Method under Infrared Spectrophotometry. It exhibits its absorption bands at wavenumbers of about 1660 cm^{-1}, 1605 cm^{-1}, 1345 cm^{-1}, 1200 cm^{-1}, and 970 cm^{-1}.

Purity (1) Specific absorbance $E^{1\%}_{1cm}$ (maximum absorption wavelength at about 354 nm): Not less than 340.

Weigh accurately about 0.05 g of Myricitrin for Assay, dried in a desiccator for 24 hours, and dissolve in methanol to make exactly 100 ml. Measure exactly 2 ml of the obtained solution, and add methanol to make exactly 100 ml. Measure the absorbance of this solution by Ultraviolet–visible Spectrophotometry.

(2) Related substances Dissolve 0.05 g of Myricitrin for Assay in 25 ml of methanol. Measure exactly 5 ml of this solution, and add an 800:200:1 mixture of water/acetonitrile/phosphoric acid to make exactly 50 ml. Use this solution as the test solution. Measure exactly 1 ml of the test solution, add 5 ml of methanol, and add an 800:200:1 mixture of water/acetonitrile/phosphoric acid to make exactly 50 ml. Use this solution as the control solution. Analyze 20 µl each of the test solution and the control solution by liquid chromatography using the operating conditions given below. Continue the chromatography for two times the retention time of the main peak, and measure the peak areas. The total area of all peaks of the test solution, other than the main peak, is not greater than the area of the main peak of the control solution.

Operating conditions
Use the operating conditions given in the Assay for Chinese Bayberry Extract in the Monographs.

Naphthalene $C_{10}H_8$ [K8690:1976] Colorless, foliaceous or rod-like crystals having a characteristic odor. Sublimes gradually at ordinary temperature. Upon ignition, burns with sooty flames.

Congealing point Not less than 79.5°C.

α-Naphthol See 1-Naphthol.

β-Naphthol See 2-Naphthol.

1-Naphthol $C_{10}H_7OH$ [K8698] Store protected from light.

2-Naphthol $C_{10}H_7OH$ [K8699] Store protected from light.

α-Naphtholbenzein See *p*-Naphtholbenzein.

***p*-Naphtholbenzein** $C_{27}H_{20}O_3$ [K8693]

α-Naphtholbenzein TS Weigh 1 g of α-naphtholbenzein, and dissolve in acetic acid for nonaqueous titration to make 100 ml.

Naphthoresorcinol See 1,3-Dihydroxynaphthalene.

α-Naphthylamine See 1-Naphthylamine.

1-Naphthylamine $C_{10}H_9N$ [K8692]

***N*-1-Naphthyl-*N′*-diethyl-ethylenediamine Oxalate**
$C_{18}H_{24}N_2O_4$ [*N,N*-Diethyl-*N′*-1-naphthylethylenediamine Oxalate, K8694:1992] A white crystalline powder. Upon exposure to light, it is gradually colored.

Content Not less than 98.0%.

Identification (1) To 0.1 g of *N*-1-Naphthyl-*N′*-diethylethylenediamine Oxalate, add 20 ml of water, and heat to dissolve. To this solution, add 1 ml of diluted acetic acid (1 in 3) and 1 ml of calcium chloride solution (1 in 10). A white precipitate is formed.

(2) Determine the absorption spectrum as directed under the Potassium Bromide Disk Method in Infrared Spectrophotometry. It exhibits absorption bands at wavenumbers of about 3340 cm^{-1}, 1720 cm^{-1}, 1580 cm^{-1}, 1530 cm^{-1}, 1410 cm^{-1}, 1280 cm^{-1}, and 770 cm^{-1}, and 720 cm^{-1}.

Melting point About 167°C.

Assay Weigh accurately about 0.5 g, add 100 ml of water, and heat to dissolve. Titrate with 0.1 mol/L sodium hydroxide. Use a potentiometer to determine the end point.

Each ml of 0.1 mol/L sodium hydroxide= 33.24 mg of $C_{18}H_{24}N_2O_4$

***N*-1-Naphthylethylenediamine Dihydrochloride**
$C_{12}H_{14}N_2{\cdot}2HCl$ [K8197] Prepare fresh before use.

Nessler's TS Weigh 10 g of potassium iodide, and dissolve in 10 ml of water. Add a saturated solution of mercury(II) chloride gradually while stirring until the red precipitate remains undissolved, and add 30 g of potassium hydroxide to dissolve. Then add 1 ml of mercury(II) chloride saturated solution and water to make 200 ml. Allow to stand, and use

the supernatant as Nessler's TS. Confirm that the solution immediately turns yellow-brown when 2 ml of the obtained Nessler's TS is added to 50 ml of water containing 0.05 mg of ammonia (NH_3).

Neutralized Ethanol See Ethanol, Neutralized.

Neutral Red $C_{15}H_{17}N_4Cl$ [K8729:1992] A dark green powder or small lumps.

Identification Determine the absorption spectrum as directed in the Potassium Bromide Disc Method under Infrared Spectrophotometry. It exhibits absorption bands at about 1620 cm^{-1}, 1500 cm^{-1}, 1360 cm^{-1}, 1320 cm^{-1}, 1200 cm^{-1}, 1140 cm^{-1}, 1010 cm^{-1}, 880 cm^{-1}, 830 cm^{-1}, and 730 cm^{-1}.

Range of color-change To 0.10 g of Neutral Red, add 80 ml of water, and warm to dissolve. After cooling to room temperature, dilute with water to make 100 ml. Add a 0.1-ml portion of the prepared test solution to 10 ml each of phosphate buffer (pH 6.8), phosphate buffer (pH 7.4), and phosphate buffer (pH 8.0). The buffer solutions turn red, orange, and yellow-orange, respectively.

Ninhydrin $C_9H_6O_4$ [K8870]

Ninhydrin TS Weigh 1 g of ninhydrin, and dissolve in water to make 1,000 ml.

Ninhydrin TS for Bacillus Natto Gum Assay

Solution 1 Dissolve 39 g of ninhydrin and 0.081 g of sodium tetrahydroborate for amino acid analysis in 979 ml of 1-methoxy-2-propanol, and mix under nitrogen gas.

Solution 2 Dissolve 204 g of lithium acetate, 123 ml of acetic acid, and 401 ml of 1-methoxy-2-propanol in water to make 1,000 ml, and mix under nitrogen gas.

Mix equal volumes of Solutions 1 and 2.

Ninhydrin–Acetic Acid TS Dissolve 2 g of ninhydrin in 50 ml of water, add 25 ml of acetate buffer (prepared by dissolving 32.8 g of sodium acetate in water and adding 10 ml of acetic acid and water to make 100 ml), and add water to make exactly 100 ml.

Ninhydrin–Ethylene Glycol Monomethyl Ether TS To 750 ml of ethylene glycol monomethyl ether, add 250 ml of acetate buffer. While passing nitrogen, add 20 g of ninhydrin first, then 0.38 g of tin(II) chloride, and dissolve them. Allow to stand in a cold, dark place for 24 hours. Store protected from light.

Ninhydrin–Hydrindantin TS Dissolve 2 g of ninhydrin in 75 ml of dimethyl sulfoxide. Add 62 mg of hydrindantin, and dissolve it. Add lithium acetate buffer to make 100 ml.

Nitric Acid HNO_3 [K8541]

Nitric Acid, Dilute Measure 10.5 ml of nitric acid, and add water to make 100 ml. (10%)

2,2′,2″-Nitrilotriethanol $(CH_2CH_2OH)_3N$ [K8663]

Nitrobenzene $C_6H_5NO_2$ [K8723]

Nitrogen N_2 Use nitrogen specified in the Japanese Pharmacopoeia.

Nitromethane CH_3NO_2 [K9523]

5-Nitroso-8-hydroxyquinoline $C_9H_6N_2O_2$ [K8715:1962] A dark greenish gray crystalline powder.

Identification Place 0.05 ml of 0.1% ethanol solution of resorcinol into a crucible, evaporate on a water bath to dryness, and cool. To the residue, add 0.05 ml of a solution prepared by dissolving 0.10 g of 5-Nitroso-8-hydroxyquinoline in 100 ml of sulfuric acid, and warm. A red-purple color develops.

Decomposing point About 245°C.

Nitrous Oxide N_2O A colorless, odorless gas. Use gas filled in a hermetic, pressure-resistant metal container.

NN Indicator Mix 0.5 g of 2-hydroxy-1-(2-hydroxy-4-sulfo-1-naphthylazo)-3-naphthoic acid and 50 g of potassium sulfate, and triturate thoroughly until it is homogeneous.

Octadecylsilanized Silica Gel for Liquid Chromatography Use products produced for liquid chromatography.

Octadecylsilanized Silica Gel for Thin-Layer Chromatography Use products made for thin-layer chromatography.

Octane C_8H_{18}

Specific gravity d_4^{20}: 0.700–0.705.

Purity Analyze 2 μl of Octane by gas chromatography using the conditions directed in the Assay for Hydroxypropyl Methylcellulose. Determine the peak area of each peak in the chromatogram, and obtain the content of octane by the Peak Area Percentage Method. The content is not less than 99.0%.

Octanoic Acid $CH_3(CH_2)_6COOH$ Use a product that is produced for amino acid analysis.

Description A colorless to light yellow, clear liquid.

Congealing point 15–17°C.

Octylsilanized Silica Gel for Liquid Chromatography Use products produced for liquid chromatography.

Orcine See Orcinol.

Orcinol $CH_3C_6H_3(OH)_2$ Colorless crystals. Turns red by oxidation in air. Soluble in water, in ethanol, and in diethyl ether. A solution of orcinol in ethanol should be prepare fresh before use.

Melting point 107–108°C.

Osmic Acid OsO_4 White to yellow crystals.

Content Not less than 57.0% of osmic acid (OsO_4).

Clarity of solution Clear. Weigh 0.5 g of Osmic Acid, transfer into a test tube with a ground-glass stopper, add 15 ml of water, shake, and allow to stand overnight.

Melting point 40–43°C

Assay Weigh accurately about 0.2 g of Osmic Acid, and add 10 ml of carbon tetrachloride, 100 ml of water, and 3 ml of diluted hydrochloric acid (2 in 3) to dissolve. Add 1 g of potassium iodide, allow to stand in a dark, cold place for 10

minutes with occasional vigorous shaking, and titrate with 0.1 mol/L sodium thiosulfate. Confirm the endpoint, using a potentiometer with a platinum electrode.

Each ml of 0.1 mol/L sodium thiosulfate solution = 6.355mg of OsO_4

Oxalic Acid See Oxalic Acid Dihydrate.

Oxalic Acid Dihydrate $HOOCCOOH \cdot 2H_2O$ [K8519]

Palladium Nitrate $Pd(NO_3)_2$ [K9069: 1957]

Palladium Nitrate TS To 0.108 g of palladium nitrate, add 10 ml of diluted nitric acid (1 in 2), dilute with water to make exactly 500 ml. Measure exactly 20 ml of this solution, and add water to make exactly 200 ml.

Palmitic Acid $C_{16}H_{32}O_2$ [K8756]

Paraffin, Liquid Use light liquid paraffin specified in the Japanese Pharmacopoeia.

Partially Hydrolyzed Saponin for Assay White crystals having slightly odor.

Identification Proceed as directed in the Potassium Bromide Disk Method under Infrared Spectrophotometry. The spectrum exhibits absorption bands at about 3240 cm^{-1}, 2920 cm^{-1}, 1640 cm^{-1}, 1150 cm^{-1}, 1080 cm^{-1}, and 1,020 cm^{-1}.

Purity Related substances Prepare a test solution by dissolving 0.010 g of Partially Hydrolyzed Saponin for Assay in 20 ml of a 65:35 mixture of 0.1% phosphoric acid/acetonitrile. Prepare a control solution by diluting 4 ml of the test solution, exactly measured, to exactly 100 ml with a 65:35 mixture of 0.1% phosphoric acid/acetonitrile. Analyze 20 µl each of the test solution and the standard solution by liquid chromatography using the conditions given below. Continue the chromatography for 30 minutes, and measure the each peak area. Remove solvent peaks from measurement. The total area of all peaks of the test solution, other than the main peak, is not greater than the area of the main peak of the control solution. The main peak appears about 10 minute after the solvent is detected.

Operating conditions

Detector: Ultraviolet spectrophotometer (determination wavelength: 210 nm).

Column: A stainless steel tube of 4–6 mm internal diameter and 15–30 cm length.

Column packing material: 5- to 10-µm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 40°C.

Mobile phase: A 65:5 mixture of 0.1% phosphoric acid/acetonitrile.

Flow rate: Adjust so that the retention time of partial hydrolyzed saponin is about 10 minutes.

Loss on drying Not more than 2.0% (105°C, 3 hours).

Pectate Lyase A water-soluble enzyme obtained from *Aspergillus* sp. It contains glycerol as an enzyme stabilizer. One unit is equivalent to the amount of enzyme required to release 1 µmol of uronic acid polymers with 4-deoxy-α-D-galact-4-enuronic acid residues at the non-reducing terminal from polygalacturonic acid as a substrate in 1 minute at 40°C and pH 10.8.

Pectate Lyase for Pectin Determination See Pectate Lyase Solution for Pectin Determination.

Pectate Lyase Solution for Pectin Determination Dissolve 120 units pectate lyase in tris buffer (pH7.0) for pectin determination to make 100 ml.

Pentaerythritol $C_5H_{12}O_4$ [K1510]

Peptone Use peptone produced for the microbial limit tests.

Peptone, Casein A grayish-yellow powder having a characteristic, non- putrefactive odor. Soluble in water, but insoluble in ethanol and in diethyl ether.

Loss on drying Not more than 7% (0.5 g, 105°C, constant weight).

Residue on ignition Not more than 15% (0.5 g).

Degree of digestion Perform the following tests for a sample solution prepared by dissolving 1 g of the sample in 10 ml of water:

(1) Overlay 1 ml of the sample solution with 0.5 ml of a solution prepared by adding 1 ml of acetic acid to 10 ml of equal-volume mixture of ethanol and water. No circular zone or precipitate is formed at the boundary surface of them, and no turbidity appears when the liquid is shaken.

(2) To 1 ml of the sample solution, add 4 ml of a saturated solution of zinc sulfate. A small quantity of precipitate (proteose) is produced.

(3) Filter the mixed solution prepared under test (2). To 1 ml of the filtrate, add 3 ml of water and 4 drops of bromine TS. A red-violet color develops.

Nitrogen content Not less than 10% (105°C, constant weight, after drying, Nitrogen Determination).

Peptone, Gelatin Use peptone prepared for the microbial limit tests.

Peptone, Meat Use peptone prepared for the microbial limit tests.

Peptone, Soybean Use peptone prepared for the microbial limit tests.

Perchloric Acid $HClO_4$ [K8223]

Periodic Acid See Periodic Acid Dihydrate.

Periodic Acid Dihydrate $HIO_4 \cdot 2H_2O$ [Periodic Acid (Dihydrate), K8284:1978] White deliquescent crystals.

Content Not less than 98.5%.

Identification Other halogens To a solution of Periodic Acid Dihydrate, add excess of sodium hydrogen carbonate, then potassium iodide solution. Iodine is liberated.

Purity (1) Other halogens Not more than 0.010% as Cl. Place 1.0 g of Periodic Acid Dihydrate into a beaker, add 100 ml of water, 8 ml of hydrogen peroxide, and 1 ml of phosphoric acid, and boil gently until the color of iodine disappears completely. After cooling, wash the wall of beaker with water, add 0.5 ml of hydrogen peroxide, and heat gently for 10 minutes. Cool and dilute exactly to 100 ml with water. Take 20 ml of this solution, add 5 ml of diluted nitric acid (1 in 3) and exactly 1 ml of 2% (w/v) silver nitrate TS, and allow to stand for 15 minutes. The turbidity of this solu-

tion is not darker than a solution prepared by adding 100 ml of water to 1 ml of Chloride Ion Standard Stock Solution and proceeding in the same manner as for the test solution.

(2) Sulfate Not more than 0.010% as SO_4. To 1.0 g of Periodic Acid Dihydrate, add 20 ml of water, 0.2 ml of 10% (w/v) sodium carbonate solution, and 10 ml of diluted hydrochloric acid (2 in 3), and evaporate on a water bath to dryness. To the residue, add 10 ml of water and 5 ml of diluted hydrochloric acid (2 in 3), and evaporate on a water bath to dryness. Repeat this procedure until the color of iodine disappears completely. Add 0.6 ml of diluted hydrochloric acid (2 in 3) and water to make exactly 50 ml. Measure exactly 25 ml of this solution, add 3 ml of ethanol and 2 ml of 10% (w/v) barium chloride solution, and allow to stand for 1 hour. The turbidity of this solution is not darker than a control solution prepared as directed below: To 0.1 ml of 10% (w/v) sodium carbonate solution, add 8 ml of hydrochloric acid (2 in 3), and evaporate on a water bath to dryness. To the residue, add 0.3 ml of hydrochloric acid (2 in 3) and exactly 0.5 ml of Sulfate Ion Standard Stock Solution, then water to dilute exactly to 25 ml. Add 3 ml of ethanol and 2 ml of 10% (w/v) barium chloride solution, and allow to stand for 1 hour.

Assay Dissolve about 1 g of Periodic Acid Dihydrate in water to make 250 ml. Place 25 ml of this solution into an iodine vial, and add 5 ml of sulfuric acid (1 in 6), 30 ml of water, and 3 g of potassium iodide. Immediately stopper tightly, and allow to stand in a dark place for 15 minutes. Titrate the liberated iodine with 0.1 mol/L sodium thiosulfate, using starch TS as the indicator. Separately, perform a blank test.

Each ml of 0.1 mol/L sodium thiosulfate = 2.8493 mg of $HIO_4 \cdot 2H_2O$

Peroxidase A red-brown powder. It is obtained from hose radish. One unit is equivalent to the amount of enzyme required to produce 1 μmol of water from hydrogen peroxide as a substrate in 1 minute at 25°C and pH 7.0.

Petroleum Benzine [K8594]

Petroleum Ether [K8593]

Petroleum Ether for Vitamin A Determination A fraction obtained by distillation of petroleum ether at 40.0–60.0°C.

***o*-Phenanthroline** See 1,10-Phenanthroline Monohydrate.

1,10-Phenanthroline Monohydrate $C_{12}H_8N_2 \cdot H_2O$ [K8789]

***o*-Phenanthroline TS** Weigh 0.15 g of *o*-phenanthroline, and dissolve in 10 ml of freshly prepared iron(II) sulfate solution (37 in 2,500). Prepare fresh before use.

Phenol C_6H_5OH [K8798]

Phenol Red $C_{19}H_{14}O_5S$ [K8800]

Phenol Red TS Weigh 0.1 g of phenol red, and dissolve in 100 ml of ethanol. Filter if necessary.

Phenol Red TS, Dilute

Solution 1 To 0.033 g of phenol red, add 1.5 ml of sodium hydroxide solution (2 in 25) and water to make 100 ml.

Solution 2 Dissolve 0.025 g of ammonium sulfate in 235 ml of water, add 105 ml of sodium hydroxide solution (2 in 25) and 135 ml of acetic acid (3 in 25), and mix.

Mix 1 volume of Solution 1 and 19 volumes of Solution 2. If necessary, adjust its pH to 4.7 with the sodium hydroxide solution or acetic acid.

Phenolphthalein $C_{20}H_{14}O_4$ [K8799]

Phenolphthalein TS Weigh 1 g of phenolphthalein, and dissolve in 100 ml of ethanol.

Phenol–Sodium Pentacyanonitrosylferrate(III) TS Dissolve 5 g of phenol and 0.025 g of sodium pentacyanonitrosylferrate(III) dihydrate in water to make 500 ml. Store in a cold, dark place.

L-Phenylalanine $C_9H_{11}NO_2$ "L-Phenylalanine"

***p*-Phenylenediamine Hydrochloride** $C_6H_4(NH_2)_2 \cdot 2HCl$
A white to light yellow or white to light red crystalline powder. Freely soluble in water.

Clarity of solution Clear (1.0 g, water 10 ml).

Molecular absorption coefficient Weigh 0.060 g of *p*-Phenylenediamine Hydrochloride, and dissolve in 100 ml of water. Measure 1.0 ml of the solution, and add phosphate buffer (pH 7) to make 50 ml. Determine the absorbance of this solution at a wavelength of 237–241 nm, using phosphate buffer (pH 7) as the reference solution. The molecular absorption coefficient is not less than 8,000.

Phenylhydrazine $C_6H_5NHN_2$ [K8795:1980] A transparent, colorless to light yellow liquid having a faint aroma.

Congealing point 18–20°C.

Phenylhydrazine Hydrochloride See Phenylhydrazinium Chloride.

Phenylhydrazine Hydrochloride–Sodium Acetate TS
Weigh 0.5 g of phenylhydrazine hydrochloride, and dissolve in 10 ml of sodium acetate solution (2 in 15). Filter if necessary. Prepare fresh before use.

Phenylhydrazinium Chloride $C_6H_5NHNH_2 \cdot HCl$ [K8203]

1-Phenyl-3-methyl-5-pyrazolone See 3-Methyl-1-phenyl-5-pyrazolone.

25% Phenyl Methyl Silicone Polymer Use a high quality product manufactured for gas chromatography.

***p*-Phenylphenol** $C_6H_5C_6H_4OH$ White crystals having a sublimation property. Soluble in ethanol, in diethyl ether, and in chloroform. Slightly soluble in petroleum ether.

Melting point 163–167°C.

Water content Not more than 0.2%.

Residue on ignition Not more than 0.20%.

***p*-Phenylphenol TS** Weigh 0.75 g of *p*-phenylphenol, and dissolve in 50 ml of sodium hydroxide solution (1 in 25). Filter if necessary. Prepare fresh before use.

Phosphate Buffer (pH3.3) Dissolve 12 g of monosodium phosphate in water to make 1,000 ml of a solution. Add phosphoric acid to adjust the pH to 3.3.

Phosphate Buffer (pH 6.2)

Solution 1 Dissolve 9.08 g of monopotassium phosphate in water to make 1,000 ml.

Solution 2 Dissolve 9.46 g of anhydrous disodium phosphate in water to make 1,000 ml.

Mix 800 ml of Solution 1 and 200 ml of Solution 2, and adjust the pH to 6.2 with either solution if necessary.

Phosphate Buffer (pH 6.8) Weigh 3.40 g of monopotassium phosphate and 3.55 g of anhydrous disodium phosphate, mix , and dissolve in water to make 1,000 ml.

Phosphate Buffer (pH 7)

Solution 1 Weigh 27.218 g of monopotassium phosphate for pH determination, and dissolve in water to make 1,000 ml.

Solution 2 Use 0.2 mol/L sodium hydroxide.

Mix 50.0 ml of Solution 1 and 29.54 ml of Solution 2, and add water to make 200 ml.

Phosphate Buffer (pH 7.1)

Solution 1 Dissolve 21.2 g of disodium phosphate in water to make 1,000 ml.

Solution 2 Dissolve 8.2 g of monopotassium phosphate in water to make 1,000 ml.

Mix 2 volumes of Solution 1 and 1 volume of Solution 2, and adjust the pH to 7.1 using either solution.

Phosphate Buffer (pH 7.4)

Solution 1 Weigh 6.80 g of monopotassium phosphate for pH determination, and dissolve in water to make 500 ml.

Solution 2 Use 0.2 mol/lmol/L sodium hydroxide.

Mix 50.0 ml of Solution 1 and 19.75 ml of Solution 2, and add water to make 100 ml.

Phosphate Buffer (pH 7.5)

Solution 1 Weigh 53.7 g of disodium phosphate, and dissolve in water to make 1,000 ml.

Solution 2 Weigh 20.4 g of monopotassium phosphate, and dissolve in water to make 1,000 ml.

Mix 21 volumes of Solution 1 and 4 volumes of Solution 2, and adjust the pH to 7.5, using either solution 1 or 2.

Phosphate Buffer (pH 7.6)

Solution 1 Weigh 4.54 g of monopotassium phosphate, and dissolve in water to make 500 ml.

Solution 2 Weigh 4.73 g of anhydrous disodium phosphate, and dissolve in water to make 500 ml.

Mix 13 volumes of Solution 1 and 87 volumes of Solution 2, and adjust the pH to 7.6, using either solution 1 or 2.

Phosphate Buffer (pH 8)

Solution 1 Weigh 23.88 g of anhydrous disodium phosphate, and dissolve in water to make 1,000 ml.

Solution 2 Weigh 9.07 g of monopotassium phosphate, and dissolve in water to make 1,000 ml.

Mix 50 parts of Solution 1 and 7 parts of Solution 2 by volume, and adjust the pH to 8 using both solutions.

Phosphomolybdic Acid $H_3(PMo_{12}O_{40})\cdot nH_2O$ [Dodecamolybdete(VI) Phosphate *n*-Hydrate, K9026:1991] Yellow crystals or crystalline powder.

Purity (1) Sulfates Not more than 0.005% as SO_4. Weigh exactly 3.0 g Phosphomolybdic Acid, add 1.5 m of diluted hydrochloric acid (2 in 3) and water to dissolve, and make 60 ml. Refer to this as solution A. Prepare a test solution: To 20 ml of solution A, add 3 ml of ethanol (95), 2 ml of barium chloride solution (1 in 10), then water to make 30 ml. Allow to stand for 1 hour. Prepare a control solution: To 20 ml of solution A, add 2 ml of barium chloride solution (1 in 10), and heat until it boils. Allow to stand for 1 hour, and filter through a filter paper for quantitative analysis (5C). To the filtrate, add 3 ml of ethanol (95). Then add 0.5 ml of Sulfate Ion Standard Stock Solution and water to make 30 ml. Compare both solutions. The test solution is not more turbid than the control solution.

(2) Calcium Not more than 0.02 %. Prepare a test solution by dissolving 1.0 g of Phosphomolybdic Acid in water to make 100 ml. Prepare a control solution by dissolving 1.0 g of Phosphomolybdic Acid in 50 ml of water and adding 1 ml of Calcium Standard Solution (0.1 mg/ml) and water to make 100 ml. Measure the absorbance of both solutions directed under Atomic Absorption Spectrophotometry. The absorbance of the test solution does not exceed the difference of the absorbances of both solutions.

Operating conditions

Light source: Calcium hollow cathode lamp.

Wavelength: 422.7 nm.

Supporting gas: Nitrous oxide.

Combustible gas: Acetylene.

Phosphoric Acid H_3PO_4 [K9005]

Phosphorous(V) Oxide P_2O_5 [K8342]

Phosphorus Trichloride PCl_3 [K8404:1962] A colorless, transparent liquid having a pungent odor. Fumes in air.

Distillation Not less than 95% (vol) is distilled at 75–78°C.

Phosphorylated Cellulose Cation Exchanger (–O–PO_3H_2 Type), Strongly Acidic Use a strongly acidic cation exchanger prepared by introducing phosphoric groups into porous cellulose.

***o*-Phthalaldehyde** $C_6H_4(CHO)_2$ Light yellow to yellow crystals.

Purity Related substances Prepare a test solution by dissolving 1 g of *o*-Phthalaldehyde in 10 ml of ethanol. Prepare a control solution by diluting exactly 1 ml of the test solution to exactly 100 ml with ethanol. Analyze 10 µl each of the test solution and the control solution by gas chromatography using the operating conditions given below. Continue the chromatography for seven times the retention time of the main peak, and measure the peak areas. Exclude the solvent peaks from measurement. The total area of all peaks of the test solution, other than the main peak, is not greater than the area of the main peak of control solution.

Operating conditions

Detector: Thermal conductivity detector.

Column: A glass tube of 3 mm internal diameter and 2 m length.

Column packing material

Liquid phase: 10% methyl silicone polymer of the amount of solid support.

Solid phase: 177- to 250-μm diatomaceous earth for gas chromatography, treated with acid and silane.

Column temperature: A constant temperature at about 180°C.

Carrier gas: Helium.

Flow rate: Adjust so that the retention time of *o*-phthalaldehyde is about 3–4 minutes at a constant rate about 50 ml/minute.

Phthalaldehyde TS Dissolve 0.040 g of *o*-phthalaldehyde in 1 ml of methanol, add 1 ml of sodium borate (1 in 50) and 0.05 ml of 2-melcaptoethanol, and mix. Store in a tightly stoppered, light-resistant bottle. Use within 1 week of the preparation.

Phthalic Acid $C_8H_6O_4$ A white crystalline powder. Freely soluble in methanol but slightly soluble in water and in diethyl ether.

Content Not less than 99.0% of phthalic acid ($C_8H_6O_4$).

Purity Other Aromatic Compounds Weigh 0.0100 g of Phthalic Acid, dissolve in 30 ml of methanol, and add diluted acetic acid (1 in 100) to make exactly 100 ml. To 10.0 ml of this solution, add a 7:3 mixture of diluted acetic acid (1 in 100)/methanol to make exactly 100 ml. Analyze the resulting solution by liquid chromatography using the operating conditions specified in Purity (6) for Benzoic Acid in the Monographs. Only one peak of phthalic acid is observed.

Assay Weigh accurately about 2 g of Phthalic Acid, dissolve in 50 ml of neutralized ethanol, and titrate with 0.1 mol/L sodium hydroxide (indicator: a few drops of phenolphthalein TS).

Each ml of 0.1 mol/L sodium hydroxide = 8.307 mg of $C_8H_6O_4$

Phthalic Anhydride $C_6H_4(CO)_2O$ [K8887]

Phytonadione $C_{31}H_{46}O_2$ Use phytonadione specified in the Japanese Pharmacopoeia.

Picric Acid See 2,4,6-Trinitrophenol.

Polyethylene Glycol 20M Use high-quality polyethylene glycol 20M produced for gas chromatography.

Polyethylene Glycol 600 A product with the average molecular weight of 560–640.

Description A colorless to pale yellow, clear liquid, or white lumps.

Identification Dissolve 0.05 g of Polyethylene Glycol 600 in 5 ml of dilute hydrochloric acid, add 1 ml of barium chloride solution (12 in 100), and mix. Filter the mixture if necessary. Add 1 ml of phosphomolybdic acid solution (1 in 10). A yellow-green precipitate is produced.

Purity (1) pH 4.0–7.0 (5 g, water 100 ml, 25°C).

(2) Viscosity (25°C) 100–150 mm^2/s. Measure the viscosity with 200 ml of the sample using a rotational viscometer.

(3) Congealing point 15–25°C.

(4) Acid Not more than 0.1% as CH_3COOH. Dissolve 10 g of Polyethylene Glycol 600 in 50 ml of carbon oxide-free water, and add 3 drops of phenolphthalein solution. Titrate with 0.1 mol/L sodium hydroxide. One ml of 0.1 mol/L sodium hydroxide is equivalent to 0.006005 g of CH_3COOH.

Water Not more than 0.3% (2 g, Direct Titration).

Average molecular weight 560–640. Place 42 g of phthalic anhydride into a 1-L light resistant stoppered bottle containing 300 ml of newly distilled pyridine, mix vigorously to dissolve, and allow to stand at least 16 hours. Measure exactly 25 ml of the obtained solution into a 200-ml pressure-resistant bottle with a stopper, add about 2.4 g of Polyethylene Glycol 600, accurately weighed, and stopper tightly. Wrap the bottle with strong cloth, and warm in a 98± 2°C water bath for 30 minutes. Maintain the bottle so that its contents are under the water. Take it out of the bath, and cool to room temperature in air. Add exactly 50 ml of 0.5 mol/L sodium hydroxide, then add 5 drops of phenolphthalein solution in pyridine (1 in 100), and titrate with 0.5 mol/L sodium hydroxide. The endpoint is when the solution remains light red for 15 seconds. Perform a blank test in the same manner as for the test solution.

$$\text{Average molecular weight} = \text{Weight (g) of the sample} \times 4{,}000/(a-b)$$

a: the volume (ml) of 0.5 mol/L sodium hydroxide consumed in the blank test,

b: the volume (ml) of 0.5 mol/L sodium hydroxide consumed in the test.

Polyethylene Glycol 6,000 Use a product produced exclusively for gas chromatography.

ε-Polylysine Hydrochloride for Assay A white to light yellow powder.

Identification Add 1 ml of methyl orange TS to a solution of 0.1 g of ε-Polylysine Hydrochloride for Assay in 100 ml of phosphate buffer (pH 6.8). A red-brown precipitate is formed.

Purity Related substances Prepare a test solution by dissolving 0.015 g of ε-Polylysine Hydrochloride for Assay in 100 ml of the same mobile phase used in the Assay for ε-Polylysine in the Monographs. Prepare a control solution by diluting 2 ml of the test solution, measured exactly, to exactly 100 ml with the mobile phase. Analyze 100 μl each of the test solution and the control solution by liquid chromatography using the operating conditions given below. Continue the chromatography for about two times the retention time of the main peak, and measure the peak areas. The total area of all peaks of the test solution, other than the main peak, is not greater than the area of the main peak of the control solution.

Operating conditions

Use the conditions specified in the Assay for ε-Polylysine in the Monographs.

Polyoxyethylene(23) Lauryl Ether Use Lauromacrogol specified in the Japanese Pharmacopoeia.

Polysorbate 20 Polysorbate 20 is mainly obtained by addition polymerization of ethylene oxide to sorbitan monolaurate. A pale yellow to yellow liquid having a slight characteristic odor.

Identification (1) To 0.5 g of Polysorbate 20, add 10 ml of water and 10 ml of sodium hydroxide TS, and boil for 5 minutes. Acidify with dilute hydrochloric acid, and oily materials are separated.

(2) Weigh 5 g of Polysorbate 20, and saponify with 50 ml of ethanolic potassium hydroxide TS as directed under the Fats and Related Substances Tests, and evaporate the ethanol completely. Add 50 ml of water, and dissolve the residue. Acidify with hydrochloric acid (indicator: methyl orange), extract twice with 30-ml portions of diethyl ether. Combine the diethyl ether layers, and wash repeatedly with 20-ml portions of water until the washing becomes neutral. Evaporate diethyl ether on the water bath. The acid value of the residue is 275–285. For saponification, use 50 ml of ethanolic potassium hydroxide TS.

Acid value Not more than 4.0.

Saponification value 43–55.

Loss on drying Not more than 3.0% (5 g, 105°C, 1 hour).

Residue on ignition Not more than 1.0%. Weigh accurately 3 g of Polysorbate 20, heat gently at first, then gradually ignite (800–1,200°C), and incinerate completely. If carbonized material remains, add hot water and leach. Filter through a filter paper for quantitative analysis (No. 5C). Ignite the residue together with the filter paper. To the residue, add filtrate, and evaporate to dryness. Ignite carefully until the carbonized material disappears. When carbonized material still remains, add 15 ml of ethanol, crush the carbonized materials with a glass rod, burn ethanol, and then re-ignite carefully. Allow to cool in a desiccator containing silica gel, and weigh accurately.

Polysorbate 80 Use polysorbate 80 specified in the Japanese Pharmacopoeia.

Porous Anion Exchanger Use a product made for ion chromatography.

Potassium Acetate CH_3COOK [K8363]

Potassium Aluminum Sulfate Dodecahydrate $AlK(SO_4)_2 \cdot 12H_2O$ [Potassium Aluminum Sulfate Dodecahydrate, K8255]

Potassium Benzylpenicillin $C_{16}H_{17}KN_2O_4S$ Use Potassium benzylpenicillin specified in the Japanese Pharmacopoeia.

Potassium Bichromate See Potassium Dichromate.

Potassium Bichromate (Standard Reagent) See Potassium Dichromate (Standard Reagent).

Potassium Bromate $KBrO_3$ [K8530]

Potassium Bromate–Potassium Bromide TS Weigh 1.4 g of potassium bromate and 8.1 g of potassium bromide, mix them, add water to dissolve, and make 100 ml.

Potassium Bromide KBr [K8506]

Potassium Bromide for Infrared Absorption Spectrophotometry A powder prepared in the following manner: Crush potassium bromide single crystals or potassium bromide, and pass through a 74-μm standard sieve. Dry the resulting powder at 120°C for 10 hours or at 500°C for 5 hours. The infrared spectrum of a disk formed with this powder shows no characteristic absorption.

Potassium Carbonate, Anhydrous K_2CO_3 [Potassium Carbonate, K8615]

Potassium Chlorate $KClO_3$ [K8207]

Potassium Chloride KCl [K8121]

Potassium Chloride–Hydrochloric Acid TS Weigh 250 g of potassium chloride, and add 8.5 ml of hydrochloric acid and 750 ml of water to dissolve it.

Potassium Chromate K_2CrO_4 [K8312]

Potassium Cyanide KCN [K8443]

Potassium Dichromate $K_2Cr_2O_7$ [K8517]

Potassium Dichromate (Standard Reagent) $K_2Cr_2O_7$ [Reference material for volumetric analysis, K8005]

Potassium Ferricyanide
See Potassium Hexacyanoferrate(III).

Potassium Ferrocyanide See Potassium Hexacyanoferrate(II).

Potassium Hexacyanoferrate(II)
See Potassium Hexacyanoferrate(II) Trihydrate.

Potassium Hexacyanoferrate(III) $K_3[Fe(CN)_6]$ [K8801]

Potassium Hexacyanoferrate(II) Trihydrate $K_4Fe(CN)_6 \cdot 3H_2O$ [K8802]

Potassium Hexahydroxoantimonate(V) $K[Sb(OH)_6]$ [K8778:1980] White granules or crystalline powder.

Identification (1) A solution of Hexahydroxoantimonate(V) (1 in 100) gives a purple flame.

(2) To 20 ml of solution prepared in Identification (1), add 10% potassium chloride solution. No precipitate is formed within 15 minutes.

(3) To 20 ml of solution prepared in Identification (1), add a few drops of ammonia solution and 10 ml of 10% ammonium chloride. No precipitate is formed within 15 minutes.

Potassium Hydrogen Phthalate $C_6H_4(COOK)(COOH)$ [K8809]

Potassium Hydrogen Phthalate for pH Determination $C_6H_4(COOK)(COOH)$ [For pH Standard Solution, K8809]

Potassium Hydrogen Pyroantimonate
See Potassium Hexahydroxoantimonate(V).

Potassium Hydrogen Pyroantimonate TS Weigh 2 g of potassium hydrogen pyroantimonate, add 100 ml of water, boil for about 5 minutes, and cool quickly. Add 10 ml of potassium hydroxide solution (3 in 20), allow to stand for 24 hours, and filter.

Potassium Hydrogen Sulfate $KHSO_4$ [K8972]

Potassium Hydroxide KOH [K8574]

Potassium Hydroxide TS, Ethanolic Weigh 35 g of potassium hydroxide, dissolve in 20 ml of water, and add ethanol to make 1,000 ml. Stopper tightly, and store.

10% Potassium Hydroxide TS, Ethanolic Weigh 10 g of potassium hydroxide, and dissolve in ethanol to make 100 ml. Prepare fresh before use.

35% Potassium Hydroxide TS, Methanolic Weigh 35 g of potassium hydroxide, dissolve in 25 ml of water, and add methanol to make 100 ml.

Potassium Iodate (Standard Reagent) KIO_3 [Standard Material for Volumetric Analysis, K8005]

Potassium Iodate TS Weigh 7.1 g of potassium iodate (standard reagent), and dissolve in water to make 1,000 ml. Store protected from light.

Potassium Iodide KI [Potassium Iodide, K8913]

Potassium Iodide TS Weigh 16.5 g of potassium iodide, and dissolve in water to make 100 ml. Store protected from light.

Potassium Iodide–Starch Paper Immerse a piece of filter paper in potassium iodide–starch TS, freshly prepared, and dry the filter paper in a clean room. Store in a glass-stoppered bottle, protected from light and moisture.

Potassium Iodide–Starch TS Weigh 0.5 g of starch, and dissolve in 50 to 60 ml of water by heating. Add 0.5 g of potassium iodide and water to dissolve, and dilute with water to make 100 ml.

Potassium Nitrate KNO_3 [K8548]

Potassium Periodate KIO_4 [K8249]

Potassium Permanganate $KMnO_4$ [K8247]

Potassium Sodium Tartrate See Potassium Sodium Tartrate Tetrahydrate.

Potassium Sodium Tartrate Tetrahydrate $NaOOCCH(OH)CH(OH)COOK \cdot 4H_2O$ [Potassium Sodium (+)-Tartrate Tetrahydrate, K8536]

Potassium Sulfate K_2SO_4 [K8962]

Potassium Tetraoxalate for pH Determination See Potassium Trihydrogen Dioxalate Dihydrate for pH Determination.

Potassium Thiocyanate KSCN [K9001]

Potassium Trihydrogen Dioxalate Dihydrate for pH Determination $KH_3(C_2O_4)_2 \cdot 2H_2O$ [K8474]

Potato Extract Use potato extract prepared for the microbial limit tests.

Powdered Cattle Bile Use a product produced for the microbial limit tests.

Propanol See 1-Propanol.

1-Propanol $CH_3CH_2CH_2OH$ [K8838]

2-Propanol $(CH_3)_2CHOH$ [K8839]

2-Propanol for Vitamin A Determination Determine the absorbance of 2-Propanol for Vitamin A Determination, using re-distilled water as the reference. It is not more than 0.01 at 320–350 nm and not more than 0.05 at 300 nm.

Propionic Acid C_2H_5COOH "Propionic Acid"

Propyl Alcohol, Iso See 2-Propanol.

Propyl Alcohol, Iso, for Vitamin A Determination See 2-Propanol for Vitamin A Determination.

Propylene Carbonate $C_4H_6O_3$ A colorless liquid.
Boiling point 240–242°C.
Water Not more than 1 mg/g.

Propylene Chlorohydrin $CH_3CH(OH)CH_2Cl$ A colorless to pale yellow liquid. Soluble in water, in ethanol, and in diethyl ether.
Content 70% of propylene chlorohydrin and 30% of 2-chloro-1-propanol.
Refractive index n_D^{20}: 1.4390–1.4410.
Specific gravity d_4^{20}: 1.111–1.115.
Boiling point 126–127°C.

Propylene Glycol $CH_3CH(OH)CH_2OH$ [K8837]

Pullulanase An enzyme (pullulan 6-glucanohydrolase, EC 3.2.1.41). It decomposes pullulan, which is obtained from the culture of bacteria (*Bacillus, Klebsiella, Sulfolobus solfataricus*). It hydrolyzes α-1,6-glucosidic linkages, producing maltotrioses.
Active Unit One unit of Pullulanase is equivalent to the amount of enzyme required to liberate 1 μmol of maltotriose from pullulan in 1 minute at pH 5.0 and 30°C.

Pullulanase TS Dissolve pullulanase in water so that its activity is 10 units per ml.

Purified Hydrochloric Acid See Hydrochloric Acid, Purified.

Purified Water Use purified water specified in the Japanese Pharmacopoeia.

Purified Water for Ion Chromatography Distilled purified water with electric conductivity not more than 1 μs/cm.

Pyridine C_5H_5N [K 8777]

Pyridine, Dehydrated C_5H_5N Measure 100 ml of pyridine, add 10 g of potassium hydroxide, and allow to stand for 24 hours. Collect the supernatant by decantation, and distill.

Pyridine for Water Determination C_5H_5N Use pyridine

containing not more than 0.1% (w/v) of water. Otherwise, use pyridine prepared in the following manner: To pyridine, add potassium hydroxide or barium oxide. Stopper tightly, and allow to stand for a couple of days. Distill the mixture, protecting from exposure to moisture. Store protected from moisture.

Pyridine–Pyrazolone TS Weigh 0.20 g of 1-phenyl-3-methyl-5-pyrazolone, add 100 ml of water of about 75°C, dissolve by shaking, and cool to room temperature (it does not have to be dissolved completely). Mix the obtained solution with a solution prepared by dissolving 0.020 g of bis(1-phenyl-3-methyl-5-pyrazolone) in 20 ml of pyridine.

Pyridine–Sodium Hydroxide TS Dissolve 1.2 g of sodium hydroxide in 200 ml of water, add 100 ml of pyridine, and mix.

1-(2-Pyridylazo)-2-naphthol $C_{15}H_{11}N_3O$ An orange-yellow or orange-red powder.

Absorbance Weigh 0.025 g of 1-(2-Pyridylazo)-2-naphthol, and dissolve in methanol to make exactly 100 ml. To 2.0 ml of this solution, add methanol to make exactly 50 ml. Measure the absorbance of this solution as directed under Ultraviolet-Visible Spectrophotometry. The absorbance at 470 nm is not less than 0.55.

Melting point 137–140°C.

Purity Clarity and color of the solution Dissolve 0.025 g of 1-(2-Pyridylazo)-2-naphthol in 100 ml of methanol. The solution is clear, orange-yellow.

Residue on ignition Not more than 1.0%.

Sensitivity To 0.2 ml of a methanol solution of 1-(2-Pyridylazo)-2-naphthol (1 in 4,000), add 50 ml of water, 30 ml of methanol, and 10 ml of acetate buffer. A yellow color develops. When 1 drop of copper(II) chloride dihydrate solution (1 in 600) is added, the solution turns red-purple. When 1 drop of diluted disodium ethylenediaminetetraacetate TS (1 in 10) is further added, the color returns to yellow.

Pyrogallol $C_6H_3(OH)_3$ [K8780]

Pyrogallol Solution, Alkaline Transfer 4.5 g of pyrogallol in a gas washing bottle, and purge air by blowing nitrogen into the bottle for 2 or 3 minutes. Add a solution prepared by dissolving 65 g of potassium hydroxide in 85 ml of water into the bottle. Purge the air completely from the bottle with nitrogen in the same manner.

Pyrrole C_4H_4NH [K8787:1961] A colorless, transparent liquid having a characteristic odor. Gradually turns brown in air.

Identification Dissolve 0.5 g of Pyrrole in 50% (vol) ethanol, and add 1 ml of sodium nitroprusside TS and 5 ml of sodium hydroxide solution (1 in 20). The solution turns green-yellow to green gradually. When boiled and made acidic with acetic acid, it turns blue.

Specific gravity 0.965–0.975.

Quinaldine Red $C_{21}H_{23}IN_2$ A crystalline powder, and soluble in ethanol. Its solution in methanol (0.005 in 1,000) exhibits absorption maximum at a wavelength of about 526 nm. The absorbance is not less than 0.5 at the maximum absorption wavelength.

Quinaldine Red TS Weigh 0.1 g of quinaldine red, and dissolve in 100 ml of acetic acid. Prepare fresh before use.

Quinoline C_9H_7O [K8279]

Rebaudioside A $C_{44}H_{70}O_{23}$ White crystals or crystalline powder.

Specific rotation $[\alpha]_D^{20}$: −20 to −24°. Prepare a test solution by dissolving 0.05 g of Rebaudioside A, previously dried at 110°C for 2 hours, in 50 ml of methanol. Measure the specific rotation of the resultant solution.

Melting point 239–244°C.

Redistilled Water Distill distilled water using a hard-glass distillation apparatus.

Red Litmus Paper See Litmus Paper, Red.

Red Phosphorus P [K8595:1961] An odorless dark red powder.

Content Not less than 98.0% of phosphorus (P).

Purity Free phosphoric acid Not more than 0.5% as H_3PO_4. Weigh accurately about 5 g of Red Phosphorus, add 10 ml of 20% sodium chloride solution, and stir well. Add 50 ml of 20% sodium chloride solution, allow to stand for 1 hour, and filter. Wash the residue on the filter paper three times with three 10-ml portions of 20% sodium chloride solution. Combine the filtrate and washings, and titrate with 0.1 mol/L sodium hydroxide. Use thymol blue TS as the indicator.

0.1 mol/L sodium hydroxide = 4.900 g H_3PO_4

Assay Weigh accurately about 0.5 g of Red Phosphorus, add 30 ml of nitric acid saturated with bromine, and allow to stand for 1 hour. Heat on a water bath until the color of bromine disappears, and cool. Add 1 g of potassium chlorate and 10 ml of hydrochloric acid, and allow to stand for 10 minutes. Heat gradually on a water bath until the solution is evaporated to about 5 ml, and add 200 ml of water. Heat for a few minutes, cool, and filter. Wash the residue on a filter paper with water, and combine the filtrate and washings. Dilute it to exactly 500 ml with water. To 25 ml of the prepared solution, exactly measured, add 0.5 g of citric acid, neutralize with ammonia solution using bromothymol blue TS as the indicator. To this solution, add 10 ml of magnesia TS gradually while stirring. Add diluted ammonia solution (1 in 10) dropwise until a precipitate is completely formed. Add about a one-tenth volume ammonia solution of the total volume, stir, and allow to stand for 3 hours. Filter it, and wash the precipitate on the filter paper with diluted ammonia solution (1 in 10). Ignite the residue, cool, and weigh accurately.

$$\text{Content (\%) of P} = \frac{\left(\text{Weight (g) of precipitate } (Mg_2P_2O_7) \times 0.2783 \times 20\right)}{\text{Weight (g) of the sample}} \times 100 - \text{Amount (\%) of free phosphoric acid} \times 0.3161$$

Resorcin See Resorcinol.

Resorcinol $C_6H_4(OH)_2$ [K9032]

D-Ribose for Assay $C_5H_{10}O_5$ White crystals or crystalline

powder.

Identification To 5 ml of boiling Fehling's TS, add 2–3 drops of a solution of D-Ribose for Assay (1 in 20). A red precipitation is produced.

Purity (1) Specific rotation $[\alpha]_D^{20}$: −18 to −22°. Weigh accurately about 1 g of D-Ribose for Assay, and add 0.2 ml of ammonia TS and water to dissolve, and make exact 50 ml. Measure the angular rotation of this solution, and calculate on the anhydrous basis.

(2) Related substances Prepare a test solution by dissolving about 0.5 g of D-Ribose for Assay in 25 ml of water. Prepare a control solution by diluting 1 ml of test solution, exactly measured, to exactly 100 ml with water. Analyze 10 µl each of the test solution and the control solution by liquid chromatography using the operating conditions given below. Continue the chromatography for two times the retention time of the main peak, and measure the peak areas. The total area of all peaks of the test solution, other than the main peak, is not greater than the area of the main peak of the control solution.

Operating conditions

Follow the operating conditions for the Assay of D-Ribose in the Monographs.

Water Not more than 1.0% (1 g, Direct Titration).

Rutin for Assay $C_{27}H_{30}O_{16}\cdot 3H_2O$ A light yellow to light yellow-green crystalline powder.

Identification Measure the absorption spectrum of Rutin for Assay as directed in the Potassium Bromide Disk Method under Infrared Spectrophotometry. It exhibits absorption bands at about 1655 cm^{-1}, 1605 cm^{-1}, 1505 cm^{-1}, 1360 cm^{-1}, 1300 cm^{-1}, 1200 cm^{-1}, and 810 cm^{-1}.

Purity (1) Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength near 350 nm): Not less than 290. Weigh accurately about 0.05 g of Rutin for Assay, previous dried at 135°C for 2 hours, and dissolve in methanol to make 100 ml. Measure exactly 2 ml of this solution, and add methanol to make exactly 100 ml. Measure the absorbance of the resultant solution in accordance with Ultraviolet-Visible Spectrophotometry.

(2) Related substance Prepare a test solution. Dissolve about 0.05 g of Rutin for Assay in 25 ml of methanol, then measure exactly 5 ml of the resultant solution, and add an 800:200:1 mixture of water/acetonitrile/phosphoric acid to make exactly 50 ml. Then prepare a control solution. Add 5 ml of methanol to 1 ml of the test solution, measured exactly, and then add an 800:200:1 mixture of water/acetonitrile/phosphoric acid to make exactly 50 ml. Analyze 20 µl each of the test solution and the control solution by liquid chromatography, using the operating conditions given below. Continue the chromatography for two times the retention time of the main peak, and measure the peak areas. The total area of all peaks, other than the main peak of the test solution and the solvent peak, is not greater than the area of the main peak of the control solution.

Operating conditions

Detector: Ultraviolet spectrophotometer (determination wavelength: 254).

Column: A stainless steel tube of 3–6 mm internal diameter and 15–25 cm length.

Column packing material: 5- to 10-µm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 40°C.

Mobil phase: An 800:200:1 mixture of water/acetonitrile/phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of rutin is about 8–12 minutes.

Salicylaldehyde HOC_6H_4CHO [K8390]

Salicylic Acid HOC_6H_4COOH [K8392]

Salicylic Acid–Methanol TS Weigh 10 g of salicylic acid, and dissolve in 100 ml of methanol for water determination. Prepare fresh before use.

Sarsasapogenin for Assay ($C_{27}H_{44}O_3$) A white, odorless crystalline powder.

Identification Dissolve 5 mg of Sarsasapogenin for Assay in 5 ml of ethyl acetate. Analyze 2 µl of this solution by thin-layer chromatography using a 2:1 mixture of hexane/ethyl acetate as the developing solvent. Use a thin-layer plate for yucca form extract, previously dried at 110°C for 1 hour. Stop the development when the solvent front ascends to a point about 8 cm above the original line, and air-dry the plate. Spray with *p*-anisaldehyde–sulfuric acid TS. After heating at 110°C for 10 minutes, examine it. A major yellow-green to blue-green spot is observed at an R_f value of about 0.55.

Purity Related substances Prepare a test solution by dissolving 0.10 g of Sarsasapogenin for Assay in 10 ml of ethyl acetate. Prepare a control solution by adding ethyl acetate to 1 ml of the test solution, exactly measured, to make exactly 50 ml. Analyze 5 µl each of the test solution and the standard solution by thin-layer chromatography as directed under Identification above. Spots, other than the major spot, from the test solution are not darker in color than the main spot from the control solution.

Water Not more than 8.0% (0.1 g, Direct Titration).

Sea Sand [K8222]

Selenium Dioxide SeO_2 [K8706:1994] White crystals. Sublimates when heated.

Residue on ignition Not more than 0.05%.

Silica Gel Use JIS Silica Gel Desiccant A for Packaging.

Silica Gel for Gas Chromatography Use silica gel prepared for gas chromatography.

Silica Gel for Liquid Chromatography Use silica gel prepared for liquid chromatography.

Silica Gel for Thin-Layer Chromatography Use silica gel of high quality prepared for thin-layer chromatography.

Silica Gel for Thin-Layer Chromatography (Containing Fluorescent Indicator) Use silica gel prepared for thin-layer chromatography to which fluorescent indicator is added.

Silicone Oil A clear, colorless, odorless liquid.

Kinematic viscosity 50–100 mm^2/s.

Silicone Resin A light-gray, translucent, almost odorless, viscous liquid or pasty substance.

Refractive index and viscosity Transfer 15 g of Silicone Resin in a Soxhlet extractor, and extract for 3 hours with 150 ml of carbon tetrachloride. Evaporate the extract on a water bath. Kinematic viscosity of this liquid is 100–1,100 mm^2/s (at 25°C), and refractive index is 1.400–1.410 (at 25°C).

Specific gravity 0.98–1.02.

Loss on drying 0.45–2.25 g (100°C, 1 hour). Perform the test on the extraction residue obtained in the Refractive Index and Viscosity Tests.

Silver Diethyldithiocarbamate See Silver *N,N*-Diethyldithiocarbamate.

Silver *N,N*-Diethyldithiocarbamate $C_5H_{10}AgNS$ [K9512]

Silver Diethyldithiocarbamate–Quinoline TS Dissolve 0.05 g of silver nitrate, ground into a fine powder, in 100 ml of quinoline, and add 0.2 g of silver diethyldithiocarbamate. Prepare fresh before use.

Silver Nitrate $AgNO_3$ [K8550]

Silver Nitrate–Ammonia TS Weigh 1 g of silver nitrate, and dissolve in 20 ml of water. Add dropwise ammonia TS while stirring until the precipitate almost dissolves, and filter. Store it in a tightly-stoppered, light-resistant container.

Silver Sulfate Ag_2SO_4 [K8965]

Silylation TS To 3 ml of *N,O*-bis(trimethylsilyl)acetamide, add 2 ml of dimethylformamide to dissolve. Prepare fresh before use.

Skimmed Milk A powder produced by almost completely dehydrating raw milk or cow's milk from which milk fat is previously removed.

Soda Lime [exclusively for carbon dioxide absorption, K8603]

Sodium Acetate See Sodium Acetate Trihydrate.

Sodium Acetate, Anhydrous CH_3COONa [Sodium Acetate, K8372]

Sodium Acetate Trihydrate $CH_3COONa{\cdot}3H_2O$ [K8371]

Sodium Borate See Sodium Tetraborate Decahydrate.

Sodium Borate for pH Determination See Sodium Tetraborate Decahydrate for pH Determination.

Sodium Bromide NaBr [K8514]

Sodium Carbonate See Sodium Carbonate Decahydrate.

Sodium Carbonate (Standard Reagent) Na_2CO_3 [Reference material for volumetric analysis, K8005]

Sodium Carbonate, Anhydrous Na_2CO_3 [Sodium Carbonate, K8625]

Sodium Carbonate Decahydrate $Na_2CO_3{\cdot}10H_2O$ [K8624]

Sodium Carbonate for pH Determination Na_2CO_3 [Exclusively for pH Standard Solution, K8625]

Sodium Chloride NaCl [K8150]

Sodium Chloride (Standard Reagent) NaCl [Standard Material for Volumetric Analysis, K8005]

Sodium Citrate See Trisodium Citrate Dihydrate.

Sodium Cobaltinitrite See Sodium Hexanitrocobaltate(III).

Sodium Cobaltinitrite TS Weigh 30 g of sodium cobaltinitrite, and dissolve in water to make 100 ml. Prepare fresh before use.

Sodium De(s)oxycholate $C_{24}H_{39}NaO_4$ A white, odorless crystalline powder.

Identification Dry Sodium De(s)oxycholate, and proceed as directed under the Potassium Bromide Disk Method in Infrared Spectrometry. The absorption bands are observed at wavenumbers of about 3400 cm^{-1}, 2940 cm^{-1}, 1562 cm^{-1}, and 1408 cm^{-1}.

Purity Related substances Prepare a sample solution by dissolving 0.10 g of Sodium De(s)oxycholate in 10 ml of methanol. Prepare a standard solution by diluting 1 ml of the sample solution, measured exactly, with methanol to make exactly 100 ml. With the sample solution and the standard solution, proceed as directed under Thin-Layer Chromatography. Spot 10 µl each of these solutions on a thin-layer plate prepared with silica-gel for thin-layer chromatography. Develop until the solvent front ascends to a point about 10 cm above the original line, using an 80:40:1 mixture of 1-butanol/methanol/ acetic acid as the developing solvent. Air-dry the thin-layer plate, spray uniformly with sulfuric acid, and heat at 105°C for 10 minutes. The spots other than the main spot from the sample solution are not darker in color than the spot from the standard solution.

Sodium Diethyldithiocarbamate See Sodium *N,N*-Diethyldithiocarbamate Trihydrate.

Sodium *N,N*-Diethyldithiocarbamate Trihydrate $(C_2H_5)_2NCS_2Na{\cdot}3H_2O$ [K8454]

Sodium Dithionite $Na_2S_2O_4$ [K8737]

Sodium Fluoride NaF [K8821]

Sodium Formate HCOONa [K8267]

Sodium Hexanitrocobaltate(III) $Na_3[Co(NO_2)_6]$ [8347]

Sodium Hydrogen Carbonate $NaHCO_3$ [K8622]

Sodium Hydrogen Carbonate for pH Determination $NaHCO_3$ [Exclusively for pH Standard Solution, K8622]

Sodium Hydrogen Sulfate See Sodium Hydrogen Sulfate Monohydrate.

Sodium Hydrogen Sulfate Monohydrate $NaHSO_4{\cdot}H_2O$ [K8973:1992] White crystals or crystalline powder. A solu-

tion of Sodium Hydrogen Sulfate Monohydrate is acidic.

Content 98.0–102.0%.

Identification To 5 ml of a solution of Sodium Hydrogen Sulfate Monohydrate (1 in 10), add 1 ml of barium chloride solution (1 in 10). A white precipitate is formed.

Assay Weigh accurately about 4 g of Sodium Hydrogen Sulfate Monohydrate, and dissolve in 50 ml of freshly boiled and cooled water. Titrate with 1 mol/L sodium hydroxide (indicator: 1–2 drops of bromothymol blue).

Each ml of 1 mol/L sodium hydroxide = 138.1 mg $NaHSO_4 \cdot H_2O$

Sodium Hydrogen Sulfite $NaHSO_3$ [K8059]

Sodium Hydrogen Sulfite TS Weigh 10 g of sodium hydrogen sulfite, and dissolve in water to make 30 ml. Prepare fresh before use.

Sodium Hydrogen Tartrate See Sodium Hydrogen Tartrate Monohydrate.

Sodium Hydrogen Tartrate Monohydrate
$HOOCCH(OH)CH(OH)COONa \cdot H_2O$
[Sodium Hydrogen (+)-Tartrate Monohydrate, K8538]

Sodium Hydrosulfite See Sodium Dithionite.

Sodium Hydroxide NaOH [K8576]

Sodium Hydroxide TS Dissolve 4.3 g of sodium hydroxide in water to make 100 ml. Store in a polyethylene bottle.

0.5 mol/L Sodium Hydroxide TS See Sodium Hydroxide TS, 0.5 mol/L.

Sodium Hydroxide TS, 0.5 mol/L Dissolve 22 g of sodium hydroxide in water, and make 1,000 ml. Store in a polyethylene bottle.

Sodium Hydroxide TS, Dilute Dissolve 4.3 g of sodium hydroxide in freshly boiled and cooled water to make 1,000 ml. Prepare fresh before use (0.1 mol/L).

5% Sodium Hydroxide TS, Methanolic Dissolve 5 g of sodium hydroxide in 5 ml of water, and add methanol to make 100 ml. Leaving to stand, and use the supernatant for testing.

Sodium Hypochlorite NaClO "Sodium Hypochlorite"
Use a product with an available chlorine of not less than 5%.

Sodium Hypochlorite TS Use a solution of 5% effective chlorine.

Sodium Hypochlorite–Sodium Hydroxide TS To sodium hypochlorite TS equivalent to 1.05 g of sodium hypochlorite (NaClO = 74.44), add 15 g of sodium hydroxide and water to make 1,000 ml. Prepare fresh before use.

Sodium Iodide NaI [K8918:1994] A white, deliquescent crystalline powder.

Content Not less than 99.5% of sodium iodide (NaI) when dried.

Identification When burned in a colorless flame, a solution of Sodium Iodide (1 in 200) gives a yellow flame.

Loss on drying Not more than 0.5% (110°C, 2 hours).

Assay Weigh accurately about 0.5 g of Sodium Iodide, previously dried, into a stoppered 300-ml flask, dissolve in 25 ml of water, and cool below 5°C. Add 35 ml of hydrochloric acid and 5 ml of chloroform, each previously cooled below 5°C. Titrate with 0.05 mol/L potassium iodide with constant shaking until the color of iodine in the water layer disappears. Stopper tightly, and shake well. Then, shake vigorously after each drop of 0.05 mol/L potassium iodide is added. The end point is when the violet color of the chloroform phase completely disappears.

Each ml of 0.05 mol/L potassium iodide = 14.99 mg of NaI

Sodium Lauryl Sulfate Use sodium lauryl sulfate specified in the Japanese Pharmacopoeia.

Sodium Lauryl Sulfate–Propylene Glycol TS Weigh 1 g of sodium lauryl sulfate, dissolve in 80 ml of water, and mix it with 20 ml of propylene glycol.

Sodium Metaperiodate $NaIO_4$ [Sodium Periodate, K8256]

Sodium Metaperiodate TS Weigh 1.25 g of sodium metaperiodate, dissolve in water to make 100 ml.

Sodium Molybdate See Disodium Molybdate(VI) Dihydrate.

Sodium Nitrite $NaNO_2$ [K8019]

Sodium Nitroprusside See Sodium Pentacyanonitrosylferrate(III) Dihydrate.

Sodium Nitroprusside TS Weigh 1 g of sodium nitroprusside, and dissolve in water to make 20 ml. Prepare fresh before use.

Sodium Oxalate (Standard Reagent) NaOCOCOONa [Standard Material for Volumetric Analysis, K8005]

Sodium Pentacyanonitrosylferrate(III) Dihydrate
$Na_2[Fe(CN)_5NO] \cdot 2H_2O$ [K8722]

Sodium Periodate TS for Glycerol Weigh 6 g of sodium metaperiodate, and dissolve in a solution which was prepared by adding 12 ml of diluted sulfuric acid (3 in 1,000) to 38 ml of freshly boiled and cooled water. Add freshly boiled and cooled water to make 100 ml. Filter if necessary.

Sodium Sulfate See Sodium Sulfate Decahydrate.

Sodium Sulfate, Anhydrous Na_2SO_4 [Sodium Sulfate, K8987]

Sodium Sulfate Decahydrate $Na_2SO_4 \cdot 10H_2O$ [K8986]

Sodium Sulfide See Sodium Sulfide Nonahydrate.

Sodium Sulfide Nonahydrate $Na_2S \cdot 9H_2O$ [K8949]

Sodium Sulfide TS Weigh 5 g of sodium sulfide and dissolve in a mixture of 10 ml of water and 30 ml of glycerol. Or, weigh 5 g of sodium hydroxide, and dissolve in a mixture of 30 ml of water and 90 ml of glycerol. Take a half volume of the prepared solution, saturate with hydrogen sulfide while cooling, and mix the resulting saturated solution with the other half volume of the solution. Store in a small, almost filled, tightly stoppered, light-resistant bottle. Use within 3 months of preparation.

Sodium Sulfite, Anhydrous Na_2SO_3 [Sodium Sulfite, K8061]

Sodium Tartrate See Sodium Tartrate Dihydrate.

Sodium Tartrate Dihydrate
$NaOOCCH(OH)CH(OH)COONa \cdot 2H_2O$ [Sodium (+)-Tartrate Dihydrate, K8540]

Sodium Tetraborate Decahydrate $Na_2B_4O_7 \cdot 10H_2O$ [K8866]

Sodium Tetraborate Decahydrate for pH Determination
$Na_2B_4O_7 \cdot 10H_2O$ [Sodium Tetraborate Decahydrate for pH Determination, K8866]

Sodium Tetrahydroborate $NaBH_4$ (for atomic absorption spectrometry)

Sodium Tetrahydroborate for Amino Acid Analysis $NaBH_4$
Use sodium tetrahydroborate produced for the amino acid analysis.
Description: A white crystalline powder.

Sodium Tetrahydroborate TS Dissolve 5 g of sodium tetrahydroborate in 500 ml of 0.1 mol/L sodium hydroxide.

Sodium Thiosulfate See Sodium Thiosulfate Pentahydrate.

Sodium Thiosulfate Pentahydrate $Na_2S_2O_3 \cdot 5H_2O$ [K8637]

Sodium *p*-Toluenesulfonchloramide Trihydrate
$C_7H_7ClNNaO_2S \cdot 3H_2O$ [K8318]

Sodium Tungstate See Sodium Tungstate(VI) Dihydrate.

Sodium Tungstate(VI) Dihydrate $Na_2WO_4 \cdot 2H_2O$ [K8612]

Solution of Ferric Ammonium Sulfate in Hydrochloric Acid (1 in 1,000) Prepare fresh before use.

D-Sorbitol $C_6H_{14}O_6$ "D-Sorbitol"

D-Sorbitol for Assay $C_6H_{14}O_6$ Weigh 80 g of "D-Sorbitol," transfer into a 500-ml flask, add 220 ml of 90% methanol, and dissolve while warming on a water bath under a reflux condenser. Cool, transfer into a 500-ml beaker, add 40 mg of "D-Sorbitol" as seed crystals, mix, and allow to stand for 72 hours. Filter the formed crystals by suction, and wash with 50 ml of methanol. Weigh 40 g of the recrystallized product, add 110 ml of 90% methanol, and repeat the process above to obtain a twice-recrystallized product, using a recrystallized product dried under reduced pressure at 80°C for 5 hours as the seed crystals. Dry the twice-recrystallized product under reduced pressure at 80°C for 5 hours.

Soybean Peptone See Peptone, Soybean.

Stannous Chloride See Tin(II) Chloride.

Stannous Chloride TS Weigh 10 g of tin(II) chloride, and add diluted sulfuric acid (3 in 200) to dissolve, and make 100 ml.

Stannous Chloride TS, Acidic Weigh 4 g of tin(II) chloride, add 125 ml of arsenic-free hydrochloric acid to dissolve, and dilute with water to make 250 ml. Store in a glass-stoppered bottle. Use within 1 month of preparation.

Stannous Chloride–Hydrochloric Acid TS for Water-soluble Annatto Weigh 40 g of tin(II) chloride, add hydrochloric acid to dissolve, and make 100 ml. Stopper tightly, and store.

Starch [K8658]

Starch TS Weigh 1 g of starch, and mix well with 10 ml of cold water. Add the mixture gradually into 200 ml of hot water while stirring, and boil until the liquid becomes translucent. Allow to cool and stand, and use the supernatant as starch TS. Prepare fresh before use.

Stearic Acid $C_{18}H_{36}O_2$ [K8585]

Stevioside for Assay $C_{38}H_{60}O_{18}$ A white powder.
Identification Dissolve 0.6 g of Stevioside for Assay in water, add 100 ml of 1-butanol, shake well, and allow to stand. Measure 5 ml of the 1-butanol layer in a test tube, and add 5 ml of anthrone TS down the inside of test tube. The boundary surface of the two layers turns to blue to green.
Purity Related substance Prepare a test solution by dissolving 0.05 g of Stevioside for Assay in 50 ml of a 4:1 mixture of acetonitrile/water. Prepare a control solution by adding a 4:1 mixture of acetonitrile/water to 1 ml of the test solution, measured exactly, to make exactly 100 ml. Analyze 20 µl each of the test solution and the control solution by liquid chromatography using the operating conditions given below. Continue the chromatography for two times the retention time of the main peak, and measure the peak areas. Exclude the solvent peak from measurement. The total area of all peaks of the test solution, other than the main peak, is not greater than the area of the main peak of the control solution.
Operating conditions
Use the operating conditions for the Assay of Stevia Extract in the Monographs. Adjust the flow rate so that the retention time of stevioside is about 10 minutes.
Loss on drying Not more than 5.0% (105°C, 2 hours).

Strong Cupric Acetate TS See Copper(II) Acetate TS, Strong.

Strongly Acidic Cation-exchange Resin See Cation-exchange Resin, Strongly Acidic.

Strongly Acidic Cation-exchange Resin (Fine) See Cation-exchange Resin, Strongly Acidic (Fine).

Strongly Acidic Cation-exchange Resin for Liquid Chromatography Use strongly acidic cation-exchange resin produced for liquid chromatography.

Strongly Acidic Phosphorylated Cellulose Cation Exchanger See Phosphorylated Cellulose Cation Exchanger (–O–PO_3H_2 Type), Strongly Acidic.

Strongly Basic Anion-exchange Resin See Anion-exchange Resin, Strongly Basic.

Strontium Nitrate $Sr(NO_3)_2$ [K8554]

Styrene–Divinylbenzene Porous Polymer for Gas Chromatography Use a product produced for gas chromatography.

Styrene–Divinylbenzene Resin for Adsorption A porous resin made as adsorbent.

Substrate Solution for Lysozyme To an appropriate amount of dried *Micrococcus luteus*, add phosphate buffer (pH 6.2) to suspend uniformly. Adjust so that its transmittance is 10% at 640 nm. Prepare fresh before use.

Sulfanilic Acid $NH_2C_6H_4SO_3H$ [K8586]

Sulfanilic Acid Azo β-Naphthol Color $C_{16}H_{11}N_2NaO_4S$ Monosodium 4-(2-hydroxy-1-naphthylazo)benzenesulfonate. An orange-red powder.

Specific absorbance $E^{1\%}_{1cm}$ (maximum absorption wavelength near 484 nm): Not less than 640. Weigh 0.0100 g of Sulfanilic Acid Azo β-Naphthol Color, previously dried for 24 hours in a vacuum desiccator, dissolve in ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Refer to this solution as solution A. Measure exactly 10 ml of solution A, and add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Measure the absorbance of this solution.

Purity Other coloring matters Measure exactly 10 ml of solution A, and add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Analyze 20 µl of this solution by liquid chromatography using the operating conditions directed under Purity (5) for Food Yellow No. 5 in the Monographs. Only one peak is observed.

Sulfanilic Acid Azo G Salt Color $C_{16}H_9N_2Na_3O_{10}S$ Trisodium 7-hydroxy-8-(4-sulfophenylazo)-1,3-naphthalenesulfonate. An orange-red powder.

Specific absorbance $E^{1\%}_{1cm}$ (maximum absorption wavelength near 475 nm): Not less than 303. Weigh 0.0100 g of Sulfanilic Acid Azo G Salt Color, previously dried for 24 hours in a vacuum desiccator, dissolve in ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Refer to this solution as solution A. Measure exactly 10 ml of solution A, add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Measure the absorbance of this solution.

Purity Other coloring matters Measure exactly 10 ml of solution A, and add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Analyze 20 µl of this solution by liquid chromatography using the operating conditions directed under Purity (5) for Food Yellow No.5 in the Monographs. Only one peak is observed.

Sulfanilic Acid Azo R Salt Color $C_{16}H_9N_2Na_3O_{10}S_3$ Trisodium 3-hydroxy-4-(4-sulfophenylazo)-2,7-naphthalene sulfonate. An orange-red powder.

Specific absorbance $E^{1\%}_{1cm}$ (maximum absorption wavelength near 488 nm): Not less than 432. Weigh 0.0100 g of Sulfanilic Acid Azo R Salt Color, previously dried for 24 hours in a vacuum desiccator, dissolve in ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Refer to this solution as solution A. Measure exactly 10 ml of solution A, and add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Measure the absorbance of this solution.

Purity Other aromatic compounds Measure exactly 10 ml of solution A, add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Analyze 20 µl of this solution by liquid chromatography using the operating conditions directed under Purity (5) for Food Yellow No.5 in Monographs. Only one peak is observed.

Sulfanilic Acid TS To 0.50 g of sulfanilic acid, add 20 ml of dilute hydrochloric acid, warm to dissolve, and add water to make 100 ml.

Sulfur Dioxide SO_2 A colorless gas having a characteristic odor. Prepare by adding dropwise sulfuric acid to a concentrated solution of sodium hydrogen sulfite.

Sulfuric Acid H_2SO_4 [K8951]

Sulfuric Acid, Dilute To 10 ml of water, add 5.7 ml of sulfuric acid gradually, cool, and add water to make 100 ml.

Sulfuric Acid for Readily Carbonizable Substances Determination To sulfuric acid whose content is previously determined by the following procedure, add water carefully to prepare 94.4–95.5% sulfuric acid (H_2SO_4). Do not use the sulfuric acid if the concentration has changed by absorbing moisture during storage.

Assay Weigh accurately about 2 g of sulfuric acid into a stoppered flask, and add 30 ml of water. Weighing should be done promptly. After cooling, titrate with 1 mol/L sodium hydroxide (indicator: 2–3 drops of bromothymol blue).

Each ml of 1 mol/L sodium hydroxide = 49.04 mg of H_2SO_4

15% Sulfuric Acid–Methanol TS To 20 ml of methanol, add 8.2 ml of sulfuric acid gradually, and cool. Add methanol to make 100 ml.

Sulfurous Acid H_2SO_3 [Sulfurous Acid Water, K8058]

Tannic Acid $C_{14}H_{10}O_9 \cdot nH_2O$ [K8629]

Tannic Acid–Acetic Acid TS Weigh 0.010 g of tannic acid, dissolve in 80 ml of acetic acid by shaking, and add 32 ml of phosphoric acid. Prepare fresh before use.

Tartaric Acid See L-Tartaric Acid.

L-Tartaric Acid HOOCCH(OH)CH(OH)COOH [L(+)-Tartaric Acid, K8532]

Tetrabase–Citric Acid TS Weigh 0.25 g of 4,4′-tetramethyldiaminodiphenylmethane and 1 g of citric acid, and mix.

Dissolve in 500 ml of water.

Tetrabutylammonium Hydrogensulfate $[(C_4H_9)_4N]HSO_4$ A white crystalline powder.

Content Not less than 98.0% of tetrabutylammonium hydrogensulfate ($[(C_4H_9)_4N]HSO_4$).

Clarity of solution A solution of 1 g of Tetrabutylammonium Hydrogensulfate (1 in 20) is almost clear.

Purity Chloride Not more than 0.001% as Cl. To a solution of 2 g of Tetrabutylammonium Hydrogensulfate (1 in 10), add 5 ml of diluted nitric acid (1 in 3) and 1 ml of silver nitrate solution (1 in 50), and allow to stand for 15 minutes. The white turbidity formed is not higher than that formed when 5 ml of diluted nitric acid (1 in 3) and 1 ml of silver nitrate solution (1 in 50) are added to 2 ml of Chloride Ion Standard Stock Solution (1 in 10) and the solution obtained is allowed to stand for 15 minutes.

Assay Weigh accurately about 0.7 g of Tetrabutylammonium Hydrogensulfate, and dissolve in 100 ml of water prepared fresh as follows: Put water in a flask, boil for 15 minutes, and cool with the flask connected to a soda lime tube to block carbon dioxide in the air. Titrate the solution prepared with 0.1 mol/L sodium hydroxide solution (indicator: bromocresol green–methyl red TS).

Each ml of 0.1 mol/L sodium hydroxide solution = 0.03395g of $[(C_4H_9)_4N]HSO_4$

Tetracycline $C_{22}H_{24}N_2O_8$ Use tetracycline hydrochloride specified in the Japanese Pharmacopoeia. The mass (potency) is expressed as the mass (potency) of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8{\cdot}HCl$).

Tetrahydrofuran C_4H_8O [K9705]

4,4′-Tetramethyldiaminodiphenylmethane $C_{17}H_{22}N_2$ White to bluish white, lustrous, foliate crystals. Slightly soluble in water, but soluble in diethyl ether, in ethanol, and in benzene.

Melting point 90–91°C.

Thin-Layer Plate for Yucca Form Extract A 10 cm × 10 cm plate coated with 5- to 7-μm silica gel for thin-layer chromatography.

2,2′-Thiodiethanol $S(CH_2CH_2OH)_2$ Use 2,2′-thiodiethanol produced for amino acid analysis.

Description A clear, colorless to pale yellow liquid.

Specific gravity 1.178–1.188.

Water Not more than 0.7% (0.1 g, coulometric titration).

β-Thujaplicin for Assay $C_{10}H_{12}O_2$

Purity (1) Boiling point 140–141°C (1.3 kPa).

(2) Melting point 51–53°C.

(3) Related substances Prepare a test solution by dissolving 0.2 g of β-Thujaplicin for Assay in ethanol to make 100 ml. Prepare the control solution by diluting 1 ml of the test solution, exactly measured, with ethanol to 100 ml. Analyze 0.5 μl each of the test solution and the control solution by gas chromatography using the operating conditions specified in the Assay for Thujaplicin in the Monographs, and measure the peak areas. Continue the chromatography for two times the retention time of the main peak. Exclude the solvent peaks from measurement. The total area of all peaks of the test solution, other than the main peak, is not greater than the area of the main peak of the control solution.

Thymol $C_{10}H_{14}O$ Use thymol specified in the Japanese Pharmacopoeia.

Thymol Blue $C_{27}H_{30}O_5S$ [K8643]

Thymol Blue TS Weigh 0.1 g of thymol blue, and dissolve in 100 ml of ethanol. Filter if necessary.

Thymolphthalein $C_{28}H_{30}O_4$ [K8642]

Thymolphthalein TS Weigh 0.1 g of thymolphthalein, and dissolve in 100 ml of ethanol. Filter if necessary.

Thymol–Sulfuric Acid TS Weigh 0.5 g of thymol, add 5 ml of sulfuric acid, and add ethanol to make 100 ml.

Tin(II) Chloride See Tin(II) Chloride Dihydrate.

Tin(II) Chloride Dihydrate $SnCl_2{\cdot}2H_2O$ [K8136]

Titanium Trichloride Solution [Titanium(III) Chloride, K8401:1961] A dark purple liquid.

Content Not less than 20% of titanium trichloride.

Identification To Titanium Trichloride Solution, add a 10-fold volume of water, then hydrogen peroxide TS in small portions. The color of the solution fades. When hydrogen peroxide TS is further added, the solution turns red-brown.

Assay Weigh accurately about 3 g, and add 250 ml of oxygen-free water and 5 ml of hydrochloric acid (2 in 3). Titrate with 0.2 mol/L iron(III) ammonium sulfate in a carbon dioxide. Use 10% ammonium thiocyanate as the indicator.

Each ml of 0.2 mol/L iron(III) ammonium sulfate = 30.85 mg of $TiCl_3$

Store in a glass-stoppered, light-resistant bottle.

***dl*-α-Tocopherol Acetate** Use tocopherol acetate specified in the Japanese Pharmacopoeia.

***d*-α-Tocopherol for Assay** $C_{29}H_{50}O_2$ A light yellow, viscous liquid.

Identification Weigh accurately about 5 mg of *d*-α-Tocopherol for Assay, dissolve in absolute ethanol to make exactly 10 ml. Measure exactly 1 ml of this solution, and add absolute ethanol to make exactly 10 ml. Measure the absorbance of this solution. The solution exhibits an absorption maximum near 292 nm.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength near 292 nm): 67–82. Weigh accurately about 5 mg of *d*-α-Tocopherol for Assay, dissolve in absolute ethanol to make exactly 10 ml. Measure exactly 1 ml of this solution, and add absolute ethanol to make exactly 10 ml. Measure the absorbance of this solution.

Purity Related substances Prepare a test solution by dissolving about 0.05 g of *d*-α-Tocopherol for Assay, weighed accurately, in hexane to make exactly 100 ml. Prepare a control solution by adding hexane to 1.5 ml of the test solution, measured exactly, to make exactly 100 ml. Analyze 20 μl each of the test solution and the control solution by liquid chromatography using the operating conditions below. Con-

tinue the chromatography for twice the retention time of the main peak and measure the peak areas. The total area of all peaks of the test solution, other than the main peak, is not greater than the area of the main peak of the control solution.

Operating conditions

Detector: Ultraviolet spectrophotometer (determination wavelength: 292 nm).

Column: A stainless steel tube of 3–6 mm internal diameter and 15–25 cm length.

Packing material of column: 5- to 10-μm silica gel for liquid chromatography.

Column temperature: Room temperature (constant).

Mobile phase: A 200:1 mixture of hexane/2-propanol.

Flow rate: Adjust the flow rate so that the retention time of the main peak is about 5 minutes.

_d_-β-Tocopherol for Assay $C_{28}H_{48}O_2$ A light yellow, viscous liquid.

Identification Weigh accurately about 5 mg of *d*-β-Tocopherol for Assay, dissolve in absolute ethanol to make exactly 10 ml. Measure exactly 1 ml of this solution, and add absolute ethanol to make exactly 10 ml. Measure the absorbance of this solution. The solution exhibits an absorption maximum near 296 nm.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength near 296 nm): 77–95. Weigh accurately about 5 mg of *d*-β-Tocopherol for Assay, dissolve in absolute ethanol to make exactly 10 ml. Measure exactly 1 ml of this solution, and add absolute ethanol to make exactly 10 ml. Measure the absorbance of this solution.

Purity Related substances Prepare a test solution by dissolving about 0.05 g of *d*-β-Tocopherol for Assay, weighed accurately, in hexane to make exactly 100 ml. Prepare a control solution by adding hexane to 1.5 ml of the test solution, measured exactly, to make exactly 100 ml. Analyze 20 μl each of the test solution and the control solution by liquid chromatography using the operating conditions below. Continue the chromatography for about twice the retention time of the main peak, and measure the peak areas. The total area of all peaks of the test solution, other than the main peak, is not greater than the area of the main peak of the control solution.

Operating conditions

Detector: Ultraviolet spectrophotometer (determination wavelength: 292 nm).

Column: A stainless steel tube of 3–6 mm internal diameter and 15–25 cm length.

Packing material of column: 5- to 10-μm silica gel for liquid chromatography

Column temperature: Room temperature (constant).

Mobile phase: A 200:1 mixture of hexane/2-propanol.

Flow rate: Adjust so that the retention time of the main peak is about 10 minutes.

_d_-γ-Tocopherol for Assay $C_{28}H_{48}O_2$ A light yellow, viscous liquid.

Identification Weigh accurately about 5 mg of *d*-γ-Tocopherol for Assay, dissolve in absolute ethanol to make exactly 10 ml. Measure exactly 1 ml of this solution, and add absolute ethanol to make exactly 10 ml. Measure the absorbance of this solution. The solution exhibits an absorption maximum near 297 nm.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength near 297 nm): 83–103. Weigh accurately about 5 mg of *d*-γ-Tocopherol for Assay, dissolve in absolute ethanol to make exactly 10 ml. Measure exactly 1 ml of this solution, and add absolute ethanol to make exactly 10 ml. Measure the absorbance of this solution.

Purity Related substances Prepare a test solution by dissolving about 0.05 g of *d*-γ-Tocopherol for Assay, weighed accurately, in hexane to make exactly 100 ml. Prepare a control solution by adding hexane to 1.5 ml of the test solution, exactly measured, to make exactly 100 ml. Analyze 20 μl each of the test solution and the control solution by liquid chromatography using the operating conditions below. Continue the chromatography for about twice the retention time of the main peak, and measure the peak areas. The total area of all peaks of the test solution, other than the main peak, is not greater than the area of the main peak of the control solution.

Operating conditions

Detector: Ultraviolet spectrophotometer (determination wavelength: 292 nm).

Column: A stainless steel tube of 3–6 mm internal diameter and 15–25 cm length.

Packing material of column: 5- to 10-μm silica gel for liquid chromatography.

Column temperature: Room temperature (constant).

Mobile phase: A 200:1 mixture of hexane/2-propanol.

Flow rate: Adjust the flow rate so that the retention time of the main peak is about 11 minutes.

_d_-δ-Tocopherol for Assay $C_{27}H_{46}O_2$ A light yellow, viscous liquid.

Identification Weigh accurately about 5 mg of *d*-δ-Tocopherol for Assay, dissolve in absolute ethanol to make exactly 10 ml. Measure exactly 1 ml of this solution, and add absolute ethanol to make exactly 10 ml. Measure the absorbance of this solution. The solution exhibits an absorption maximum near 298 nm.

Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength near 298 nm): 83–101. Weigh accurately about 5 mg of *d*-δ-Tocopherol for Assay, dissolve in absolute ethanol to make exactly 10 ml. Measure exactly 1 ml of this solution, and add absolute ethanol to make exactly 10 ml. Measure the absorbance of this solution.

Purity Related substances Prepare a test solution by dissolving about 0.05 g of *d*-δ-Tocopherol for Assay, weighed accurately, in hexane to make exactly 100 ml. Prepare a control solution by adding hexane to 3 ml of the test solution, measured exactly, to make exactly 100 ml. Analyze 20 μl each of the test solution and the control solution by liquid chromatography using the conditions below. Continue the chromatography for about twice the retention time of the main peak, and measure the peak areas. The total area of all peaks of the test solution, other than the main peak, is not greater than the area of the main peak for the control solution.

Operating conditions

Detector: Ultraviolet spectrophotometer (determination wavelength: 292 nm).

Column: A stainless steel tube of 3–6 mm internal diameter and 15–25 cm length.

Packing material for column: 5- to 10-μm silica gel for liquid chromatography.

Column temperature: Room temperature (constant).

Mobile phase: A 200:1 mixture of hexane/2-propanol.
Flow rate: Adjust the flow rate so that the retention time of the main peak is about 20 minutes.

Toluene $C_6H_5CH_3$ [K8680]

***o*-Toluenesulfonamide** $C_7H_9NO_2S$ Colorless crystals, or a white crystalline powder.

Melting point 157–160°C.

Purity *p*-Toluenesulfonamide Analyze a solution of *o*-Toluenesulfonamide in ethyl acetate (1 in 5,000) by gas chromatography using the operating conditions specified in the Purity (6) for Sodium Saccharin in the Monographs. Only one peak of *o*-toluenesulfonamide is observed.

Trichloroacetic Acid CCl_3COOH [K8667]

Trichloroacetic Acid TS Dissolve 18 g of anhydrous sodium acetate, 110 ml of 1 mol/L trichloroacetic acid solution, and 19 ml of acetic acid in about 600 ml of water. Adjust the pH to 4.0 with 1 mol/L sodium hydroxide, and dilute with water to make 1,000 ml.

Triethanolamine See 2,2′,2″-Nitrilotriethanol.

Trifluoroacetic Acid CF_3COOH A colorless, transparent liquid having pungent odor. Very soluble in water.

Content Not less than 99.0% of trifluoroacetic acid (CF_3COOH).

Identification (1) Trifluoroacetic Acid is acidic.

(2) Proceed as directed under the Liquid Film Method in Infrared Spectrophotometry. The spectrum exhibits absorption bands at wavenumbers of about 3180 cm^{-1}, 1785 cm^{-1}, 1458 cm^{-1}, 1170 cm^{-1}, 811 cm^{-1} and 687 cm^{-1}.

Purity Unvolatile matter Not more than 0.02%. Weigh 10.0 g of Trifluoroacetic Acid, evaporate, and dry at 100°C for 2 hours. Cool in a desiccator for about 30 minutes, and weigh the residue.

Assay Weigh accurately about 3 g of Trifluoroacetic Acid, and add 30 ml of water. Titrate with 1 mol/L sodium hydroxide (indicator: phenolphthalein).

Each ml of 1 mol/L sodium hydroxide = 114.0 mg of CF_3COOH

Trimethylaminopropyl-bonded Silica Gel Use trimethylaminopropyl-bonded silica gel produced for ion-exchange absorbent.

Trimethylchlorosilane $(CH_3)_3SiCl$ [Chlorotrimethylsilane, K9555:1992] A colorless or almost colorless liquid. Fumes in humid air. Very soluble in diethyl ether, and reacts to water and ethanol.

Identification Determine the spectrum of Trimethylchlorosilane as directed in the Potassium Bromide Disk Method under Infrared Spectrophotometry. The spectrum exhibits absorption bands at wavenumbers of about 2970 cm^{-1}, 1410 cm^{-1}, 1260 cm^{-1}, 850 cm^{-1}, 760 cm^{-1} and 700 cm^{-1}.

Purity Analyze 1 µl of Trimethylchlorosilane by gas chromatography. Measure the peak area of each peak. The percentage of main peak obtained by the Peak Area Percentage Method is not less than 98%.

Operating conditions
Detector: Thermal conductivity detector.
Column: A glass or stainless steel tube of 2–4 mm internal diameter and 2–3 m length.
Column packing material
Liquid phase: 25% phenyl methyl silicone polymer (15–20% of the amount of support).
Support: 180- to 250-µm diatomaceous earth for gas chromatography.
Column temperature: A constant temperature of 70–80°C.
Vaporizer temperature: A constant temperature of 80–100°C.
Detector temperature: A constant temperature of 80–100°C.
Carrier gas: Helium.
Flow rate: A constant rate of 30–40 ml/minute.

2,2,4-Trimethylpentane $(CH_3)_2CH(CH_2)_4CH_3$ A colorless liquid. Practically insoluble in water, and miscible in chloroform and in diethyl ether.

Purity Measure the absorbance of this substance as directed in Ultraviolet-Visible Spectrophotometry. Use water as the reference. The absorbance is not more than 0.050 at 230 nm, 0.010 at 250 nm, and 0.005 at 280 nm.

2,2,4-Trimethylpentane for Ultraviolet Absorption Spectrum Measurement $CH_3C(CH_3)_2CH_2CH(CH_3)CH_3$

Absorbance Prepare a test solution as follows. To 180 ml of 2,2,4-Trimethylpentane for Ultraviolet Absorption Spectrum Measurement, add 1 ml of hexadecane for ultraviolet absorption spectrum measurement. Evaporate the mixture on a water bath under nitrogen until the residue is reduced to 1 ml. To the residue, add 2,2,4-Trimethylpentane for Ultraviolet Absorption Spectrum Measurement to dissolve, and make exactly 25 ml. Using this solution as the test solution. Measure the absorbance of the test solution in a 5-cm path length cell, using 2,2,4-Trimethylpentane for Ultraviolet Absorption Spectrum Measurement as the reference solution. It is not more than 0.01 (absorbance/cm light pass length) at 280–400 nm.

2,4,6-Trinitrophenol $(NO_2)_3C_6H_2OH$ [K8759:1984]
Light yellow, odorless crystals. Sublimes when heated gradually, and burns vigorously when heated strongly.

Melting point 121–123°C.

Triphenylchloromethane $(C_6H_5)_3CCl$ [K8674:1978]
White to grayish white or yellowish crystals or crystalline powder.

Identification (1) To 5 ml of a saturated solution of Triphenylchloromethane in acetic acid, add 1 ml of water. A white precipitate is formed.

(2) To 5 ml of a saturated solution of Triphenylchloromethane in acetic acid, add 1 ml of hydrochloric acid. A yellow precipitate is formed.

Melting point 105–113°C.

Triphenylphosphine Oxide $C_{18}H_{15}OP$ A very slightly brownish white powder.

Purity (1) Melting point 156–158°C.

(2) Clarity and color of solution Light brown, clear (1 g, 10 ml acetone).

(3) Related substances Weigh accurately 0.01 g of Tri-

phenylphosphine Oxide, previously dried for 24 hours in a vacuum desiccator, and dissolve in methanol to make exactly 100 ml. Measure exactly 1 ml of this solution, and add a 67:33 mixture of acetonitrile/water to make exactly 100 ml. Use the resultant solution as the test solution. Measure exactly 2 ml of the test solution, and add a 67:33 mixture of acetonitrile/water to make exactly 100 ml. Use this solution as the control solution. Analyze 20 µl each of the test solution and the control solution by liquid chromatography using the operating conditions specified in Purity (6) for Sucralose in the Monographs. Continue the chromatography for two times the retention time of the main peak and measure peak areas. The total area of all peaks of the test solution, other than the main peak, is not greater than the area of the main peak of the control solution.

Tris Buffer (pH 7.0) for Pectin Determination Dissolve 6.055 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 0.147 g of calcium chloride dihydrate in about 750 ml of water, adjust the pH to 7.0 with 1 mol/L hydrochloric acid. Add water to make exactly 1,000 ml.

Tris(hydroxymethyl)aminomethane See 2-Amino-2-hydroxymethyl-1,3-propanediol.

Trisodium Citrate See Trisodium Citrate Dihydrate.

Trisodium Citrate Dihydrate $Na_3C_6H_5O_7 \cdot 2H_2O$ [K8288]

Trisodium 7-Hydroxy-1,3,6-naphthalenetrisulfonate $C_{10}H_5Na_3O_{10}S_3$ A white to whitish powder.

Specific absorbance $E^{1\%}_{1cm}$ (maximum absorption wavelength near 288 nm): Not less than 105. Weigh 0.0100 g of Trisodium 7-Hydroxy-1,3,6-naphthalenetrisulfonate, dried previously for 24 hours in a vacuum desiccator, dissolve in ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Refer to this solution as solution A. Measure exactly 10 ml of solution A, add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. This solution exhibits absorption maxima at wavelengths of 240 nm, 288 nm, and 344 nm.

Purity Other aromatic substances Measure exactly 10 ml of solution A, add ammonium acetate solution (3 in 2,000) to make exactly 100 ml. Analyze 20 µl of this solution by liquid chromatography using the operating conditions directed under Purity (6) for Food Red No. 2 in the Monographs. Only one peak is observed.

Urea NH_2CONH_2 [K8731]

Vanadic Acid–Molybdic Acid TS To 1.12 g of ammonium metavanadate, add about 300 ml of warm water to dissolve, and add 250 ml of nitric acid. Mix this solution with a solution prepared by dissolving 27 g of powdered ammonium molybdate in about 400 ml of warm water. After cooling, add water to make 1,000 ml. Store in a colored bottle, and use 3 or 4 days after preparation.

Vanillin $C_8H_8O_3$ [K9544]

Vinyl Acetate $CH_3COOCHCH_2$ A colorless liquid. Soluble in toluene.

Refractive index n_D^{20}: 1.393–1.397.

Water Determination TS Weigh 63 g of iodine, dissolve in 100 ml of pyridine for water determination, and cool in ice. Pass dry sulfur dioxide through the solution until the weight of the solution increases by 32.3 g. Then, add methanol for water determination to make 500 ml, and allow to stand for 24 hours or longer. Since this solution deteriorates with time, standardize before use. Store in a cold place, protected from light and moisture.

Standardization Transfer 25 ml of methanol for water determination into a dry titration flask, and add carefully Water Determination TS to the end point as directed in the Water Determination Test. Add quickly about 50 mg of water, accurately weighed, and titrate with Water Determination TS, protecting from moisture. The number of mg (f) of water (H_2O) equivalent to 1 ml of Water Determination TS is obtained by the formula:

$$f = \frac{\text{Weight (mg) of added water } (H_2O)}{\left(\dfrac{\text{Volume (ml)}}{\text{of Water Determination TS consumed}}\right)}$$

Weakly Acidic Cation-exchange Resin (fine) See Cation-exchange Resin, Weakly Acidic (fine).

Weakly Basic Anion-exchange Resin See Anion-exchange Resin, Weakly Basic.

Weakly Basic Diethylaminoethyl-Cellulose Anion Exchanger See DEAE-Cellulose Anion Exchanger (–O–C_2H_4-$N(C_2H_5)_2$ Type), Weakly Basic.

White Sugar Use sucrose specified in the Japanese Pharmacopoeia.

Wijs TS Weigh 7.9 g of iodine trichloride and 8.9 g of iodine, separately dissolve in a small volume of acetic acid. Mix the two solutions, and add acetic acid to make 1,000 ml. Store in a light-resistant, glass-container.

Xylene $C_6H_4(CH_3)_2$ [K8271]

***o*-Xylene** $C_6H_4(CH_3)_2$ A clear, colorless liquid.

Refractive index n_D^{20}: 1.501–1.506.

Specific gravity d_4^{20}: 0.875–0.885.

Distillation test 143–146°C, not less than 95% vol.

Xylene Cyanol FF [K8272]

Xylenol Orange $C_{31}H_{30}N_2Na_2O_{13}S$ [K9563]

Xylenol Orange TS Weigh 0.1 g of xylenol orange, and dissolve in water to make 100 ml.

Yeast Extract A reddish-yellow to brown powder having a characteristic, non- putrefactive odor. It is obtained from output of yeast (*Saccharomyces*), peptone-like, water-soluble substances, through clarification process under appropriate conditions, followed by evaporation to dryness. 1 g of Yeast Extract is obtained from 7.5 g or more of yeast. It dissolves in water to make a yellow-to-brown, weakly-acidic solution. Carbohydrates are not added.

Purity (1) Chloride Not more than 5% (as NaCl).

(2) Coagulable proteins On heating Yeast Extract solution

(1 in 20) to boiling, no precipitate is observed.

Loss on drying Not more than 5% (105°C, constant weight).

Residue on ignition Not more than 15% (0.5 g).

Nitrogen content 7.2–9.5% (105°C, constant weight, after drying, Nitrogen Determination).

Yellow Mercuric Oxide See Mercuric Oxide, Yellow.

Zeolite for Gas Chromatography Use natural or synthetic zeolite prepared for gas chromatography.

Zinc Zn [K8012]

Zinc, Arsenic-free See Zinc for Arsenic Analysis.

Zinc (Standard Reagent) Zn [Standard Material for Volumetric Analysis, K 8005]

Zinc Acetate See Zinc Acetate Dihydrate.

Zinc Acetate Dihydrate $Zn(CH_3COO)_2 \cdot 2H_2O$ [K8356]

Zinc Chloride $ZnCl_2$ [K8111]

Zinc Dust See Zinc powder.

Zinc for Arsenic Analysis [Zinc for Arsenic Analysis, K8012] Use 1,000- to 1,410-μm zinc. Porous zinc should not be used because it dissolves too rapidly in general. Suitable one is such that when the operation is finished, a small amount remains and hydrogen gas still evolves.

Zinc Iodide–Starch TS Boil 100 ml of water, and add 5 ml of potassium iodide solution (3 in 20) and 10 ml of zinc chloride solution (1 in 5). Keep boiling, and with continuous stirring, add a uniform suspension prepared by adding 30 ml of cold water to 5 g of starch.. Continue to boil for 2 minutes, and cool. Stopper tightly, and store in a cold place.

Zinc Powder Zn [K8013]

Zinc Sulfate See Zinc Sulfate Heptahydrate.

Zinc Sulfate Heptahydrate $ZnSO_4 \cdot 7H_2O$ [Zinc Sulfate Heptahydrate, K8953]

2. Volumetric Solutions

0.1 mol/L Ammonium Thiocyanate
This solution contains 7.612 g of ammonium thiocyanate (NH_4SCN, molecular weight: 76.12) per 1,000 ml.

Weigh about 8 g of ammonium thiocyanate, and dissolve in 1,000 ml of water. This solution can be replaced by 0.1 mol/L potassium thiocyanate.

Standardization Measure exactly 30 ml of 0.1 mol/L silver nitrate, and transfer into a flask with a ground-glass stopper. Add 50 ml of water, 2 ml of nitric acid, and 2 ml of ferric ammonium sulfate TS, and titrate with the prepared ammonium thiocyanate solution while shaking until a red-brown color persists.

0.01 mol/L Bismuth Nitrate
This solution contains 4.851 g of bismuth nitrate ($Bi(NO_3)_3 \cdot 5H_2O$, molecular weight: 485.07) per 1,000 ml.

Weigh 4.86 g of bismuth nitrate, dissolve in 60 ml of diluted nitric acid (1 in 10), and add water to make 1,000 ml.

Standardization Measure exactly 25 ml of the prepared bismuth nitrate solution, add 50 ml of water, and titrate with 0.01 mol/L EDTA (indicator: 1 drop of xylenol orange TS) until the color of the solution changes from red to yellow.

0.1 mol/L Ceric Ammonium Sulfate
This solution contains 63.26 g of ceric ammonium sulfate ($Ce(NH_4)_4(SO_4)_4 \cdot 2H_2O$, molecular weight: 632.55) per 1,000 ml.

Weigh 64 g of ceric ammonium sulfate, and dissolve in 0.5 mol/L sulfuric acid to make 1,000 ml. Standardize before use.

Standardization Measure exactly 25 ml of the prepared ceric ammonium sulfate solution, add 20 ml of water and 20 ml of diluted sulfuric acid (1 in 20), dissolve 1 g of potassium iodide, and titrate immediately with 0.1 mol/L sodium thiosulfate. When the color of the solution changes to light yellow near the endpoint, add 3 ml of starch TS as the indicator, and continue the titration until the blue color of the solution disappears. Perform a blank test, and make any necessary correction.

0.01 mol/L Ceric Ammonium Sulfate
This solution contains 6.326 g of ceric ammonium sulfate ($Ce(NH_4)_4(SO_4)_4 \cdot 2H_2O$, molecular weight: 632.55) per 1,000 ml.
Dilute 0.1 mol/L ceric ammonium sulfate with 0.5 mol/L sulfuric acid to 10 times its original volume.

0.1 mol/L Ceric Sulfate
This solution contains 33.22 g of ceric sulfate ($Ce(SO_4)_2$, molecular weight: 332.24) per 1,000 ml.

Weigh 55 g of ceric ammonium sulfate, transfer into a beaker, and mix with 31 ml of sulfuric acid. Add a few 20-ml portions of water carefully to dissolve. Cover the beaker, and allow to stand overnight. Filter through a glass filter, and add water to make 1,000 ml.

Standardization Weigh accurately about 0.2 g of arsenic trioxide (standard reagent), previously dried at 100°C for 1 hour, add 25 ml of sodium hydroxide solution (2 in 25), and dissolve while shaking. Add 100 ml of water, 10 ml of diluted sulfuric acid (1 in 3), 2 drops of *o*-phenanthroline TS, and 2 drops of a solution of osmic acid in 0.05 mol/L sulfuric acid (1 in 400), and titrate with the prepared ceric sulfate solution until the red color of the solution changes to light blue.

$$\text{Normality factor} = \frac{\text{Weight (g) of arsenic trioxide} \times 1{,}000}{\left(\text{Volume (ml) of } 0.1\,\text{mol/L ceric sulfate consumed}\right) \times 4.946}$$

0.1 mol/L EDTA
This solution contains 37.22 g of disodium ethylenediaminetetraacetate ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$, molecular weight: 372.24) per 1,000 ml.

Weigh 38 g of disodium ethylenediaminetetraacetate, and dissolve in freshly boiled and cooled water to make 1,000 ml.

Standardization Measure exactly 20 ml of the prepared EDTA solution, add 2 ml of ammonia–ammonium chloride buffer (pH 10.7) and water to make about 100 ml, and titrate with 0.05 mol/L zinc chloride (indicator: 5 drops of eriochrome black T TS).

$$\text{Normality factor} = \frac{\left(\begin{array}{c}\text{Volume (ml) of}\\ \text{0.05 mol/L zinc chloride consumed}\end{array}\right)}{\text{Volume (ml) of 0.1 mol/L EDTA} \times 2}$$

0.05 mol/L EDTA

This solution contains 18.61 g of disodium ethylenediaminetetraacetate ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$, molecular weight: 372.24) per 1,000 ml.

Weigh 18.7 g of disodium ethylenediaminetetraacetate, and dissolve in freshly boiled and cooled water to make 1,000 ml.

Standardization Measure exactly 20 ml of the prepared EDTA solution, add 2 ml of ammonia–ammonium chloride buffer (pH 10.7) and water to make about 100 ml, and titrate with 0.025 mol/L zinc chloride (indicator: 5 drops of eriochrome black T TS).

$$\text{Normality factor} = \frac{\left(\begin{array}{c}\text{Volume (ml) of}\\ \text{0.025 mol/L zinc chloride consumed}\end{array}\right)}{\text{Volume (ml) of 0.05 mol/L of EDTA} \times 2}$$

0.02 mol/L EDTA

This solution contains 7.445 g of disodium ethylenediaminetetraacetate ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$, molecular weight: 372.24) per 1,000 ml.

Prepare as directed under 0.05 mol/L EDTA, using 7.5 g of disodium ethylenediaminetetraacetate.

Standardization Measure exactly 25 ml of the prepared EDTA solution, add 2 ml of ammonia–ammonium chloride buffer (pH 10.7) and water to make about 100 ml, and titrate with 0.025 mol/L zinc chloride (indicator: 3 drops of eriochrome black T TS).

0.01 mol/L EDTA

This solution contains 3.722 g of disodium ethylenediaminetetraacetate ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$, molecular weight: 372.24) per 1,000 ml.

Prepare as directed under 0.05 mol/L EDTA, using 3.8 g of disodium ethylenediaminetetraacetate.

Standardization Measure exactly 50 ml of the prepared EDTA solution, add 2 ml of ammonia–ammonium chloride buffer (pH 10.7) and water to make about 100 ml, and titrate with 0.025 mol/L zinc chloride (indicator: 3 drops of eriochrome black T TS).

0.5 mol/L Ethanolic Potassium Hydroxide

See 0.5 mol/L Potassium Hydroxide, Ethanolic.

0.1 mol/L Ethanolic Potassium Hydroxide

See 0.1 mol/L Potassium Hydroxide, Ethanolic.

0.02 mol/L Ethanolic Potassium Hydroxide

See 0.02 mol/L Potassium Hydroxide, Ethanolic.

0.1 mol/L Ferric Ammonium Sulfate

This solution contains 48.22 g of ferric ammonium sulfate ($FeNH_4(SO_4)_2 \cdot 12H_2O$, molecular weight: 482.19) per 1,000 ml.

Weigh 49 g of ferric ammonium sulfate, dissolve in a cooled mixture of 6 ml of sulfuric acid and 300 ml of water, and add water to make 1,000 ml.

Standardization Measure exactly 25 ml of the prepared ferric ammonium sulfate solution into an iodine-flask, add 5 ml of hydrochloric acid and shake. Add and dissolve 2 g of potassium iodide, stopper tightly, and allow to stand for 10 minutes. Add 50 ml of water, titrate liberated iodine with 0.1 mol/L sodium thiosulfate. When the color of the solution changes to light yellow near the end point, add 3 ml of starch TS as the indicator, and continue the titration until the blue color of the solution disappears. Perform a blank test in the same manner, and make any necessary correction. Store protected from light, and restandardize frequently.

0.1 mol/L Ferrous Ammonium Sulfate

This solution contains 39.21 g of ferrous ammonium sulfate ($Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, molecular weight: 392.14) per 1,000 ml.

Weigh 40 g of ferrous ammonium sulfate, dissolve in 100 ml of cooled, diluted sulfuric acid (1 in 2), and add water to make 1,000 ml.

Standardization Measure exactly 25 ml of the prepared ferrous ammonium sulfate solution, and titrate with 0.1 mol/L ceric sulfate (indicator: 2 drops of *o*-phenanthroline TS) until the red color of the solution changes to light blue.

15 mol/L Formic Acid

This solution contains 690.4 g of formic acid (HCOOH, molecular weight: 46.03) per 1,000 ml.

Weigh 705 g of formic acid, and add water to make 1,000 ml.

Standardization Measure exactly 1 ml of the prepared formic acid solution, add water to make 50 ml, and titrate with 0.5 mol/L sodium hydroxide (indicator: 3 drops of phenolphthalein TS).

6 mol/L Hydrochloric Acid

This solution contains 218.8 g of hydrochloric acid (HCl, molecular weight: 36.46) per 1,000 ml.

Prepare and standardize, as directed under 1 mol/L Hydrochloric Acid, using 540 ml of hydrochloric acid.

1 mol/L Hydrochloric Acid

This solution contains 36.46 g of hydrochloric acid (HCl, molecular weight: 36.46) per 1,000 ml.

Measure 90 ml of hydrochloric acid, and add water to make 1,000 ml.

Standardization Weigh accurately about 1.5 g of sodium carbonate (standard reagent), previously dried at about 270°C for 1 hour, dissolve in 100 ml of water, and titrate with the prepared hydrochloric acid solution (indicator: 2 drops of bromophenol blue TS). Near the end point, boil to expel the carbon dioxide, and immediately continue the titration.

Each ml of 1 mol/L hydrochloric acid = 52.99 mg of Na_2CO_3

0.5 mol/L Hydrochloric Acid
This solution contains 18.23 g of hydrochloric acid (HCl, molecular weight: 36.46) per 1,000 ml.

Prepare and standardize, as directed under 1 mol/L Hydrochloric Acid, using 45.0 ml of hydrochloric acid.

0.2 mol/L Hydrochloric Acid
This solution contains 7.292 g of hydrochloric acid (HCl, molecular weight: 36.46) per 1,000 ml.

Prepare by diluting 1 mol/L hydrochloric acid with water to 5 times its original volume, or prepare as directed under 1 mol/L Hydrochloric Acid, using 18 ml of hydrochloric acid. Standardize as directed under 1 mol/L Hydrochloric Acid.

0.1 mol/L Hydrochloric Acid
This solution contains 3.646 g of hydrochloric acid (HCl, molecular weight: 36.46) per 1,000 ml.

Prepare by diluting 1 mol/L hydrochloric acid with water to 10 times its original volume, or prepare as directed under 1 mol/L Hydrochloric Acid, using 9.0 ml of hydrochloric acid. Standardize as directed under 1 mol/L Hydrochloric Acid.

0.02 mol/L Hydrochloric Acid
This solution contains 0.7292 g of hydrochloric acid (HCl, molecular weight: 36.46) per 1,000 ml.

Dilute 0.1 mol/L hydrochloric acid with water to 5 times its original volume, and standardize as directed under 1 mol/L Hydrochloric Acid.

0.01 mol/L Hydrochloric Acid
This solution contains 0.3646 g of hydrochloric acid (HCl, molecular weight: 36.46) per 1,000 ml.

Dilute 0.1 mol/L hydrochloric acid with water to 10 times its original volume, and standardize as directed under 1 mol/L Hydrochloric Acid.

0.5 mol/L Hydroxylamine Hydrochloride
This solution contains 34.75 g of hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$, molecular weight: 69.49) per 1,000 ml.

Weigh exactly 35 g of hydroxylamine hydrochloride, add 40 ml of water, and dissolve by heating to about 65°C. Cool, add 15 ml of bromophenol blue–sodium hydroxide TS, and add ethanol to make exactly 1,000 ml. Prepare fresh before use.

0.05 mol/L Iodine
This solution contains 12.69 g of iodine (I, atomic weight: 126.90) per 1,000 ml.

Weigh about 14 g iodine, dissolve in 100 ml of potassium iodide solution (9 in 25), and add 3 drops of hydrochloric acid and water to make 1,000 ml. Store in a glass-stoppered bottle, and restandardize frequently.

Standardization Weigh accurately about 0.15 g of arsenic trioxide (standard reagent), previously powdered and dried at 100°C to constant weight, and dissolve in 20 ml of 1 mol/L sodium hydroxide by heating if necessary. Add about 40 ml of water and 2 drops of methyl orange TS, and add diluted hydrochloric acid (1 in 4) until the yellow color of the solution changes to light pink. Add 2 g of sodium hydrogen carbonate, about 50 ml of water, and 3 ml of starch TS, and titrate with the prepared iodine solution until a persistent blue color is produced.

Each ml of 0.05 mol/L Iodine = 4.946 mg of As_2O_3

0.05 mol/L Iodine for Sodium Hydrosulfite
This solution contains 12.69 g of iodine (I, atomic weight: 126.90) per 1,000 ml.

Weigh about 13 g of iodine, dissolve in the solution, previously prepared by dissolving 40 g of potassium iodide in 25 ml of water, and add 0.5 ml of hydrochloric acid and water to make 1,000 ml. Store in a brown bottle in a dark place.

Standardization Measure exactly 25 ml of the prepared iodine solution for sodium hydrosulfite, and titrate with 0.1 mol/L sodium thiosulfate (indicator: starch TS). Add the indicator after the color of the solution changes to pale yellow.

0.1 mol/L Magnesium Acetate
This solution contains 21.45 g of magnesium acetate ($Mg(CH_3COO)_2{\cdot}4H_2O$, molecular weight: 214.46) per 1,000 ml.

Weigh 21.5 g of magnesium acetate, and dissolve in water to make 1,000 ml.

Standardization Measure exactly 10 ml of the prepared magnesium acetate solution, add 50 ml of water and 3 ml of ammonia–ammonium chloride buffer (pH 10.7), and titrate with 0.05 mol/L EDTA (indicator: 3 drops of eriochrome black T TS).

0.05 mol/L Magnesium Chloride
This solution contains 10.17 g of magnesium chloride ($MgCl_2{\cdot}6H_2O$, molecular weight: 203.30) per 1,000 ml.

Weigh 10.2 g of magnesium chloride, and dissolve in freshly boiled and cooled water to make 1,000 ml.

Standardization Measure exactly 25 ml of the prepared magnesium chloride solution, add 50 ml of water, 3 ml of ammonia–ammonium chloride buffer (pH 10.7), and 0.04 g of eriochrome black T-sodium chloride indicator. Titrate with 0.05 mol/L EDTA. Near the endpoint, titrate slowly until the red-purple color of the solution changes to blue purple.

0.05 mol/L Oxalic Acid
This solution contains 6.303 g of oxalic acid ($C_2H_2O_4{\cdot}2H_2O$, molecular weight: 126.07) per 1,000 ml.

Weigh 6.45 g of oxalic acid, and dissolve in water to make 1,000 ml. Store in a glass-stoppered, light-resistant bottle.

Standardization Measure exactly 25 ml of the prepared oxalic acid solution, add 200 ml of diluted sulfuric acid (1 in 20), and heat to about 70°C. Titrate with freshly standardized 0.02 mol/L potassium permanganate while hot.

0.1 mol/L Perchloric Acid
This solution contains 10.05 g of perchloric acid ($HClO_4$, molecular weight: 100.46) per 1,000 ml.

Measure about 8.5 ml of perchloric acid, transfer into a 1,000-ml volumetric flask, add 950 ml of acetic acid, and shake well. Add 15 ml of acetic anhydride in 1-ml portions while shaking well, and add acetic acid to make 1,000 ml. Allow to stand overnight.

Standardization Weigh accurately about 0.4 g of potassium hydrogen phthalate, previously dried at 120°C for 1 hour, and dissolve in 50 ml of acetic acid while heating on a water bath. Titrate with the prepared perchloric acid solution (indicator: 1 ml of crystal violet–acetic acid TS) until the purple color of the solution changes to blue.

$$\text{Normality factor} = \frac{\left(\begin{array}{c}\text{Weight (g) of}\\ \text{potassium hydrogen phthalate}\end{array}\right) \times 1{,}000 \times 10}{\left(\begin{array}{c}\text{Volume (ml) of}\\ \text{0.1 mol/L perchloric acid consumed}\end{array}\right) \times 204.22}$$

1/60 mol/L Potassium Dichromate
This solution contains 4.903 g of potassium dichromate ($K_2Cr_2O_7$, molecular weight: 294.18) per 1,000 ml.

Weigh exactly 4.903 g of potassium dichromate (standard reagent), previously powdered and dried at 120°C to constant weight, and dissolve in water to make exactly 1,000 ml.

1 mol/L Potassium Hydroxide
This solution contains 56.11 g of potassium hydroxide (KOH, molecular weight: 56.11) per 1,000 ml.

Weigh about 70 g of potassium hydroxide, and proceed as directed under 1 mol/L Sodium Hydroxide to prepare and standardize the solution.

0.5 mol/L Potassium Hydroxide
This solution contains 28.05 g of potassium hydroxide (KOH, molecular weight: 56.11) per 1,000 ml.

Prepare by diluting 1 mol/L potassium hydroxide with freshly boiled and cooled water to 2 times its original volume, or prepare as directed under 1 mol/L Potassium Hydroxide, using about 35 g of potassium hydroxide. Standardize as directed under 1 mol/L Potassium Hydroxide.

0.1 mol/L Potassium Hydroxide
This solution contains 5.611 g of potassium hydroxide (KOH, molecular weight: 56.11) per 1,000 ml.

Prepare by diluting 1 mol/L potassium hydroxide with freshly boiled and cooled water to 10 times its original volume, or prepare as directed under 1 mol/L Potassium Hydroxide, using about 7 g of potassium hydroxide. Standardize as directed under 1 mol/L Potassium Hydroxide.

0.5 mol/L Potassium Hydroxide, Ethanolic
This solution contains 28.05 g of potassium hydroxide (KOH, molecular weight: 56.11) per 1,000 ml.

Weigh about 35 g of potassium hydroxide, dissolve in 20 ml of water, and add aldehyde-free ethanol to make 1,000 ml. Transfer the solution into a bottle tightly stoppered with a ground-glass or rubber stopper, allow to stand for 24 hours, and decant quickly the supernatant into another bottle. Stopper tightly with a rubber stopper, and store protected from light.

Standardization Measure exactly 25 ml of 0.5 mol/L hydrochloric acid, add 50 ml of water, and titrate with the prepared ethanolic potassium hydroxide solution (indicator: 2 drops of phenolphthalein TS).

0.1 mol/L Potassium Hydroxide, Ethanolic
This solution contains 5.611 g of potassium hydroxide (KOH, molecular weight: 56.11) per 1,000 ml.

Prepare and standardize, as directed under 0.5 mol/L Ethanolic Potassium Hydroxide, using about 7 g of potassium hydroxide.

0.02 mol/L Potassium Hydroxide, Ethanolic
This solution contains 1.122 g of potassium hydroxide (KOH, molecular weight: 56.11) per 1,000 ml.

Dilute 0.1 mol/L ethanolic potassium hydroxide with aldehyde-free ethanol to 5 times its original volume. Standardize as directed under 0.5 mol/L Potassium Hydroxide, Ethanolic.

0.02 mol/L Potassium Permanganate
This solution contains 3.161 g of potassium permanganate ($KMnO_4$, molecular weight: 158.03) per 1,000 ml.

Weigh about 3.3 g of potassium permanganate, dissolve in 1,000 ml of water, and boil for about 15 minutes. Allow to stand in a tightly stoppered flask for at least 2 days, and filter through a glass-filter (G4). Store in a light-resistant, glass-stoppered bottle, and restandardize frequently.

Standardization Weigh accurately about 0.2 g of sodium oxalate (standard reagent), previously dried at 110°C to constant weight, and dissolve in about 250 ml of water. Add 7 ml of sulfuric acid, heat to about 70°C, and titrate with the prepared potassium permanganate solution while hot.

Each ml of 0.02 mol/L $KMnO_4$ = 6.700 mg of $Na_2C_2O_4$

0.1 mol/L Silver Nitrate
This solution contains 16.99 g of silver nitrate ($AgNO_3$, molecular weight: 169.87) per 1,000 ml.

Weigh about 17.5 g of silver nitrate, and dissolve in 1,000 ml of water. Store protected from light.

Standardization Measure exactly 25 ml of 0.1 mol/L sodium chloride, and add 50 ml of water and 1 ml of potassium chromate solution (1 in 20). While shaking, titrate with the prepared silver nitrate solution until a persistent light red-brown color is produced.

0.1 mol/L Sodium Acetate
This solution contains 13.61 g of sodium acetate ($CH_3COONa{\cdot}3H_2O$, molecular weight: 136.08) per 1,000 ml.

Weigh 8.2 g of anhydrous sodium acetate, and dissolve in acetic acid to make 1,000 ml.

Standardization Measure exactly 25 ml of the prepared sodium acetate solution, add 50 ml of acetic acid, and titrate with 0.1 mol/L perchloric acid (indicator: 1 ml of α-naphtholbenzein) until the color of the solution changes from yellow-brown through yellow to green. Perform a blank test, and make any necessary correction.

0.1 mol/L Sodium Chloride
This solution contains 5.844 g of sodium chloride (NaCl, molecular weight: 58.44) per 1,000 ml.

Weigh exactly 5.844 g of sodium chloride (standard reagent), previously dried at 110°C for 2 hours, and dissolve in water to make exactly 1,000 ml.

1 mol/L Sodium Hydroxide
This solution contains 40.00 g of sodium hydroxide (NaOH, molecular weight: 40.00) per 1,000 ml.

Weigh 45 g of sodium hydroxide, dissolve in about 950 ml of water, and add a freshly prepared saturated solution of barium hydroxide until no more precipitate is formed. Shake the mixture well, stopper tightly, and allow to stand overnight. Collect the supernatant by decantation or filtration. Store in a bottle tightly stoppered with a rubber stopper, or in a bottle equipped with an absorption tube of carbon dioxide (soda lime), and restandardize frequently.

Standardization Weigh accurately about 5 g of potassium

hydrogen phthalate, previously powdered and dried at 100°C for 3 hours, dissolve in 75 ml of freshly boiled and cooled water, and titrate with the prepared sodium hydroxide solution (indicator: 2 drops of phenolphthalein TS).

0.5 mol/L Sodium Hydroxide
This solution contains 20.00 g of sodium hydroxide (NaOH, molecular weight: 40.00) per 1,000 ml.

Using about 22 g of sodium hydroxide, prepare, standardize, and store, as directed under 1 mol/L Sodium Hydroxide. Restandardize frequently.

0.25 mol/L Sodium Hydroxide
This solution contains 9.999 g of sodium hydroxide (NaOH, molecular weight: 40.00) per 1,000 ml.

Prepare by diluting 1 mol/L sodium hydroxide with freshly boiled and cooled water to 4 times its original volume, or prepare as directed under 1 mol/L Sodium Hydroxide, using about 11 g of sodium hydroxide. Standardize and store, as directed under 1 mol/L Sodium Hydroxide. Restandardize frequently.

0.2 mol/L Sodium Hydroxide
This solution contains 7.999 g of sodium hydroxide (NaOH, molecular weight: 40.00) per 1,000 ml.

Prepare by diluting 1 mol/L sodium hydroxide with freshly boiled and cooled water to 5 times its original volume, or prepare as directed under 1 mol/L Sodium Hydroxide, using about 9 g of sodium hydroxide. Standardize and store, as directed under 1 mol/L Sodium Hydroxide. Restandardize frequently.

0.1 mol/L Sodium Hydroxide
This solution contains 4.000g of sodium hydroxide (NaOH, molecular weight: 40.00) per 1,000 ml.

Prepare by diluting 1 mol/L sodium hydroxide with freshly boiled and cooled water to 10 times its original volume, or prepare as directed under 1 mol/L Sodium Hydroxide, using about 4.5 g of sodium hydroxide. Standardize and store, as directed under 1 mol/L Sodium Hydroxide. Restandardize frequently.

0.05 mol/L Sodium Hydroxide
This solution contains 2.000 g of sodium hydroxide (NaOH, molecular weight: 40.00) per 1,000 ml.

Dilute 1 mol/L sodium hydroxide with freshly boiled and cooled water to 20 times its original volume. Standardize and store, as directed under 1 mol/L Sodium Hydroxide. Restandardize frequently.

0.02 mol/L Sodium Hydroxide
This solution contains 0.7999 g of sodium hydroxide (NaOH, molecular weight: 40.00) per 1,000 ml.

Dilute 0.1 mol/L sodium hydroxide with freshly boiled and cooled water to 5 times its original volume. Standardize and store, as directed under 1 mol/L Sodium Hydroxide. Restandardize frequently.

0.01 mol/L Sodium Hydroxide
This solution contains 0.400 g of sodium hydroxide (NaOH, molecular weight: 40.00) per 1,000 ml.

Dilute 0.1 mol/L sodium hydroxide with freshly boiled and cooled water to 10 times its original volume. Standardize and store, as directed under 1 mol/L sodium hydroxide. Restandardize frequently.

0.1 mol/L Sodium Thiosulfate
This solution contains 24.82 g of sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$, molecular weight: 248.19) per 1,000 ml.

Weigh about 26 g of sodium thiosulfate and 0.2 g of anhydrous sodium carbonate, and dissolve in freshly boiled and cooled water to make 1,000 ml. Restandardize frequently.

Standardization Titrate 0.05 mol/L iodine with the prepared sodium thiosulfate solution. Alternatively, titrate 1/60 mol/L potassium dichromate with the prepared sodium thiosulfate solution as follows:

Measure exactly 30 ml of 1/60 mol/L potassium dichromate, transfer into a flask with a ground-glass stopper, and add 50 ml of water, 2 g of potassium iodide, and 5 ml of hydrochloric acid. Stopper tightly, and allow to stand for 10 minutes. Add 100 ml of water, and titrate with the prepared sodium thiosulfate solution (indicator: 4 ml of starch TS).

0.01 mol/L Sodium Thiosulfate
This solution contains 2.482 g of sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$, molecular weight: 248.19) per 1,000 ml.

Dilute 0.1 mol/L sodium thiosulfate with freshly boiled and cooled water to 10 times its original volume. Standardize as directed under 0.1 mol/L Sodium Thiosulfate before use.

0.005 mol/L Sodium Thiosulfate
This solution contains 1.241 g of sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$, molecular weight: 248.19) per 1,000 ml. Dilute 0.1 mol/L sodium thiosulfate with freshly boiled and cooled water to 20 times its original volume. Standardize as directed under 0.1 mol/L Sodium Thiosulfate before use.

0.5 mol/L Sulfuric Acid
This solution contains 49.04 g of sulfuric acid (H_2SO_4, molecular weight: 98.08) per 1,000 ml.

To about 1,000 ml of water, add slowly 30 ml of sulfuric acid while stirring, and allow to cool to 20°C.

Standardization Standardize as directed under 1 mol/L Hydrochloric Acid, or as follows:

Measure exactly 20 ml of the prepared sulfuric acid solution, and transfer into a 500-ml beaker. Add 250 ml of water and 1 ml of hydrochloric acid, and heat to boil. Add slowly warm barium chloride solution (3 in 25), while stirring continuously, until no more precipitate is formed. Heat the mixture on a water bath for 1 hour, collect the precipitate by filtering through a filter paper for quantitative analysis, wash with hot water until the washings do not respond to the test for Chloride, and dry together with the filter paper. Then, ignite to constant weight. Weigh the residue accurately, and calculate as $BaSO_4$.

0.25 mol/L Sulfuric Acid
This solution contains 24.52 g of sulfuric acid (H_2SO_4, molecular weight: 98.08) per 1,000 ml.

Prepare and standardize, as directed under 0.5 mol/L Sulfuric Acid, using 15 ml of sulfuric acid.

0.1 mol/L Sulfuric Acid
This solution contains 9.808 g of sulfuric acid (H_2SO_4, molecular weight: 98.08) per 1,000 ml.

Prepare and standardize, as directed under 0.5 mol/L Sulfuric Acid, using 6 ml of sulfuric acid.

0.05 mol/L Sulfuric Acid
This solution contains 0.4904 g of sulfuric acid (H_2SO_4, molecular weight: 98.08) per 1,000 ml.

Prepare by diluting 0.5 mol/L sulfuric acid with water to 10 times its original volume, or prepare as directed under 0.5 mol/L Sulfuric Acid, using 3 ml of sulfuric acid. Standardize as directed under 0.5 mol/L Sulfuric Acid.

0.005 mol/L Sulfuric Acid
This solution contains 0.4904 g of sulfuric acid (H_2SO_4, molecular weight: 98.08) per 1,000 ml.

Dilute 0.05 mol/L sulfuric acid with water to 10 times its original volume, and standardize as directed under 0.5 mol/L Sulfuric Acid.

0.1 mol/L Titanium Trichloride
This solution contains 15.42 g of titanium trichloride ($TiCl_3$, molecular weight: 154.24) per 1,000 ml.

Measure 75 ml of titanium trichloride solution, and add 75 ml of hydrochloric acid and freshly boiled and cooled water to make 1,000 ml. Transfer into a light-resistant bottle equipped with a burette, replace the air in the bottle with hydrogen, allow to stand for 2 days, and use. Standardize before use.

Standardization Weigh 3 g of ferrous ammonium sulfate, transfer into a wide-mouthed 500-ml flask, and, while passing carbon dioxide, add 50 ml of freshly boiled and cooled water to dissolve. Add 25 ml of diluted sulfuric acid (27 in 100), and add quickly 40 ml of 0.02 mol/L potassium permanganate, exactly measured, while passing carbon dioxide. Titrate with the prepared titanium trichloride solution to almost the end point, add immediately 5 g of ammonium thiocyanate, and continue the titration with the prepared titanium trichloride solution until the color of the solution disappears. Perform a blank test, and make any necessary correction.

$$\text{Normality factor} = \frac{\left(\begin{array}{c}\text{Volume (ml) of}\\ \text{0.02 mol/L potassium permanganate added}\end{array}\right)}{\left(\begin{array}{c}\text{Volume (ml) of}\\ \text{0.1 mol/L titanium trichloride consumed}\end{array}\right)}$$

0.1 mol/L Zinc Acetate
This solution contains 21.95 g of zinc acetate ($Zn(CH_3COO)_2{\cdot}2H_2O$, molecular weight: 219.53) per 1,000 ml.

Weigh about 22 g of zinc acetate, dissolve in 100 ml of water and 10 ml of diluted acetic acid (1 in 20), and add water to make 1,000 ml.

Standardization Measure exactly 20 ml of the prepared zinc acetate solution, and add 6 ml of ammonia–ammonium chloride buffer (pH 10.7) and water to make about 100 ml. Titrate with 0.1 mol/L EDTA (indicator: 3 drops of eriochrome black T TS).

0.02 mol/L Zinc Acetate
This solution contains 4.391 g of zinc acetate ($Zn(CH_3COO)_2{\cdot}2H_2O$, molecular weight: 219.53) per 1,000 ml.

Weigh 4.43 g of zinc acetate, dissolve in 20 ml of water and 2 ml of diluted acetic acid (1 in 20), and add water to make 1,000 ml.

Standardization Measure exactly 25 ml of the prepared zinc acetate solution, and add 2 ml of ammonia–ammonium chloride buffer (pH 10.7) and water to make about 100 ml. Titrate with 0.02 mol/L EDTA (indicator: 3 drops of eriochrome black T TS).

0.01 mol/L Zinc Acetate
This solution contains 2.195 g of zinc acetate ($Zn(CH_3COO)_2{\cdot}2H_2O$, molecular weight: 219.53) per 1,000 ml.

Weigh about 2 g of zinc acetate, and dissolve in water to make 1,000 ml.

Standardization Measure exactly 25 ml of the prepared zinc acetate, and add 2 ml of ammonia–ammonium chloride buffer (pH 10.7) and water to make about 100 ml. Titrate with 0.01 mol/L EDTA (indicator: 3 drops of eriochrome black T TS).

0.05 mol/L Zinc Chloride
The solution contains 6.816 g of zinc chloride ($ZnCl_2$, molecular weight: 136.32) per 1,000 ml.

Weigh accurately about 1.6 g of zinc (standard reagent), transfer to a beaker, and add 30 ml of diluted hydrochloric acid (1 in 4). Cover the beaker with a watch glass, and allow to stand. After the generation of hydrogen gas becomes gentle, dissolve by heating gently on a water bath. Wash the watch glass and the inside wall of the beaker with water, and evaporate to almost dryness on a water bath. Cool, and add water to make exactly 500 ml.

0.025 mol/L Zinc Chloride
The solution contains 3.408 g of zinc chloride ($ZnCl_2$, molecular weight: 136.32) per 1,000 ml.

Weigh accurately about 1.6 g of zinc (standard reagent), and proceed as directed under 0.05 mol/L Zinc Chloride. Cool, and add water to make exactly 1,000 ml.

0.1 mol/L Zinc Sulfate
This solution contains 28.76 g of zinc sulfate ($ZnSO_4{\cdot}7H_2O$, molecular weight: 287.58) per 1,000 ml.

Weigh 28.8 g of zinc sulfate, and dissolve in water to make 1,000 ml.

Standardization Measure exactly 25 ml of the prepared zinc sulfate, and add 5 ml of ammonia–ammonium chloride buffer (pH 10.7) and 0.04 g of eriochrome black T-sodium chloride indicator. Titrate with 0.1 mol/L EDTA until the red-purple color of the solution changes to blue-purple.

0.01 mol/L Zinc Sulfate
This solution contains 2.876 g of zinc sulfate ($ZnSO_4{\cdot}7H_2O$, molecular weight: 287.58) per 1,000 ml.

Weigh 2.9 g of zinc sulfate, and dissolve in water to make 1,000 ml.

Standardization Weigh accurately about 0.5 g of aluminum, add 20 ml of hydrochloric acid, dissolve while heating gently, and add water to make exactly 1,000 ml. Measure exactly 10 ml of the solution, transfer into a beaker containing 90 ml of water and 3 ml of hydrochloric acid, and add 1 drop of methyl orange TS and 25 ml of 0.02 mol/L EDTA. Add dropwise ammonia TS until the red color of the solution changes to orange-yellow, add 10 ml of ammonium acetate

buffer and 10 ml of diammonium phosphate buffer, boil for 5 minutes, and cool quickly. Mix 3 drops of xylenol orange TS, and add dropwise the prepared zinc sulfate solution until the yellow color of the solution changes to reddish yellow. Add 2 g of sodium fluoride, boil for 2 to 5 minutes, cool quickly, and titrate the liberated EDTA with the 0.01 mol/L zinc sulfate until the yellow color of the solution changes to reddish yellow. Calculate the weight (T mg/ml) of aluminum oxide (Al_2O_3) equivalent to 1 ml of 0.01 mol/L zinc sulfate by the following formula:

$$T\ (mg/ml) = \frac{18.895 \times \text{Weight (g) of aluminum}}{\left(\begin{array}{c}\text{Volume (ml) of} \\ \text{0.01 mol/L zinc sulfate consumed}\end{array}\right)}$$

3. Standard Solutions

Aluminum Standard Stock Solution
Weigh 1.0 g of aluminum, add 60 ml of diluted hydrochloric acid (1 in 2), and heat to dissolve. Cool, and add water to make 1,000 ml. Measure exactly 10 ml of this solution, add 30 ml of water and 5 ml of ammonium acetate buffer (pH 3.0), adjust the pH to about 3 by adding ammonia TS dropwise. Add 0.5 ml of Cu–PAN TS, and titrate with 0.01 mol/L EDTA while boiling until the red color of the solution changes to yellow and maintain more than one minute. Perform a blank test in the same manner, and make any necessary correction.

Each ml of 0.01 mol/L EDTA = 0.26982 mg of Al

Ammonium Standard Solution
Weigh exactly 2.97 g of ammonium chloride, and add water to make 1,000 ml. Measure exactly 10 ml of this solution, and add water to make 1,000 ml. Each ml of this solution contains 0.01 mg ammonium (NH_4).

Arsenic Standard Stock Solution
Weigh exactly 0.10 g of arsenic trioxide, previously very finely powdered and dried at 105°C for 4 hours, and dissolve in 5 ml of sodium hydroxide solution (1 in 5). Neutralize with diluted sulfuric acid (1 in 20), and add 10 ml of diluted sulfuric acid (1 in 20) and freshly boiled and cooled water to make exactly 1,000 ml. Each ml of this solution contains 0.1 mg of arsenic trioxide (As_2O_3).

Arsenic Standard Solution
Measure exactly 10 ml of Arsenic Standard Stock Solution, add 10 ml of diluted sulfuric acid (1 in 20), and add freshly boiled and cooled water to make exactly 1,000 ml. Each ml of this solution contains 1 µg of arsenic trioxide (As_2O_3). Prepare fresh before use, and store in a ground glass-stoppered bottle.

Barium Standard Solution
Weigh exactly 1.779 g of barium chloride, and dissolve in water to make exactly 1,000 ml. Each ml of this solution contains 1 mg of barium (Ba).

Bromide Ion Standard Stock Solution
Weigh exactly 0.129 g of sodium bromide, previously dried at 110°C for 2 hours, and dissolve in water to make exactly 1,000 ml. Each ml of this solution contains 100 µg of Bromide ion (Br^-).

Calcium Standard Solution (0.1 mg/ml)
To 2.50 g of calcium carbonate, add 100 ml of diluted hydrochloric acid (1 in 10). Heat it gently without boiling, cool, and add water to make 1,000 ml. To 10 ml of this solution, add water to make 100 ml.

Chloride Ion Standard Stock Solution
Weigh exactly 0.165 g of sodium chloride (standard reagent), previously dried at 500–600°C for 1 hour, and dissolve in water to make exactly 1,000 ml. Each ml of this solution contains 100 µg of chloride ion (Cl^-).

Chromium Standard Solution
Weigh exactly 0.934 g of potassium chromate, and dissolve in 1 drop of sodium hydroxide solution (1 in 10) and water to make exactly 1,000 ml. Measure exactly 10 ml of the solution, and add 1 drop of sodium hydroxide solution (1 in 10) and water to make exactly 1,000 ml. Each ml of this solution contains 2.5 µg of chromium (Cr).

Cyanide Standard Stock Solution
Weigh 2.5 g of potassium cyanide, and dissolve in water to make exactly 1,000 ml. Standardize before use. Stopper tightly, and store in a cold, dark place.

Standardization Measure exactly 100 ml of the prepared cyanide standard stock solution, and titrate with 0.1 mol/L silver nitrate (indicator: 0.5 ml of *p*-dimethylamino benzylidenerhodanine TS) until a red color develops.

Each ml of 0.1 mol/L silver nitrate = 5.204 mg of CN

Cyanide Standard Solution
Measure exactly an amount of Cyanide Standard Stock Solution equivalent to 10 mg of cyanide (CN), and add 100 ml of sodium hydroxide solution (1 in 25) and water to make exactly 1,000 ml. Prepare fresh before use. Each ml of this solution contains 0.01 mg of cyanide (CN).

Dilute Formaldehyde Standard Solution
See Formaldehyde Standard Solution, Dilute.

Dimethylamine Hydrochloride Standard Solution
Weigh exactly 1.116 g of dimethylamine hydrochloride, and dissolve in water to make exactly 1,000 ml. Measure exactly 1 ml of the solution, and add water to make exactly 1,000 ml. Each ml of this solution contains the equivalent of 1 µg of dimethylformamide (C_3H_7NO).

Formaldehyde Standard Solution, Dilute
Weigh exactly 0.54 g of formalin (equivalent to 37% HCHO), and add water to make exactly 1,000 ml. Measure exactly 10 ml of the solution, and add water to make exactly 1,000 ml. Each ml of this solution contains 2 µg of formaldehyde (HCHO). Prepare fresh before use.

Iodide Ion Standard Stock Solution
Weigh exactly 0.118 g of sodium iodide, previously dried at 110°C for 2 hours, and dissolve in water to make exactly 1,000 ml. Each ml of this solution contains 100 µg of Iodide ion (I^-).

Iron Standard Solution
Weigh exactly 8.63 g of ferric ammonium sulfate, and dissolve in 20 ml of diluted nitric acid (1 in 10) and water to make exactly 1,000 ml. Measure exactly 10 ml of the solution, and add 20 ml of diluted nitric acid (1 in 10) and water to make exactly 1,000 ml. Each ml of this solution contains 0.01 mg of iron (Fe). Store protected from light.

Lead Standard Stock Solution
Weigh exactly 0.1599 g of lead nitrate, dissolve in 10 ml of diluted nitric acid (1 in 10), and add water to make exactly 1,000 ml. Each ml of this solution contains 0.1 mg of lead (Pb). For the preparation and storage of this solution, use glass instruments free from soluble lead salts.

Lead Standard Solution
Measure exactly 10 ml of Lead Standard Stock Solution, and add water to make exactly 100 ml. Each ml of this solution contains 10 μg of lead (Pb). Prepare fresh before use.

Lithium Lactate Standard Solution
Weigh exactly 0.1066 g of lithium lactate, previously dried at 105°C for 4 hours, and dissolve in water to make exactly 1,000 ml. Each ml of this solution contains 0.1 mg of lactic acid ($C_3H_6O_3$). Prepare fresh before use.

Manganese Standard Solution
Weigh exactly 0.2877 g of potassium permanganate, dissolve in 100 ml of water and 1 ml of sulfuric acid, add 0.5 g of sodium hydrogen sulfite, and boil. Cool, and add water to make exactly 200 ml. Measure exactly 20 ml of the solution, and add water to make exactly 1,000 ml. Each ml of this solution contains 0.01 mg of manganese (Mn).

Matching Fluids
According to the table below, transfer the prescribed volumes of Colorimetric Standard Stock Solutions and water into a test tube, and mix them. To transfer them, use a burette or pipette with precise graduations of 0.1 ml or less. The preparation of each Colorimetric Standard Stock Solution (CSSS) is given below.

Colorimetric Standard Stock Solution (CSSS)
Prepare each CSSS as directed below, and store in a ground glass-stoppered bottle.

Cobaltous Chloride CSSS
Weigh about 65 g of cobaltous chloride, and dissolve in diluted hydrochloric acid (1 in 40) to make 1,000 ml. Measure exactly 5 ml of this solution, transfer into a 250-ml flask with a ground-glass stopper, add 5 ml of hydrogen peroxide TS and 15 ml of sodium hydroxide solution (1 in 5), and boil for 10 minutes. Cool, and add 2 g of potassium iodide and 20 ml of diluted sulfuric acid (1 in 4). After the precipitate is dissolved, titrate with 0.1 mol/L sodium thiosulfate (indicator: starch TS). Each ml of 0.1 mol/L sodium thiosulfate is equivalent to 23.79 mg of cobaltous chloride ($CoCl_2 \cdot 6H_2O$, molecular weight: 237.93). To the remaining portion of the cobaltous chloride solution, add diluted hydrochloric acid (1 in 40) to make a solution containing 59.5 mg of cobaltous chloride ($CoCl_2 \cdot 6H_2O$) per ml.

Cupric Sulfate CSSS
Weigh about 65 g of cupric sulfate, and dissolve in diluted hydrochloric acid (1 in 40) to make 1,000 ml. Measure exactly 10 ml of the solution, transfer into a 250-ml flask with a ground-glass stopper, and add 40 ml of water. Add 4 ml of diluted acetic acid (1 in 4) and 3 g of potassium iodide, and titrate with 0.1 mol/L sodium thiosulfate (indicator: starch TS). Each ml of 0.1 mol/L sodium thiosulfate is equivalent to 24.97 mg of cupric sulfate ($CuSO_4 \cdot 5H_2O$, molecular weight: 249.69). To the remaining portion of the cupric sulfate solution, add diluted hydrochloric acid (1 in 40) to make a solution containing 62.4 mg of cupric sulfate ($CuSO_4 \cdot 5H_2O$) per ml.

Symbol for Matching Fluid	Volume (ml) of Cobaltous Chloride CSSS	Volume (ml) of Ferric Chloride CSSS	Volume (ml) of Cupric Sulfate CSSS	Volume (ml) of Water
A	0.1	0.4	0.1	4.4
B	0.3	0.9	0.3	3.5
C	0.1	0.6	0.1	4.2
D	0.3	0.6	0.4	3.7
E	0.4	1.2	0.3	3.1
F	0.3	1.2	0.0	3.5
G	0.5	1.2	0.2	3.1
H	0.2	1.5	0.0	3.3
I	0.4	2.2	0.1	2.3
J	0.4	3.5	0.1	1.0
K	0.5	4.5	0.0	0.0
L	0.8	3.8	0.1	0.3
M	0.1	2.0	0.1	2.8
N	0.0	4.9	0.1	0.0
O	0.1	4.8	0.1	0.0
P	0.2	0.4	0.1	4.3
Q	0.2	0.3	0.1	4.4
R	0.3	0.4	0.2	4.1
S	0.2	0.1	0.0	4.7
T	0.5	0.5	0.4	3.6

Ferric Chloride CSSS
Weigh about 55 g of ferric chloride, and dissolve in diluted hydrochloric acid (1 in 40) to make 1,000 ml. Measure exactly 10 ml of the solution, transfer into a 250-ml flask with a ground-glass stopper, and add 15 ml of water and 3 g of potassium iodide. Stopper tightly, and allow to stand in a dark place for 15 minutes. Add 100 ml of water, and titrate with 0.1 mol/L sodium thiosulfate (indicator: starch TS). Each ml of 0.1 mol/L sodium thiosulfate is equivalent to 27.03 mg of ferric chloride ($FeCl_3 \cdot 6H_2O$, molecular weight: 270.30). To the remaining portion of the ferric chloride solution, add diluted hydrochloric acid (1 in 40) to make a solution containing 45.0 mg of ferric chloride ($FeCl_3 \cdot 6H_2O$) per ml.

Mercury Standard Solution
Weigh exactly 0.135 g of mercuric chloride, and dissolve in 10 ml of diluted nitric acid (1 in 10) and water to make exactly 1,000 ml. Measure exactly 10 ml of the solution, and add 10 ml of diluted nitric acid (1 in 10) and water to make exactly 1,000 ml. Measure exactly 10 ml of the resulting solution, and add 10 ml of diluted nitric acid (1 in 10) and water to make exactly 100 ml. Each ml of this solution contains 0.1 μg of mercury (Hg). Prepare fresh before use.

Monopotassium Phosphate Standard Solution
Weigh exactly 4.394 g of monopotassium phosphate, and dissolve in water to make exactly 1,000 ml. Each ml of this solution contains 1 mg of phosphorus (P).

Nickel Standard Solution
Weigh exactly 6.73 g of ammonium nickel sulfate, dissolve in water to make exactly 1,000 ml. Measure exactly 5 ml of the solution, and add water to make exactly 1,000 ml. Each ml of this solution contains 0.005 mg of nickel (Ni).

Nitrate Ion Standard Stock Solution
See Nitrate Standard Solution.

Nitrate Standard Solution
Weigh exactly 1.631 g of potassium nitrate and dissolve in water to make exactly 1,000 ml. Measure exactly 10 ml of the solution, and add water to make exactly 100 ml. Each ml of this solution contains 0.1 mg of nitrate (NO_3).

Phosphate Standard Solution
Weigh exactly 0.1433 g of monopotassium phosphate, and dissolve in water to make exactly 100 ml. Measure exactly 10 ml of the solution, and add water to make exactly 1,000 ml. Each ml of this solution contains 0.01 mg of the phosphate (PO_4).

Potassium Standard Solution (0.1 mg/ml)
To 1.91 g of potassium chloride, add water to make 1,000 ml. To 10 ml of this solution, add water to male 100 ml.

Sodium Standard Solution (0.1 mg/ml)
Dissolve 2.54 g of sodium chloride in water to make 1,000 ml. To 10 ml of this solution, add water to make 100 ml.

Strontium Standard Solution (5.0 mg/ml)
Dissolve 2.42 g of strontium nitrate in water to make 200 ml.

Sulfate Ion Standard Stock Solution
Weigh exactly 0.148 g of sodium sulfate, previously dried at 110°C for 2 hours, and dissolve in water to make exactly 1,000 ml. Each ml of this solution contains 100 μg of sulfate ion (SO_4^{2-}).

Tyrosine Standard Solution
Weigh exactly 0.050 g of Tyrosine Reference Standard, previously dried at 105°C for 3 hours, and dissolve in 0.1 mol/L hydrochloric acid to make exactly 50 ml. Measure exactly 5 ml of this solution, add 0.1 mol/L hydrochloric acid to make exactly 100 ml.

Water–Methanol Standard Solution
Measure 500 ml of methanol for water determination, transfer into a dry 1,000-ml volumetric flask, add 2 ml of water, and add methanol for water determination again to make 1,000 ml. The standardization of this solution is done immediately after water determination TS is standardized. Store in a cold place, protected from light and moisture.

Standardization According to the procedure directed under Water Determination, transfer 25 ml of methanol for water determination into a dry titration flask, and add carefully water determination TS to the end point. Add exactly10 ml of water determination TS, and titrate with Water-Methanol Standard Solution to the end point. Calculate the number of mg of water (H_2O), f′, contained in 1 ml of Water-Methanol Standard Solution by the following formula:

$$f' = \frac{f \times 10}{\left(\text{Volume (ml) of Water–Methanol Standard Solution consumed}\right)}$$

f = The number of mg of water (H_2O) equivalent to 1 ml of water determination TS.

Zinc Standard Solution
Weigh exactly 4.40 g of zinc sulfate, and dissolve in water to make exactly 1,000 ml. Measure exactly 10 ml of the solution, and add water to make exactly 1,000 ml. Each ml of this solution contains 0.01 mg of zinc (Zn).

4. Reference Standards

(1) For Reference Standards listed in this section, use products manufactured by persons who are registered with the Minister of Health, Labour and Welfare, as specified by the Minister.

Food Blue No. 1 Reference Standard
Food Blue No. 2 Reference Standard
Food Green No. 3 Reference Standard
Food Red No. 2 Reference Standard
Food Red No. 3 Reference Standard
Food Red No. 40 Reference Standard
Food Red No. 102 Reference Standard
Food Red No. 104 Reference Standard
Food Red No. 105 Reference Standard
Food Red No. 106 Reference Standard

Food Yellow No. 4 Reference Standard
Food Yellow No. 5 Reference Standard
Natamycin Reference Standard
Xylitol Reference Standard

(2) ***p*-Aminobenzoylglutamic Acid Reference Standard**
Use Reference Standard specified in the Japanese Pharmacopoeia.

(3) **Cyanocobalamin Reference Standard**
Use Reference Standard specified in the Japanese Pharmacopoeia

(4) **Folic Acid Reference Standard**
Use Reference Standard specified in the Japanese Pharmacopoeia.

(5) **Glycyrrhizic Acid Reference Standard**
Use Reference Standard specified in the Japanese Pharmacopoeia.

(6) **Lysozyme Reference Standard**
Use Reference Standard specified in the Japanese Pharmacopoeia.

(7) **Nicotinamide Reference Standard**
Use Reference Standard specified in the Japanese Pharmacopoeia.

(8) **Riboflavin Reference Standard**
Use Reference Standard specified in the Japanese Pharmacopoeia.

(9) **Saccharated Pepsin Reference Standard**
Use Reference Standard specified in the Japanese Pharmacopoeia.

(10) **Thiamine Hydrochloride Reference Standard**
Use Reference Standard specified in the Japanese Pharmacopoeia.

(11) ***dl*-α-Tocopherol Reference Standard**
Use Reference Standard specified in the Japanese Pharmacopoeia.

(12) **Tyrosine Reference Standard**
Use Reference Standard specified in the Japanese Pharmacopoeia.

5. Thermometers

Unless otherwise specified, use rod thermometers with an immersion line or total immersion mercury-filled rod thermometers calibrated under the Japanese Industrial Standards. For the tests directed under Congealing Point, Boiling Point and Distillation Range Tests, and Melting Point (Class 1 substances), use thermometers with an immersion line. The rod thermometers with an immersion line are shown in the following table.

Standards for Thermometers with an Immersion Line

	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
Liquid	Mercury	Mercury	Mercury	Mercury	Mercury	Mercury
Gas filled above liquid	Nitrogen or Argon	Nitrogen or Argon	Nitrogen or Argon	Nitrogen or Argon	Nitrogen or Argon	Nitrogen or Argon
Temperature range	–17 to 50°C	40 to 100°C	90 to 150°C	140 to 200°C	190 to 250°C	240 to 320°C
Minimum graduation	0.2°C	0.2°C	0.2°C	0.2°C	0.2°C	0.2°C
Longer graduation lines	each 1°C	each 1°C	each 1°C	each 1°C	each 1°C	each 1°C
Graduation number	each 2°C	each 2°C	each 2°C	each 2°C	each 2°C	each 2°C
Total length (mm)	280–300	280–300	280–300	280–300	280–300	280–300
Stem diameter (mm)	6.0 ± 0.3	6.0 ± 0.3	6.0 ± 0.3	6.0 ± 0.3	6.0 ± 0.3	6.0 ± 0.3
Bulb length (mm)	12–18	12–18	12–18	12–18	12–18	12–18
Distance from the bottom of bulb to the lowest graduation line (mm)	75–90	75–90	75–90	75–90	75–90	75–90
Distance from the top of thermometer to the highest graduation line (mm)	35–65	35–65	35–65	35–65	35–65	35–65
Distance from the bottom of bulb to immersion line (mm)	58–62	58–62	58–62	58–62	58–62	58–62
Form of top of thermometer	loop	loop	loop	loop	loop	loop
Test temperature	–15°C 15°C 45°C	45°C 70°C 95°C	95°C 120°C 145°C	145°C 170°C 195°C	195°C 220°C 245°C	245°C 280°C 315°C
Allowable limit of error	0.2°C	0.2°C	0.2°C	0.2°C	0.3°C (0.2°C when the temperature tested is 195°C)	0.4°C (0.5°C when the temperature tested is 315°C)

Note. For auxiliary thermometers, use appropriate types of mercury thermometers with a temperature range of 0°C to 360°C and a minimum graduation of not more than 1°C.

6. Filter Papers

Use filter papers conforming to the specifications given below. Unless otherwise specified, when the term "filter paper" is given alone, use filter papers for qualitative analysis. Filter papers must be stored, protected from gases and other contaminants.

Filter Paper for Qualitative Analysis
Use filter papers conforming to the specifications for filter papers for qualitative analysis under the Japanese Industrial Standards (for chemical analysis).

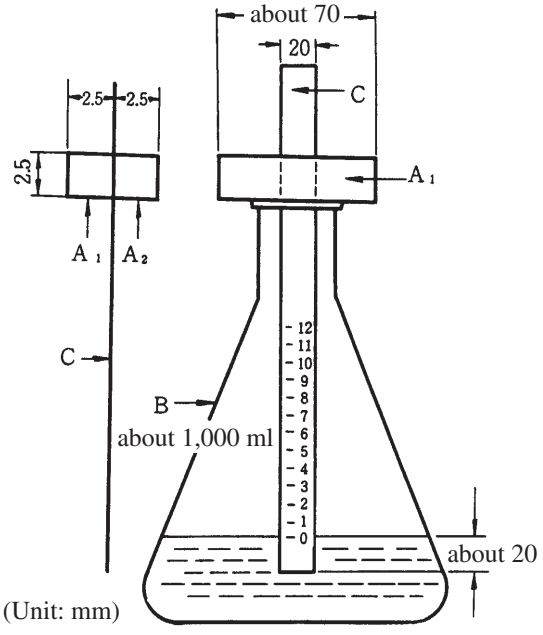
Filter Paper for Quantitative Analysis
Use filter papers conforming to the specifications for filter papers for quantitative analysis under the Japanese Industrial Standards (for chemical analysis).

Filter Paper for Chromatography
Use filter papers conforming to the specifications for filter papers for the quantitative analysis and the specifications given in the table below. The tests for α-cellulose content, copper value, pH, ash content, filtration time, and wet burst strength should be performed as directed under the Japanese Industrial Standards. The test for water absorption should be performed as directed below.

Class	No. 1	No. 2	No. 3	No. 4
α-Cellulose content (%)	Not less than 90	Not less than 95	Not less than 95	Not less than 95
Copper value (%)	Not more than 1.6	Not more than 1.4	Not more than 1.4	Not more than 1.4
pH	5–8	5–8	5–8	5–8
Ash content (%)	Not more than 0.02	Not more than 0.12	Not more than 0.12	Not more than 0.12
Filtration time (sec)	330 ± 132	240 ± 96	120 ± 48	100 ± 40
Wet burst strength (cm)	Not less than 13	Not less than 20	Not less than 12	Not less than 15
Water absorption (cm)	6 ± 1.2	5.5 ± 1.1	7 ± 1.4	7.5 ± 1.5

Test for Water Absorption

Apparatus
Use the apparatus illustrated below.



A_1 and A_2: Glass block to hold the filter paper
B: Erlenmeyer flask (Capacity: about 1,000 ml)
C: Sample filter paper

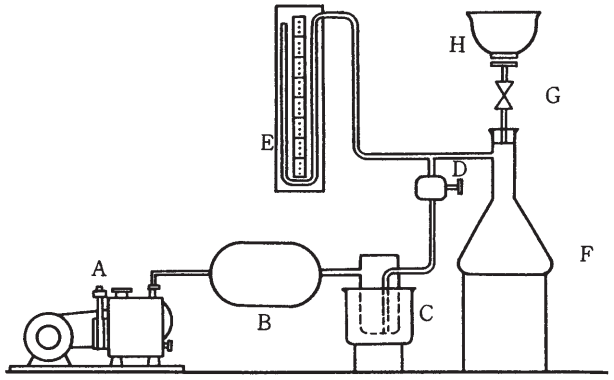
Procedure
Transfer about 300 ml of distilled water into Erlenmeyer flask B, and place 2 pieces of glass blocks (A_1 and A_2) in parallel on the mouth of the Erlenmeyer flask to hold the filter paper. Insert a sample filter paper, previously marked with 1-cm graduations using a pencil, between the two glass blocks. Gently slip down the filter paper in water until its lower edge reaches the surface of the water, then quickly slip it down until the zero mark is at the water-level, and fix the filter paper. Measure the height of water absorbed by the filter paper in 10 minutes.

Membrane Filter
Use membrane filters conforming to the specifications given in the following table. The tests for thickness should be performed, according to the testing methods for paper thickness and paper density under the Japanese Industrial Standards. The tests for water flow rate and bubble point should be performed as directed below:

Pore diameter (μm)	Thickness (μm)	Water flow rate (ml/min/cm^2)	Bubble point (N/mm^2)
1.0 or 1.2	100–170	150–300	5.9×10^{-2}–14.7×10^{-2}
0.45	130–170	20–60	16.7×10^{-2}–34.3×10^{-2}
0.10	90–150	1.0–5.0	49.0×10^{-2}–294.2×10^{-2}
0.05	70–150	0.1–2.0	98.1×10^{-2}–490.3×10^{-2}

Water Flow Rate Test

Apparatus
Use the apparatus illustrated in the following figure:



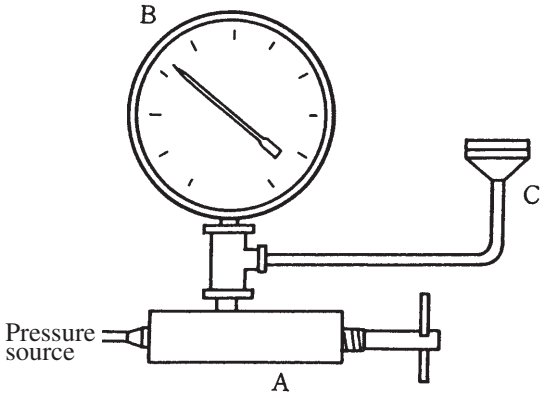
A: Vacuum pump
B: Reservoir (Capacity: not less than 10 liters)
C: Cold trap
D: Vacuum regulator
E: Manometer
F: Suction filter bottle (Capacity: 1–4 liters)
G: Valve
H: Filter device (1,000-ml container, equipped with a filter holder 47 mm in internal diameter, supported by a stainless steel screen)

Procedure
Close valve G and open vacuum regulator D fully to reduce the pressure in the system with vacuum pump A. Then, using D, adjust the pressure in the system to 69 ± 0.7 kPa. Place the sample membrane filter into the filter holder. The filter should be previously moistened with water, taking care not to allow air to enter the filter. Then assemble the filter device. Measure 500 ml of water, previously filtered twice through a membrane filter with the same pore size as the sample filter or with a smaller pore size, and pour into the filter device. Open valve G, measure the time it takes to finish filtering, and calculate the water flow rate by the formula:

$$\text{Water flow rate (ml/min/cm}^2\text{)} = \frac{500\ (\text{ml}) \times 60}{\text{Filtration time (sec)} \times \text{Effective filtration area (cm}^2\text{)}}$$

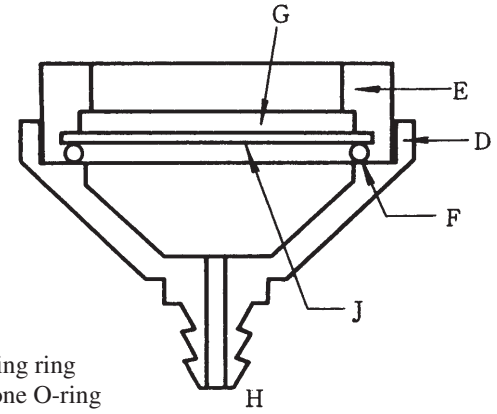
Bubble Point Test

Apparatus
Use the apparatus illustrated in Figures 1 and 2.



A: Regulator
B: Pressure gauge
C: Filter holder (9.5±0.5 cm² in effective filtration area, illustrated in Figure 2.)

Fig. 1



D: Base
E: Locking ring
F: Silicone O-ring
G: Supporting disk
H: Air inlet
J: Sample membrane filter

Fig. 2

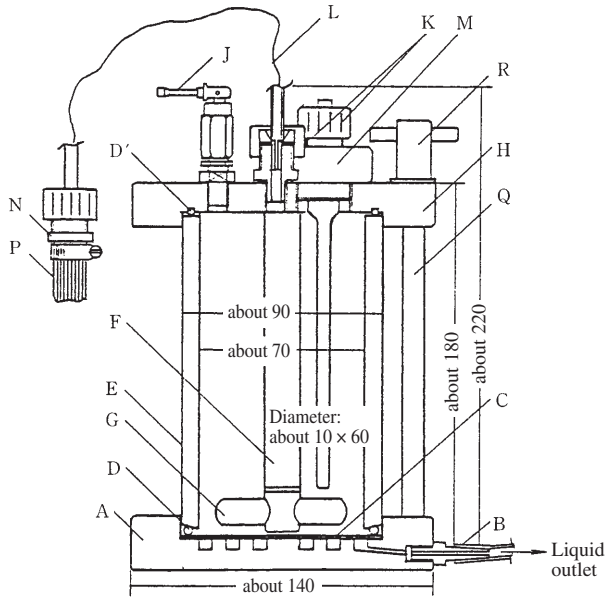
Procedure
Moisten completely the sample membrane filter with water, fit it in the filter holder. Put water into the holder until 2 to 3 mm above supporting disk G. Adjust the pressure to a point not exceeding the expected bubble point, using regulator A, and increase the pressure at a rate of 0.14×10^{-2}N/mm² per second. Regard the pressure at which a stable effervescence occurs at the center of the sample membrane filter.

7. Filters

Glass Filter
Use a glass filter conforming to the specifications for chemical analysis-grade glass filters under the Japanese Industrial Standards.

Pressure Filter
Operate a pressure filter as directed in the procedure given below.

Apparatus
Generally, apparatus is as illustrated below.



(Unit: mm)

A: Base plate
B: Liquid outlet tube
C: Supporting screen
D, D′: Silicone O-ring
E: Cell
F: Supporting column for stirrer
G: Stirrer
H: Cover
J: Safety valve
K: Tube joint cap
L: Pressure-resistant tube
M: Sample inlet
N: Pressure source connector
P: Pressure-resistant hose
Q: Clamping shaft
R: Cross nut for clamping

Procedure
Attach liquid outlet tube B to base plate A, place the membrane filter on supporting screen C, and attach silicone O-ring D to the surface of the membrane filter. Place cell E on D, attach silicone O-ring D′ to cover H, to which stirring apparatus F and G, safety valve J, and other parts are attached, and place on E. Set up clamping shaft Q to H, and tighten uniformly with cross nut R. Place the pressure filter on the stirrer, and pour the sample liquid through sample inlet M. Connect the pressure source (such as a nitrogen cylinder) and the pressure filter, using pressure-resistant hose P and pressure-resistant tube L, increase gradually the pressure to the specified level, and filter the sample. During filtration, stir slowly to the extent that effervescence ceases.

8. Sieves

Use sieves conforming to the specifications for sieves under the Japanese Industrial Standards.

9. Detector Tube Type Gas-Measuring Instruments

Use a detector tube type gas-measuring device that meets the Japan Industrial Standards.

10. Bertrand Table

Bertrand Table (1/2)

Sugar Weight (mg)	Mass of Copper Equivalent to Each Sugar (mg)					Sugar Weight (mg)	Mass of Copper Equivalent to Each Sugar (mg)				
	Invert Sugar	Glucose	Galactose	Maltose	Lactose		Invert Sugar	Glucose	Galactose	Maltose	Lactose
10	20.6	20.4	19.3	11.2	14.4	33	64.8	64.6	61.5	36.5	46.1
11	22.6	22.4	21.2	12.3	15.8	34	66.7	66.5	63.3	37.6	47.4
12	24.6	24.3	23.0	13.4	17.2	35	68.5	68.3	65.0	38.7	48.7
13	26.5	26.3	24.9	14.5	18.6	36	70.3	70.1	66.8	39.8	50.1
14	28.5	28.3	26.7	15.6	20.0	37	72.2	72.0	68.6	40.9	51.4
15	30.5	30.2	28.6	16.7	21.4	38	74.0	73.8	70.4	41.9	52.7
16	32.5	32.2	30.5	17.8	22.8	39	75.9	75.7	72.1	43.0	54.1
17	34.5	34.2	32.3	18.9	24.2	40	77.7	77.5	73.9	44.1	55.4
18	36.4	36.2	34.2	20.0	25.6	41	79.5	79.3	75.6	45.2	56.7
19	38.4	38.1	36.0	21.1	27.0	42	81.2	81.1	77.4	46.3	58.0
20	40.4	40.1	37.9	22.2	28.4	43	83.0	82.9	79.1	47.4	59.3
21	42.3	42.0	39.7	23.3	29.8	44	84.4	84.7	80.8	48.5	60.6
22	44.2	43.9	41.6	24.4	31.1	45	86.5	86.4	82.5	49.5	61.9
23	46.1	45.8	43.4	25.5	32.5	46	88.3	88.2	84.3	50.6	63.3
24	48.0	47.7	45.2	26.6	33.9	47	90.1	90.0	86.6	51.7	64.6
25	49.8	49.6	47.0	27.7	35.2	48	91.9	91.8	87.7	52.8	65.9
26	51.7	51.5	48.9	28.9	36.6	49	93.6	93.6	89.5	53.9	67.2
27	53.6	53.4	50.7	30.0	38.0	50	95.4	95.4	91.2	55.0	68.5
28	55.5	55.3	52.5	31.1	39.4	51	97.1	97.1	92.9	56.1	69.8
29	57.4	57.2	54.4	32.2	40.7	52	98.8	98.9	94.6	57.1	71.1
30	59.3	59.1	56.2	33.3	42.1	53	100.6	100.6	96.3	58.2	72.4
31	61.1	60.9	58.0	34.4	43.4	54	102.2	102.3	98.0	59.3	73.7
32	63.0	62.8	59.7	35.5	44.8	55	104.0	104.1	99.7	60.3	74.9

(Continued)

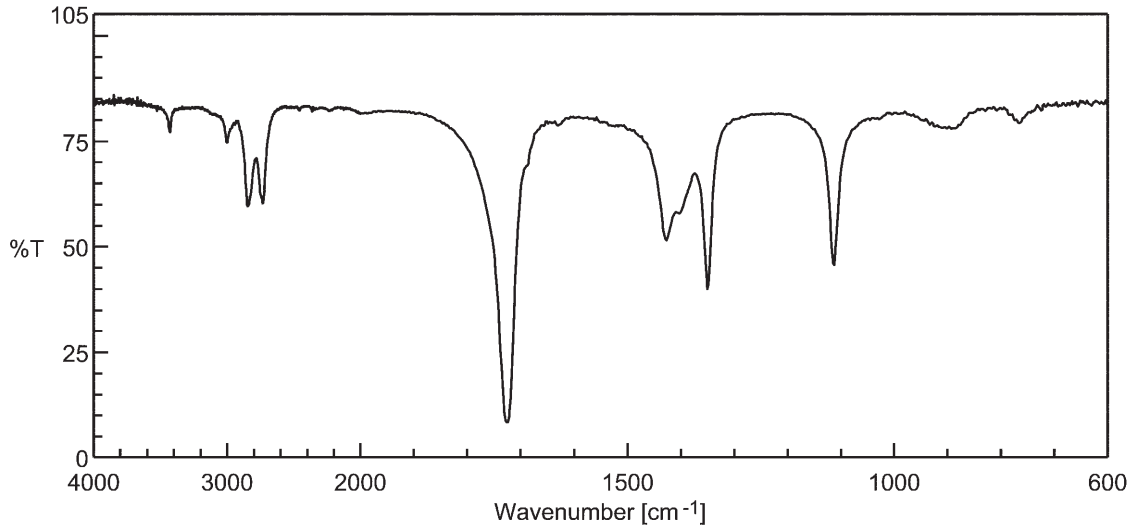
Bertrand Table (2/2)

Sugar Weight (mg)	Mass of Copper Equivalent to Each Sugar (mg)					Sugar Weight (mg)	Mass of Copper Equivalent to Each Sugar (mg)				
	Invert Sugar	Glucose	Galactose	Maltose	Lactose		Invert Sugar	Glucose	Galactose	Maltose	Lactose
56	105.7	105.8	101.5	61.4	76.2	79	143.7	144.5	139.7	86.1	105.4
57	107.4	107.6	103.2	62.5	77.5	80	145.3	146.1	141.3	87.2	106.7
58	109.2	109.3	104.9	63.5	78.8	81	146.9	147.7	142.9	88.3	107.9
59	110.9	111.1	106.2	64.6	80.1	82	148.5	149.3	144.6	89.4	109.2
60	112.6	112.8	108.3	65.7	81.4	83	150.0	150.9	146.2	90.4	110.4
61	114.3	114.5	110.0	66.8	82.7	84	151.6	152.5	147.8	91.5	111.7
62	115.9	116.2	111.6	67.9	83.9	85	153.2	154.0	149.4	92.6	112.9
63	117.6	117.9	113.3	68.9	85.2	86	154.8	155.6	151.1	93.7	114.1
64	119.2	119.6	115.0	70.0	86.5	87	156.4	157.2	152.7	94.8	115.4
65	120.9	121.3	116.6	71.1	87.7	88	157.9	158.3	154.3	95.8	116.6
66	122.6	123.0	118.3	72.2	89.0	89	159.5	160.4	156.0	96.9	117.9
67	124.2	124.7	120.0	73.3	90.3	90	161.1	162.0	157.6	98.0	119.1
68	125.9	126.4	121.7	74.3	91.6	91	162.6	163.6	159.2	99.0	120.3
69	127.5	128.1	123.3	75.4	92.8	92	164.2	165.2	160.8	100.1	121.6
70	129.2	129.8	125.0	76.5	94.1	93	165.7	166.7	162.4	101.1	122.8
71	130.8	131.4	126.6	77.6	95.4	94	167.3	168.3	164.0	102.2	124.0
72	132.4	133.1	128.3	78.6	96.7	95	168.8	169.9	165.6	103.2	125.2
73	134.0	134.7	130.0	79.7	98.0	96	170.3	171.5	167.2	104.2	126.5
74	135.6	136.3	131.5	80.8	99.1	97	171.9	173.1	168.8	105.3	127.7
75	137.2	137.9	133.1	81.8	100.4	98	173.4	174.6	170.4	106.3	128.9
76	138.9	139.6	134.8	82.9	101.7	99	175.0	176.2	172.0	107.4	130.2
77	140.5	141.2	136.4	84.0	102.9	100	176.5	177.8	173.6	108.4	131.4
78	142.1	142.8	138.0	85.1	104.2						

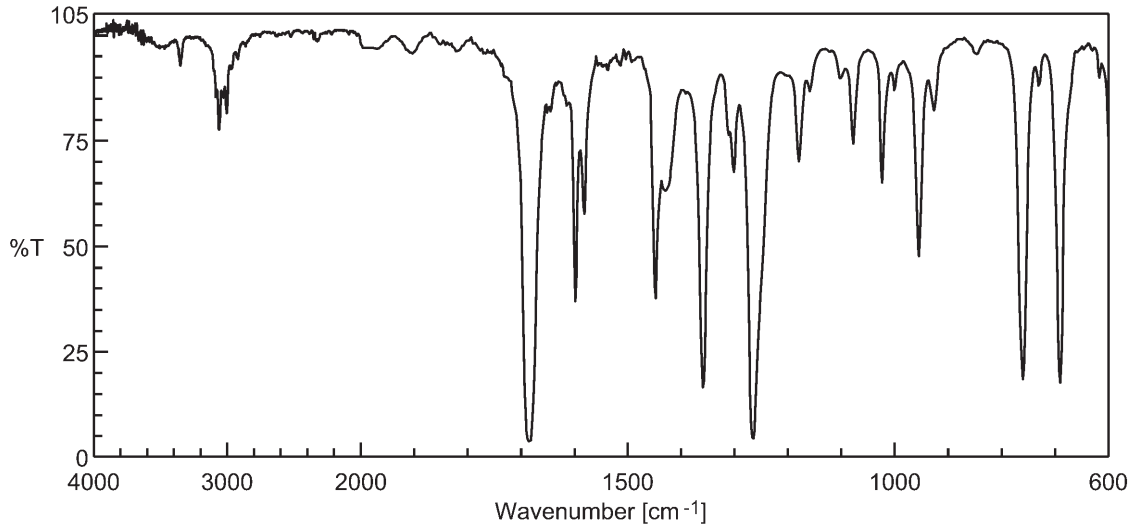
11. Infrared Reference Spectra

The infrared reference spectra contained in this section were obtained at a resolution of 4 cm^{-1}, using a Fourier-transform infrared spectrophotometer, under the conditions specified in the individual monographs. The horizontal axis indicates the wavenumber (cm^{-1}) and the vertical axis indicates the transmittance (%). As a reference, a potassium bromide disk without any sample was used in the potassium bromide disk method (10 mm in diameter), and an optical plate was used in the paste, thin film , and liquid film methods.

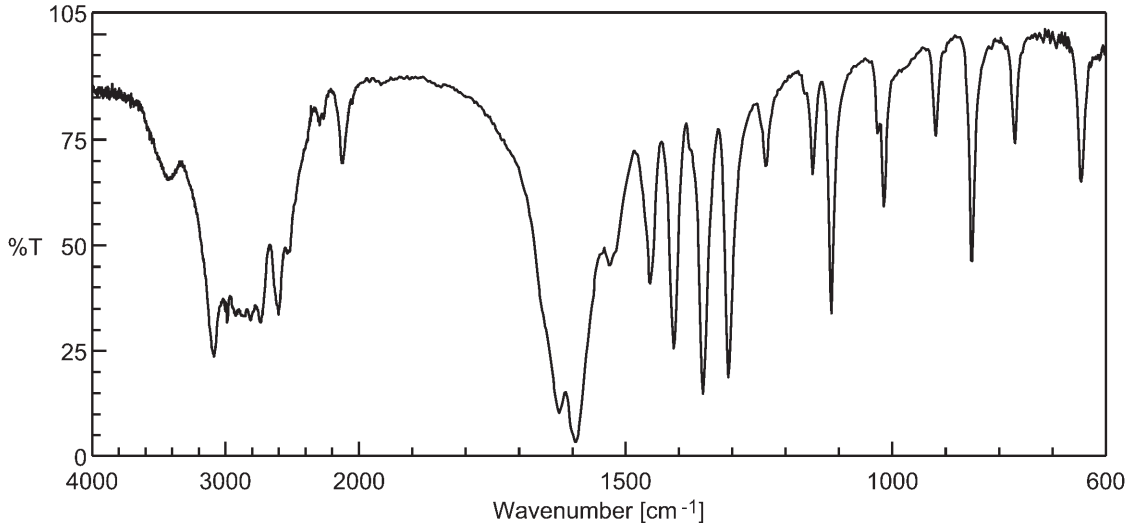
Acetaldehyde



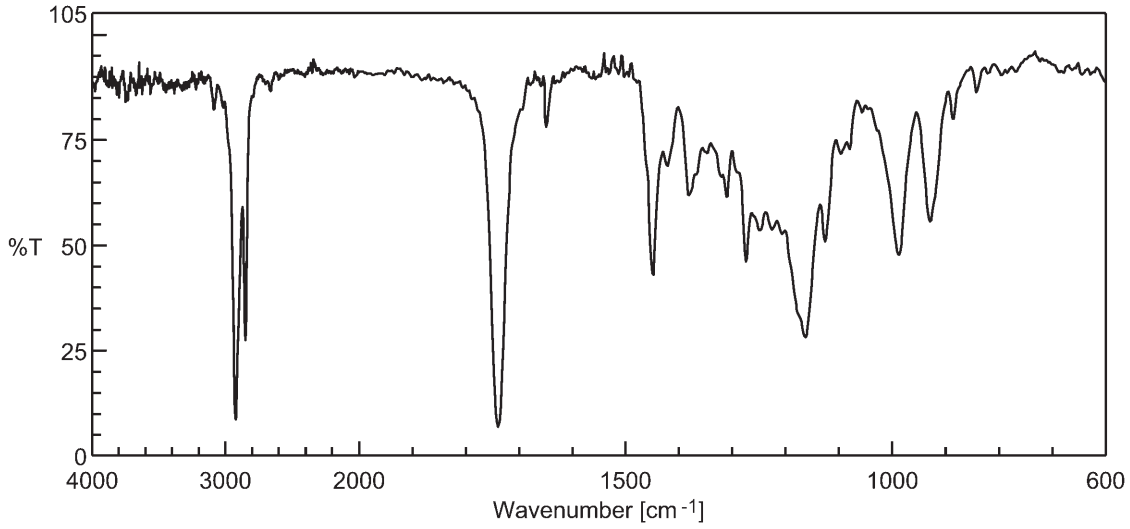
Acetophenone



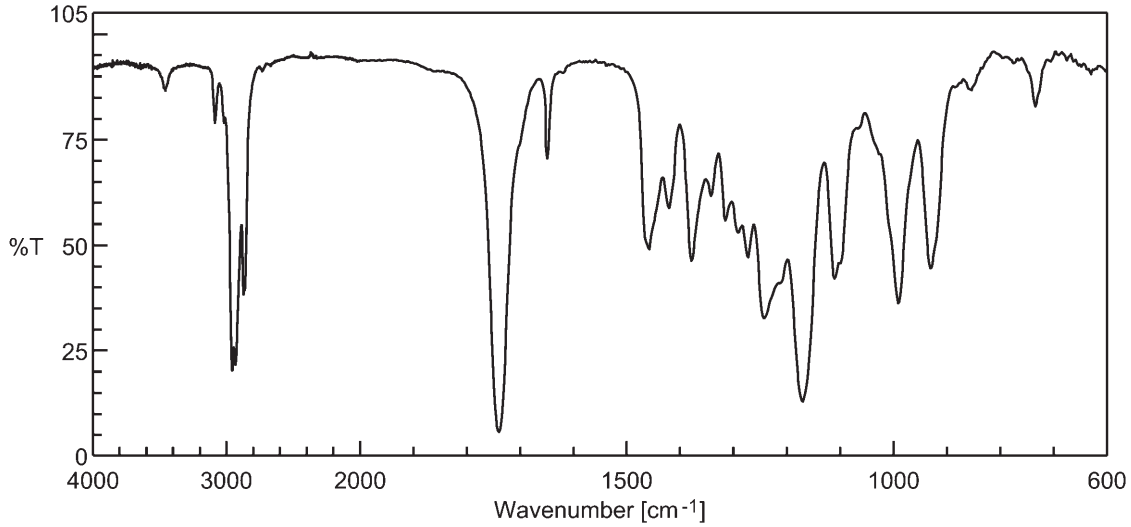
DL-Alanine



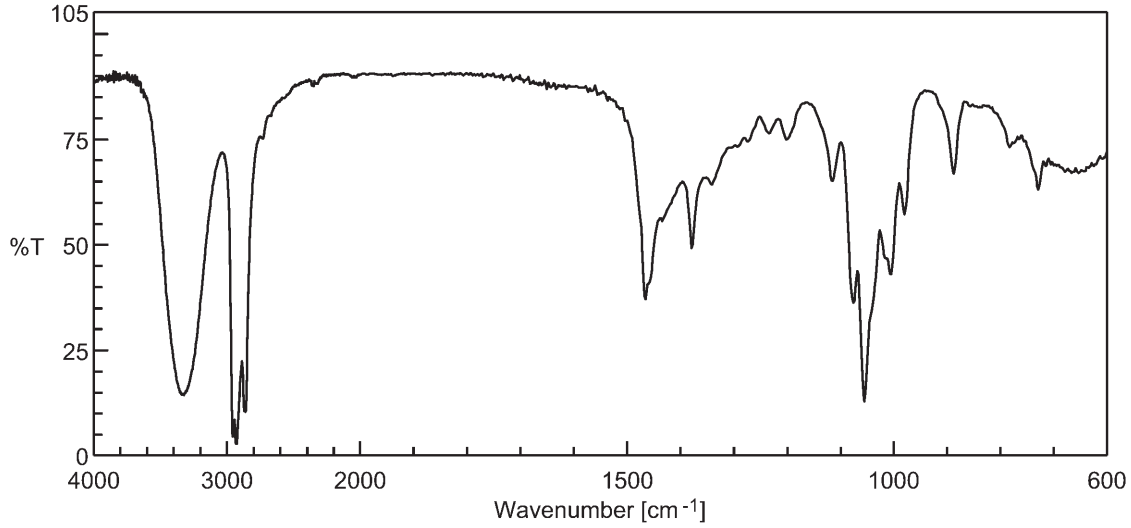
Allyl Cyclohexylpropionate



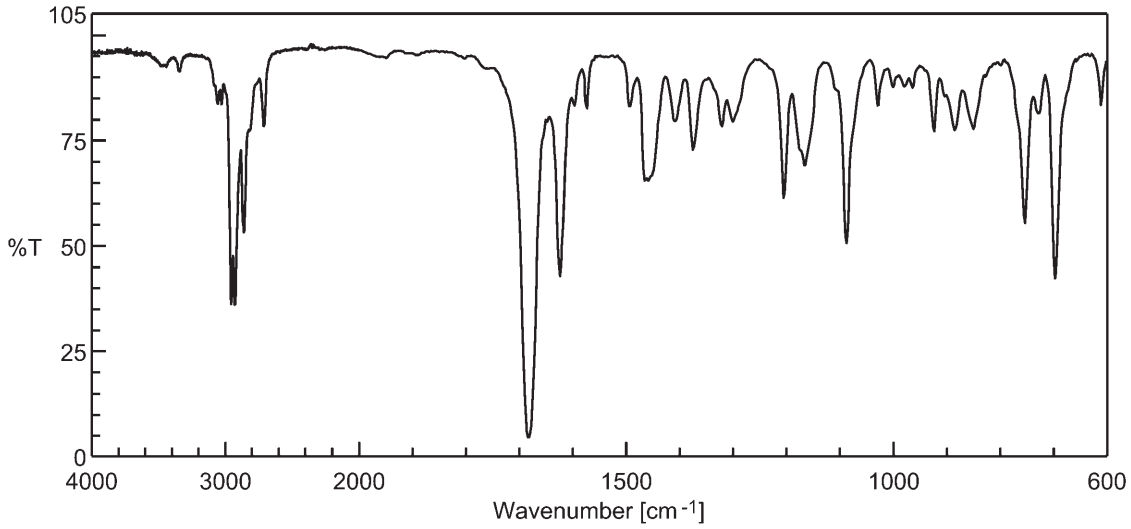
Allyl Hexanoate



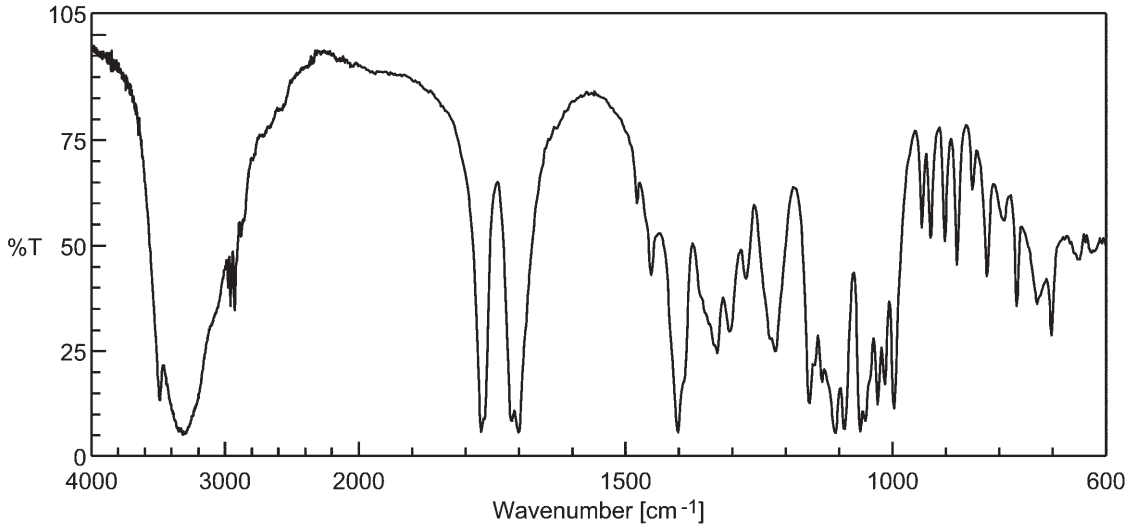
Amyl Alcohol



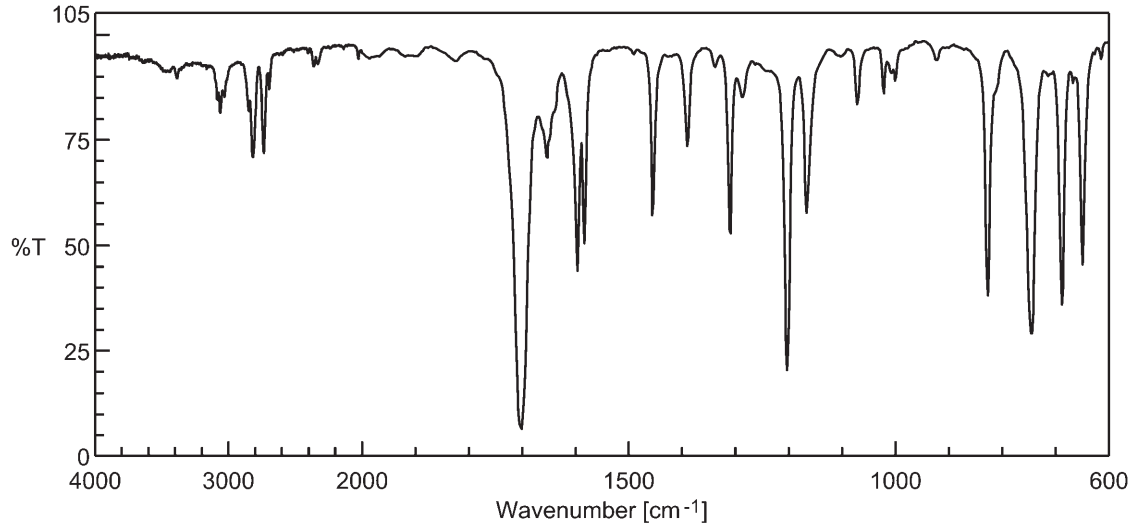
α-Amylcinnamaldehyde



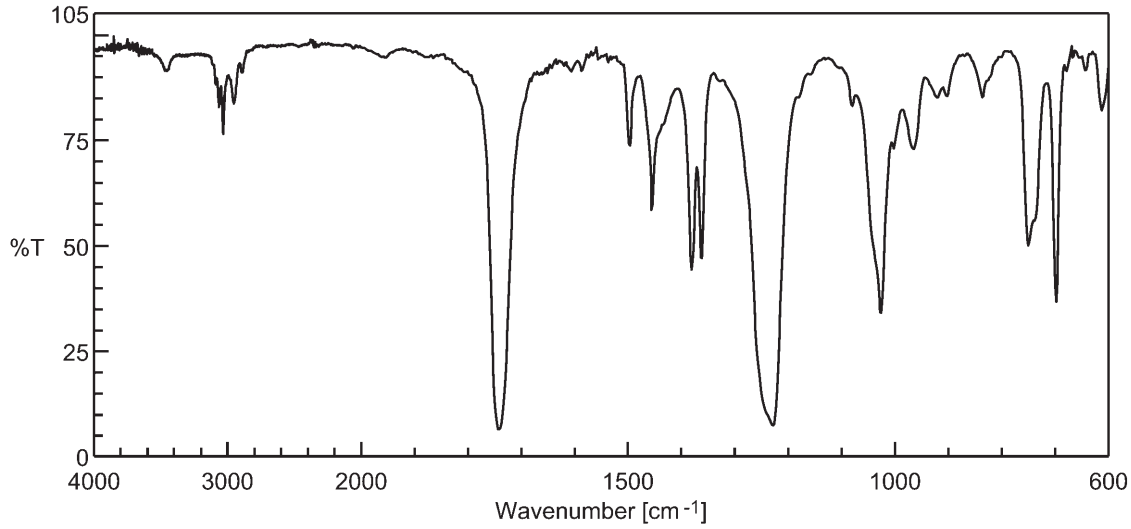
L-Ascorbic Acid 2-Glucoside



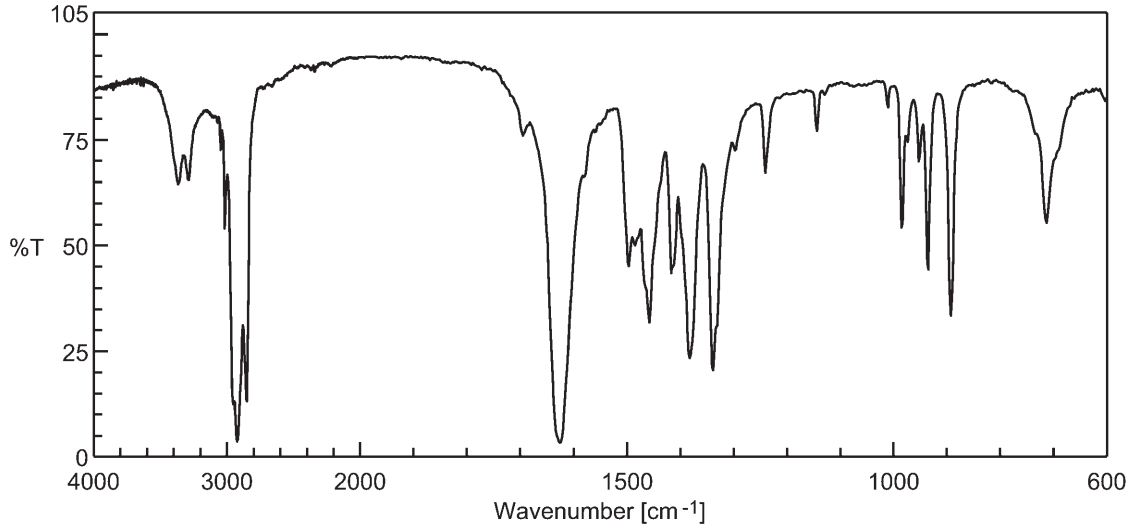
Benzaldehyde



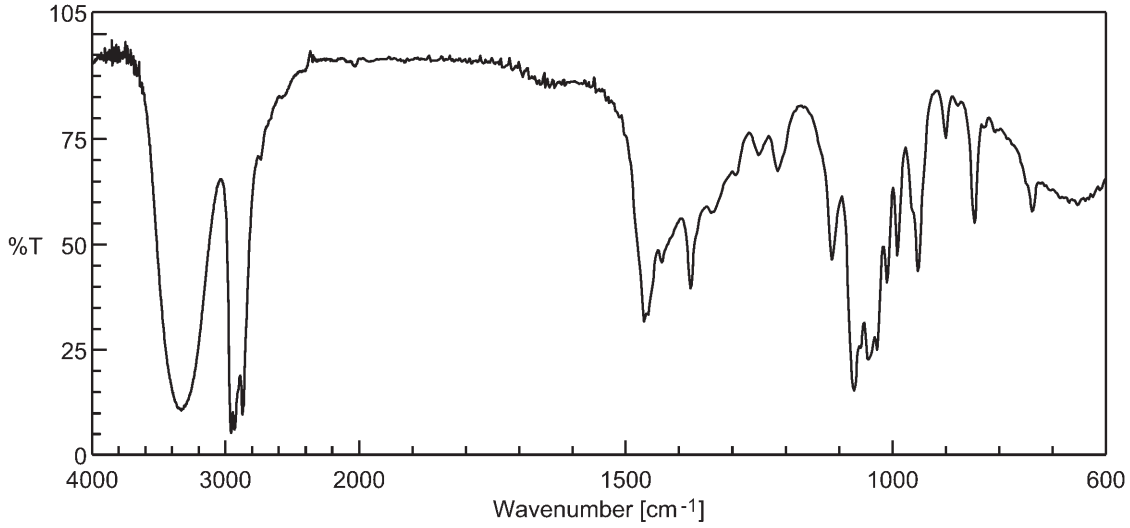
Benzyl Acetate



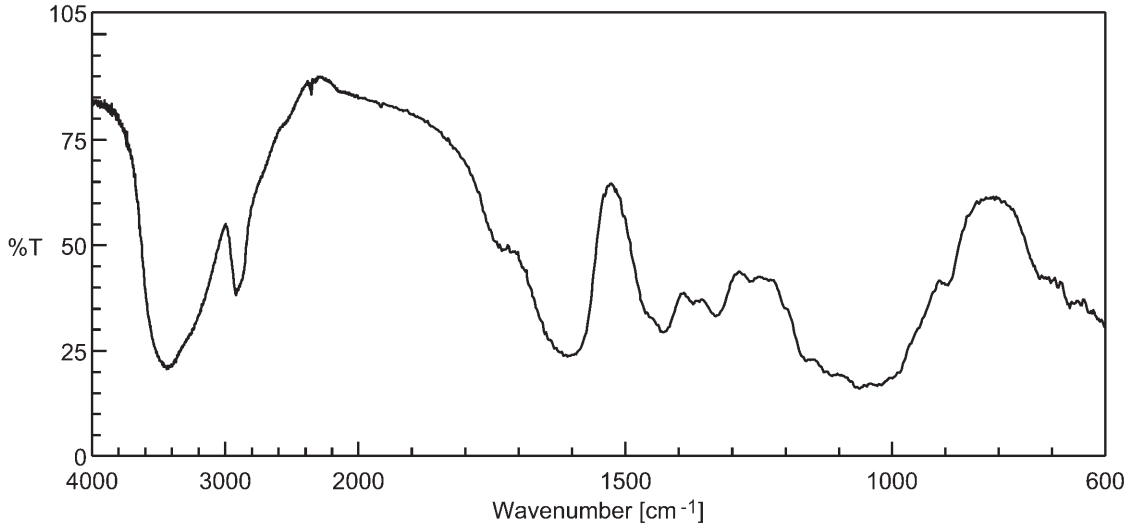
Betaine



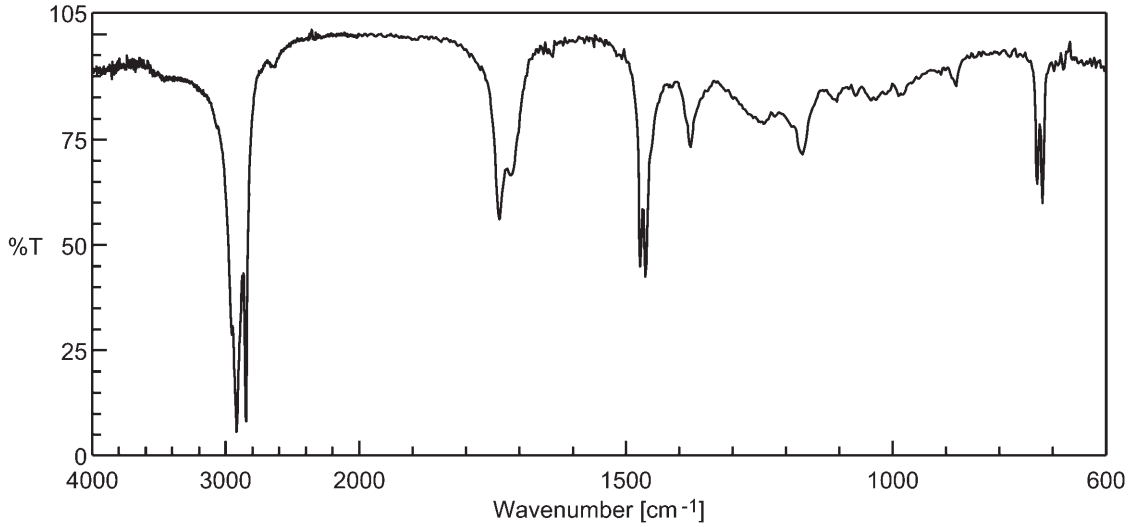
Butanol



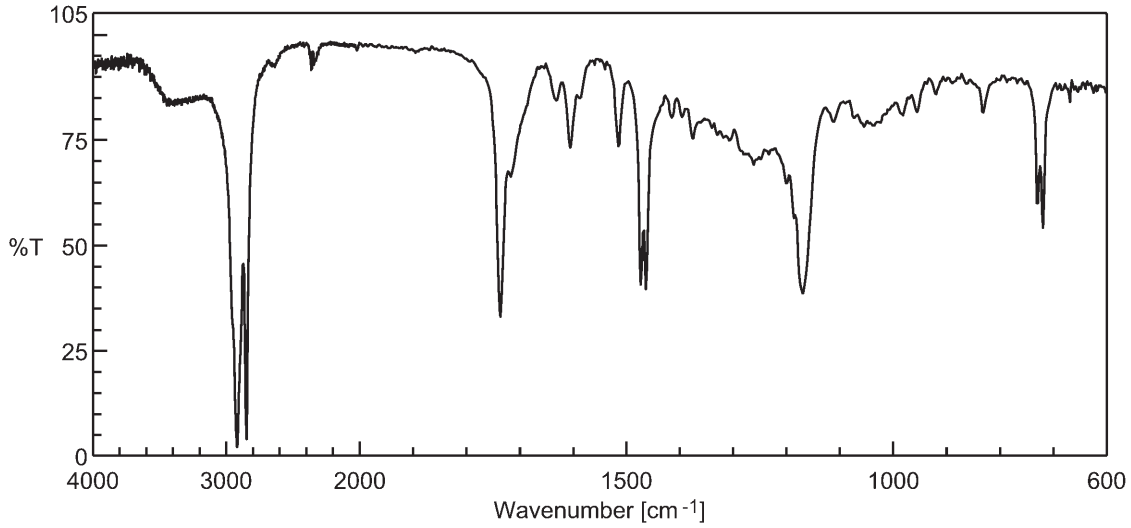
Calcium Carboxymethylcellulose



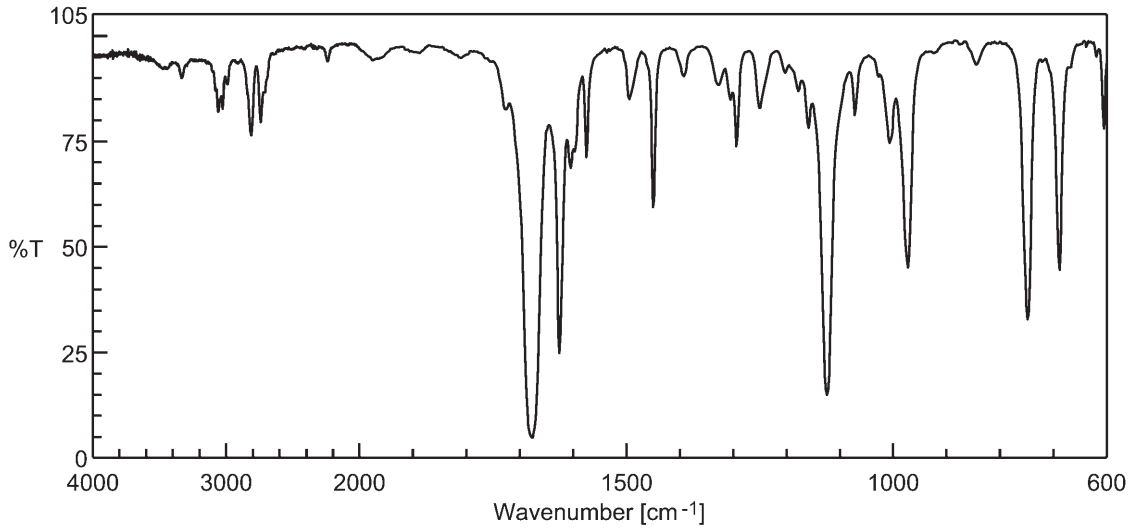
Candelilla Wax



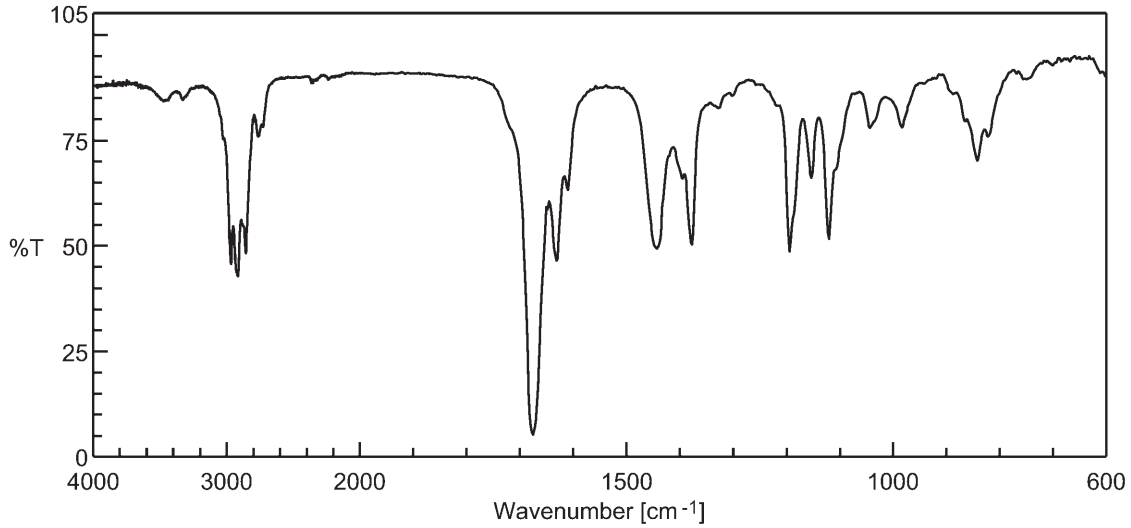
Carnauba Wax



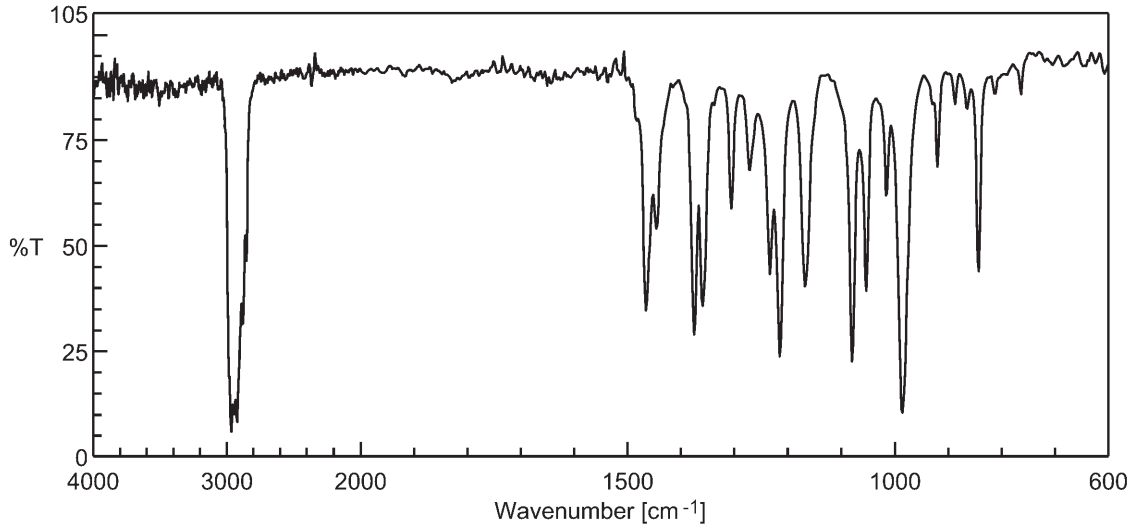
Cinnamaldehyde



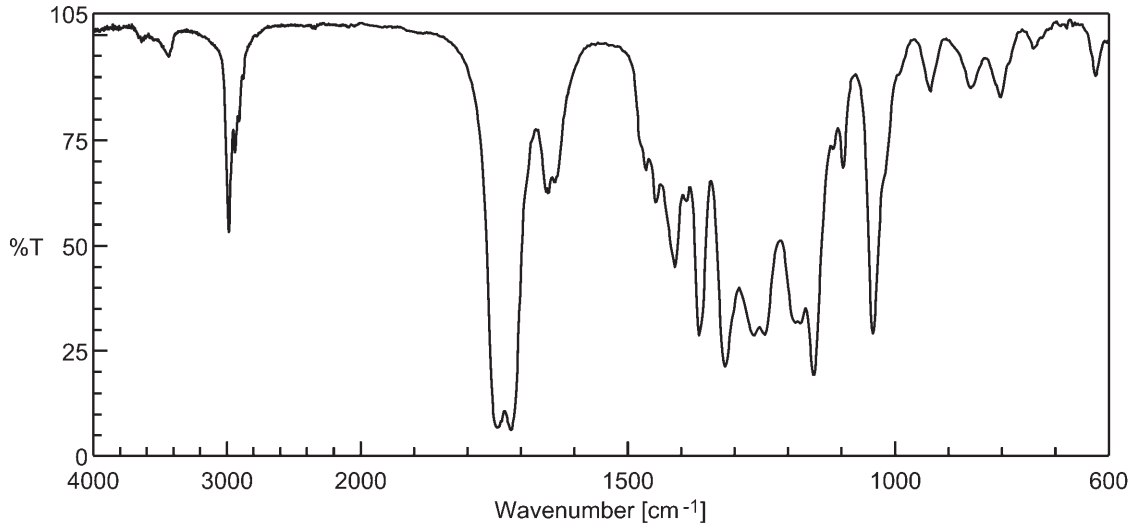
Citral



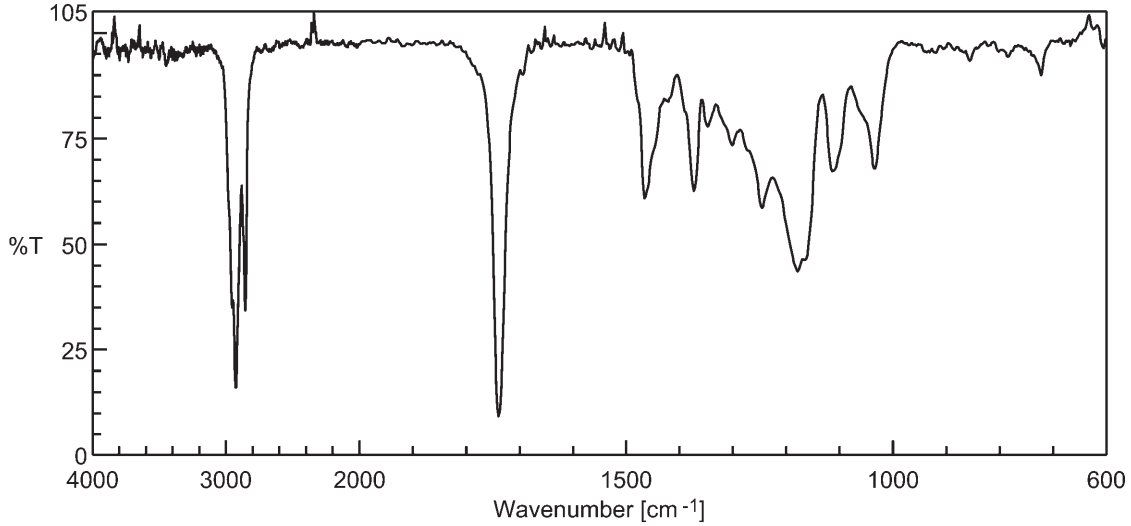
1,8-Cineole



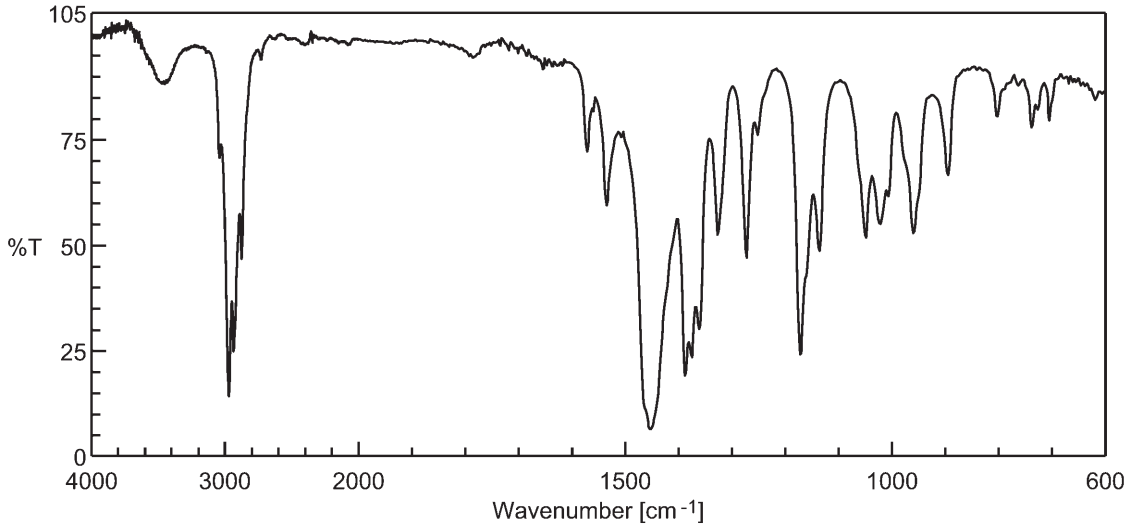
Ethyl Acetoacetate



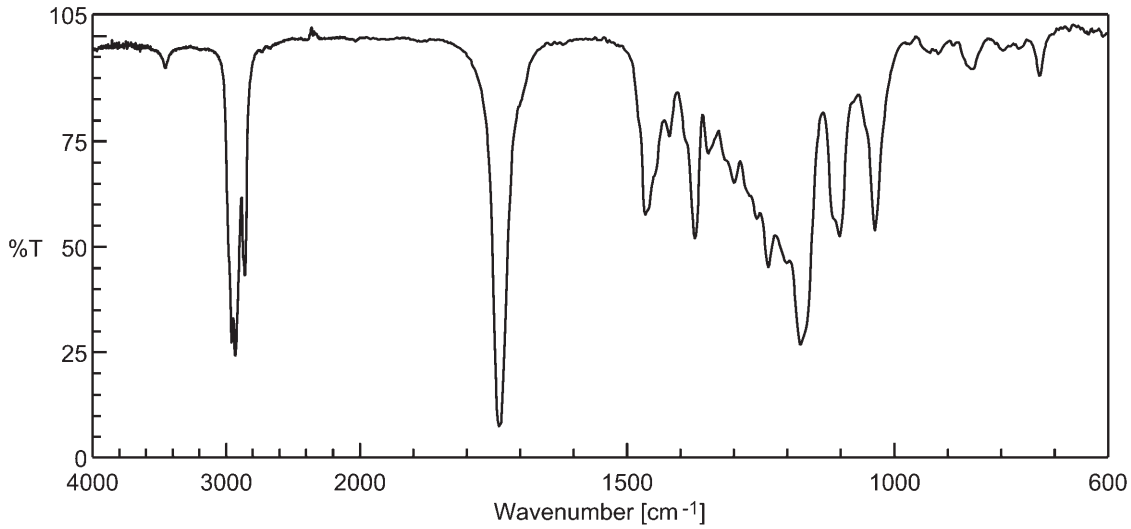
Ethyl Decanoate



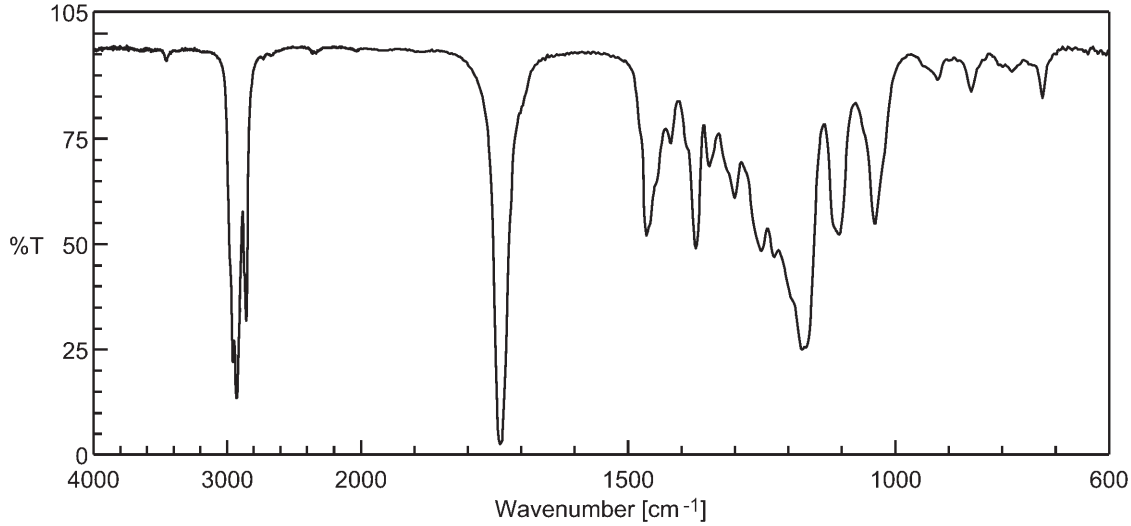
2-Ethyl-3,(5 or 6)-dimethylpyrazine



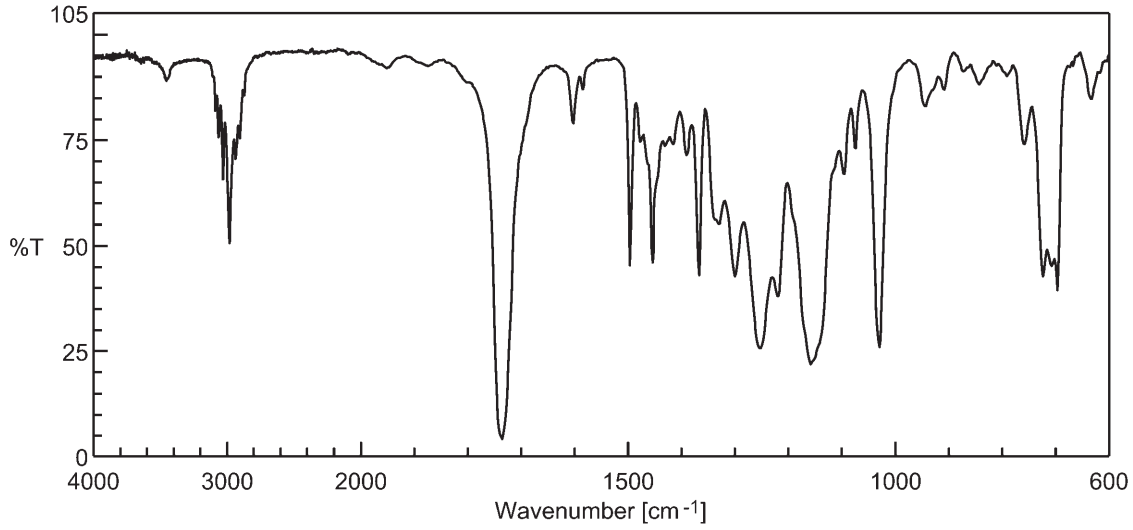
Ethyl Heptanoate



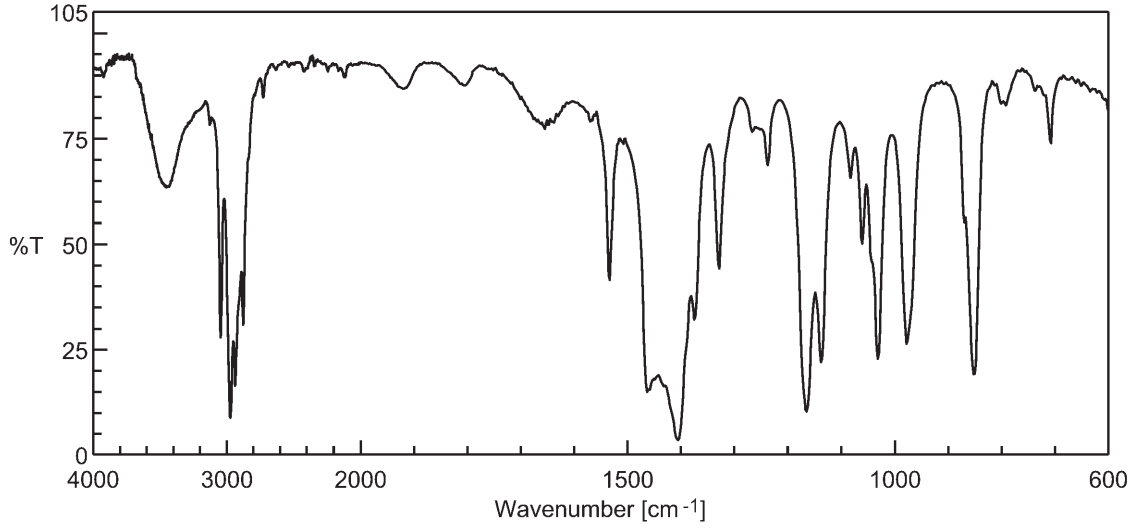
Ethyl Octanoate



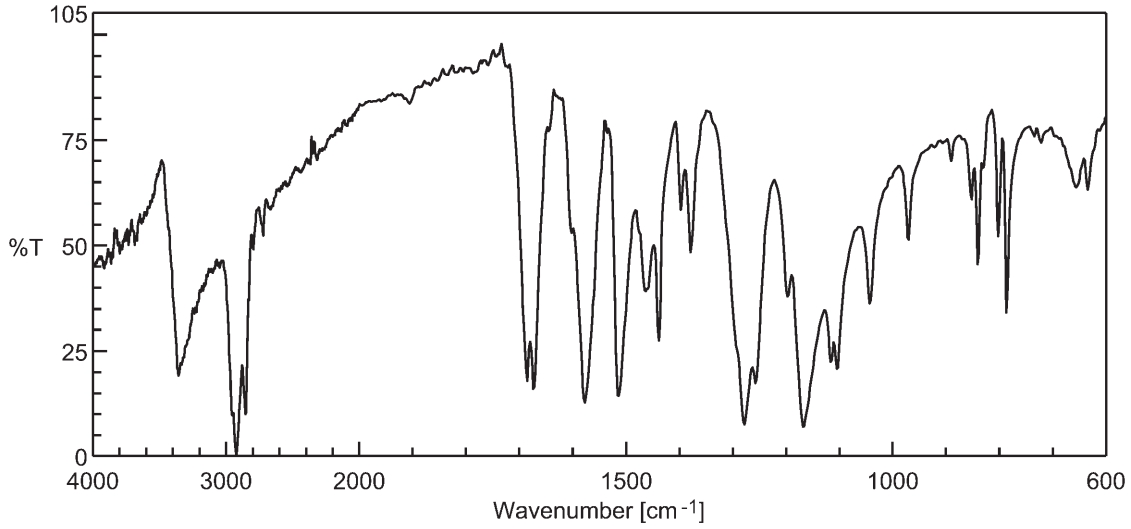
Ethyl Phenylacetate



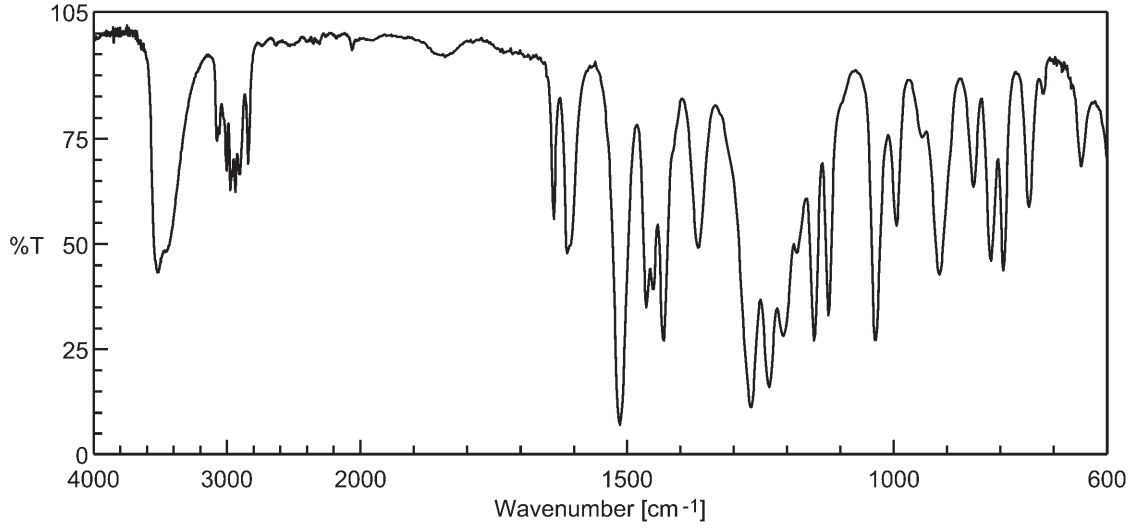
2-Ethyl-3-methylpyrazine



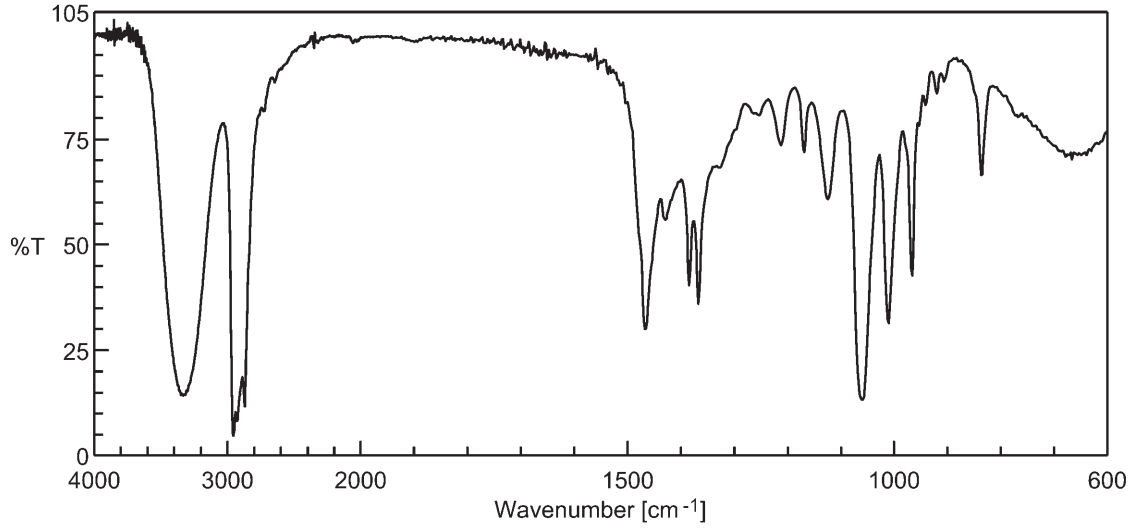
Ethylvanillin



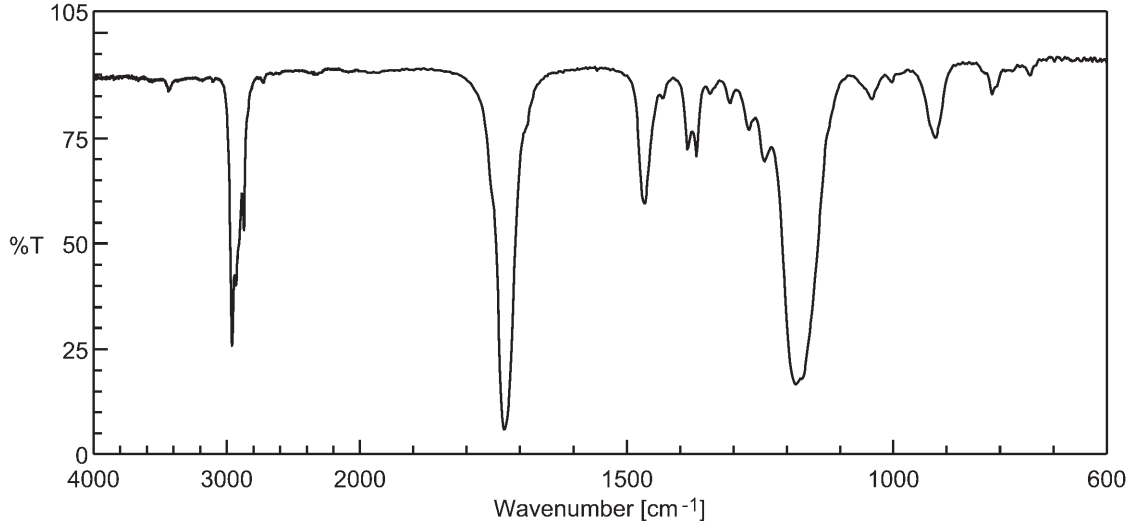
Eugenol



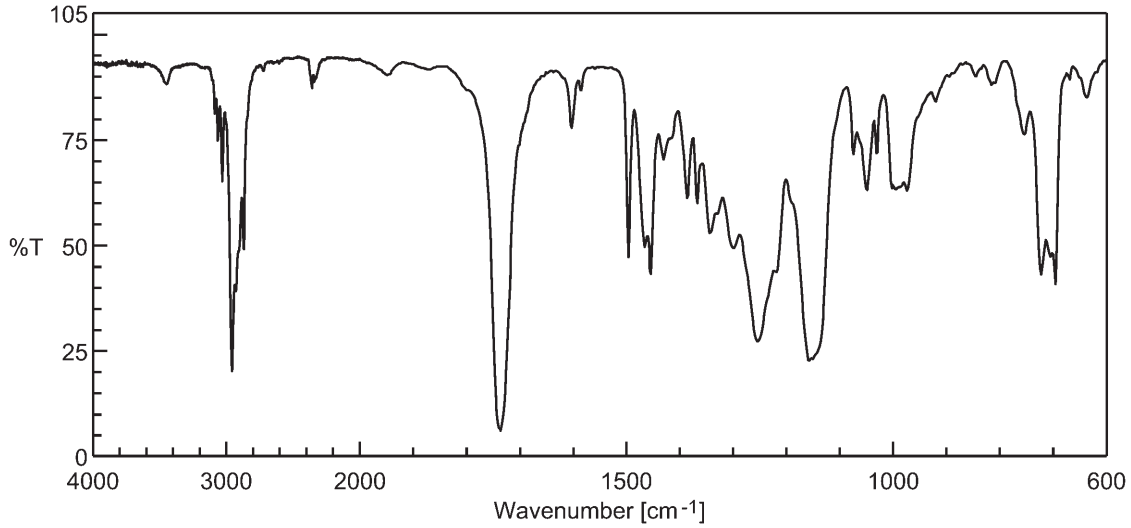
Isoamyl Alcohol



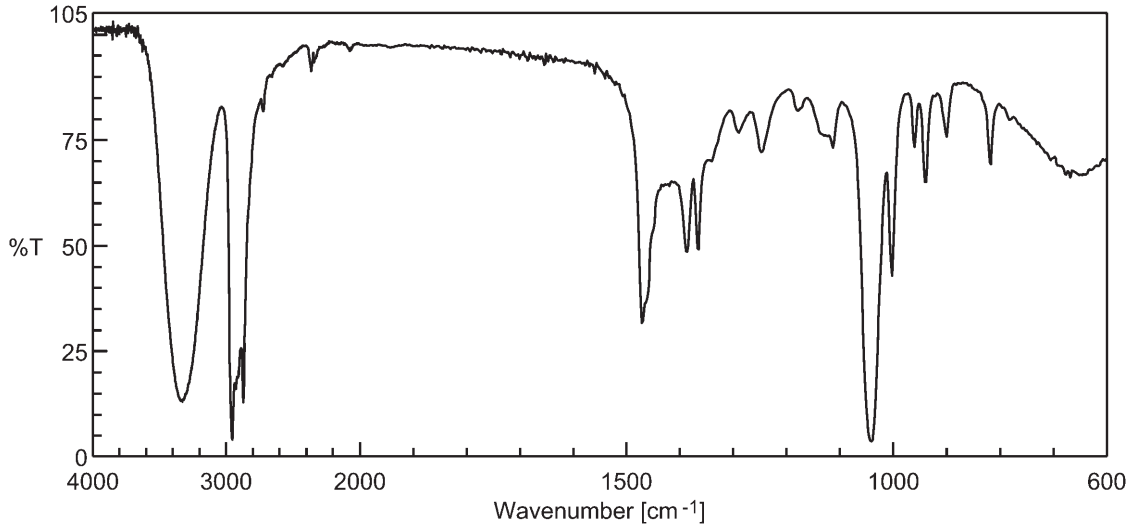
Isoamyl Formate



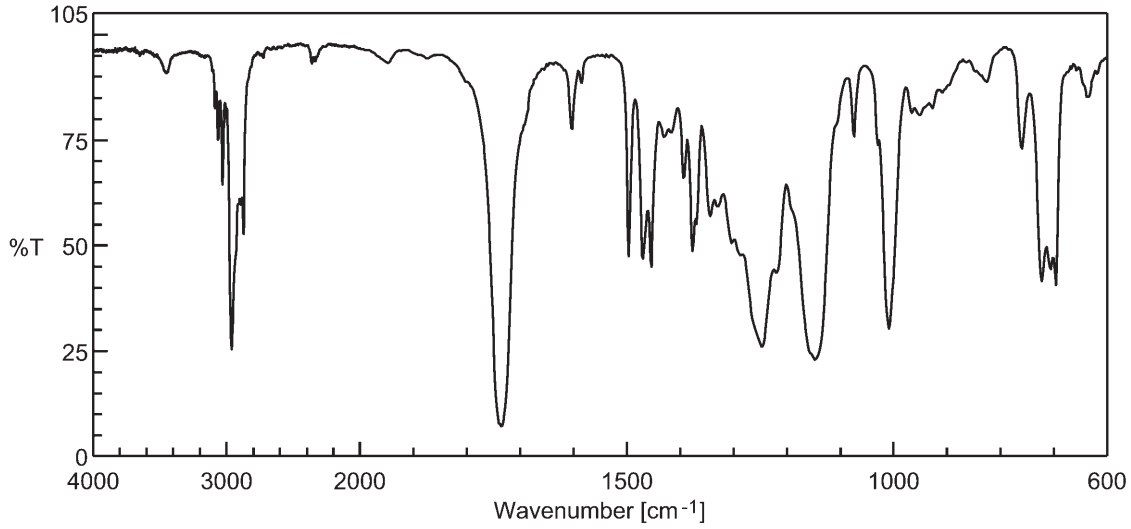
Isoamyl Phenylacetate



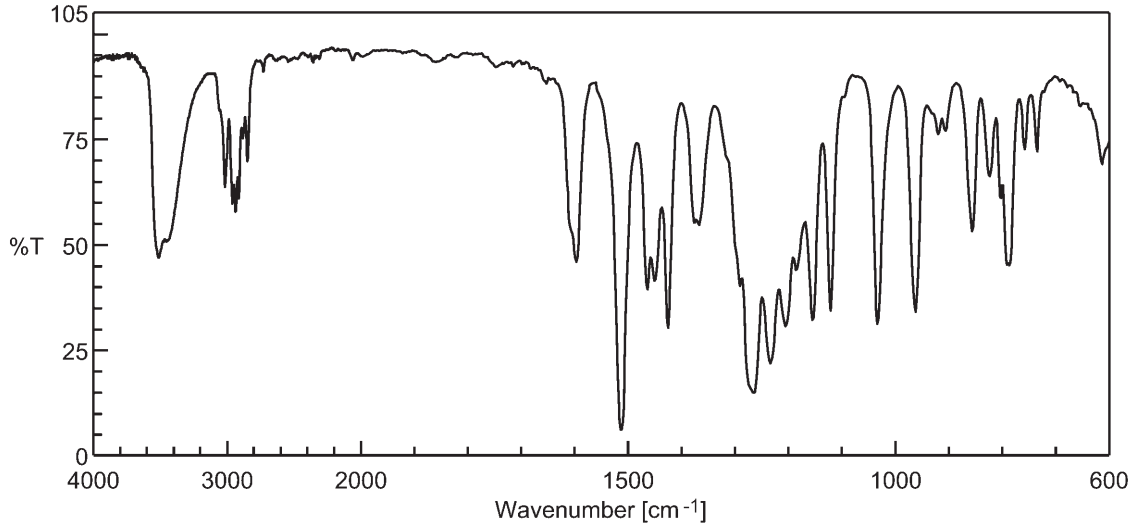
Isobutanol



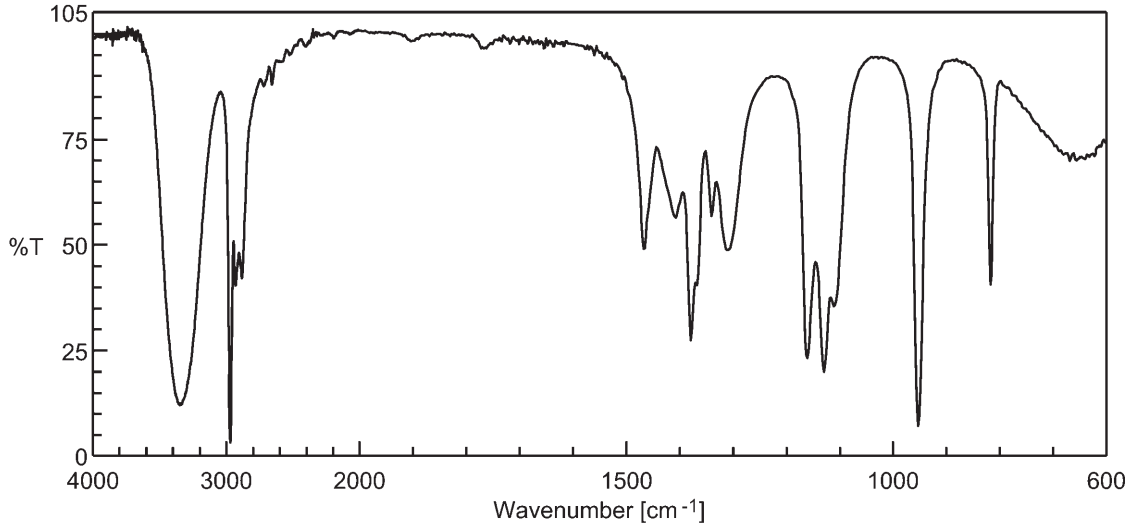
Isobutyl Phenylacetate



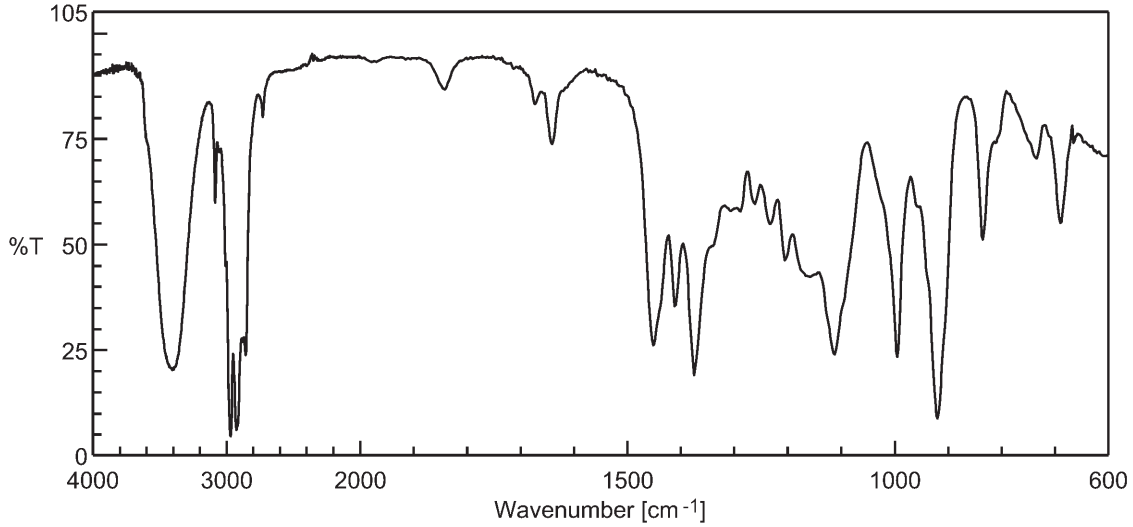
Isoeugenol



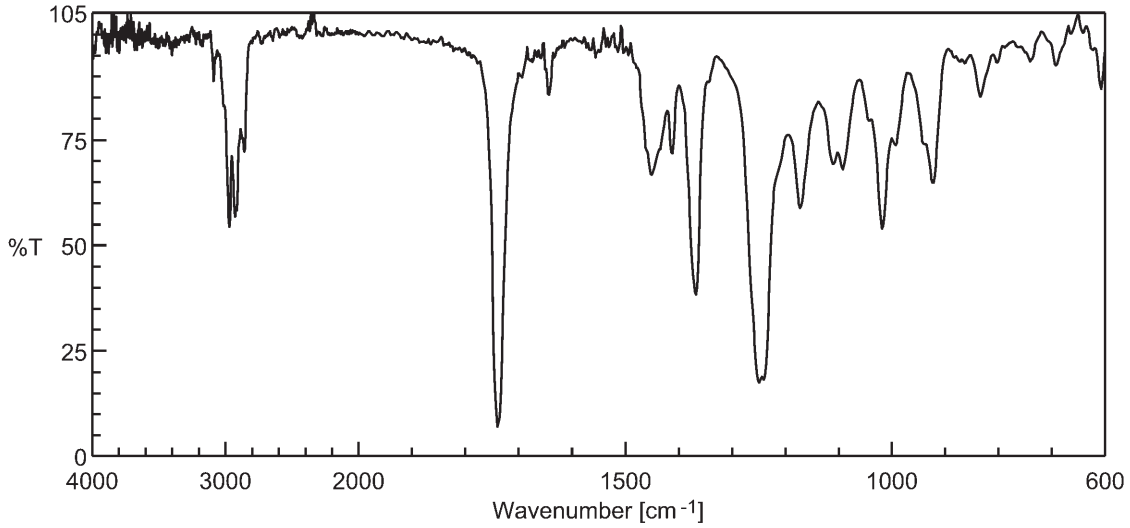
Isopropanol



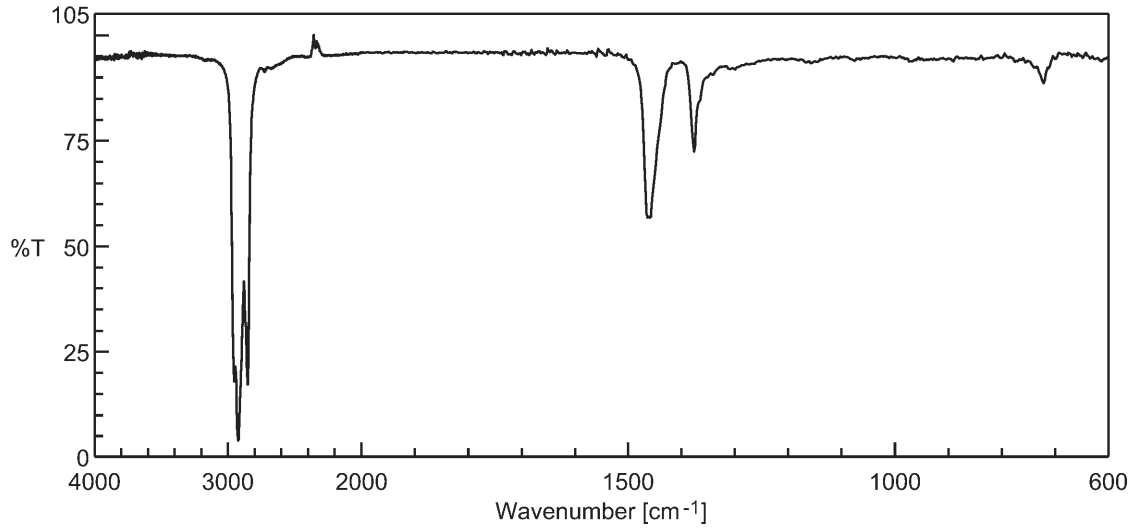
Linalool



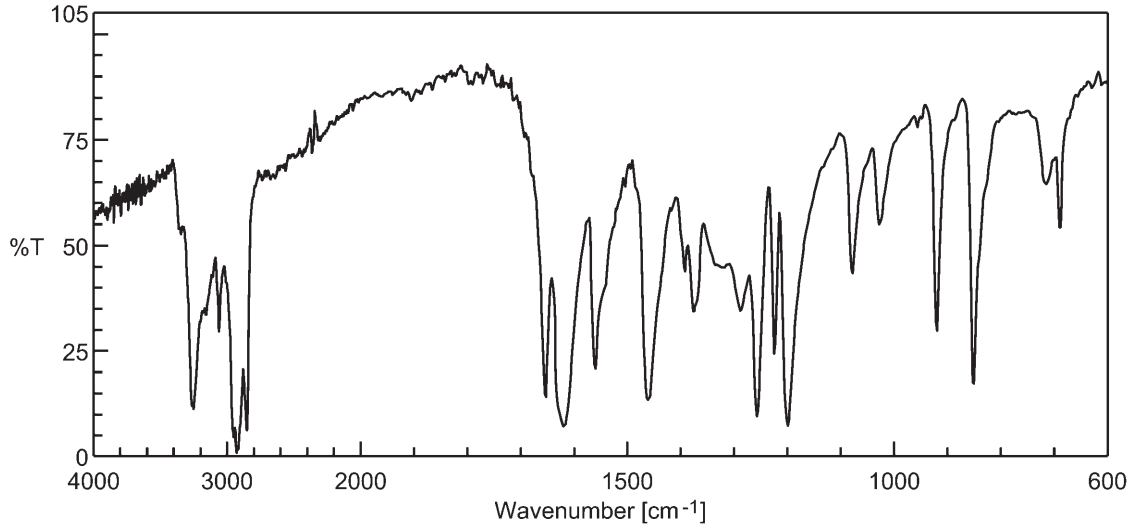
Linalyl Acetate



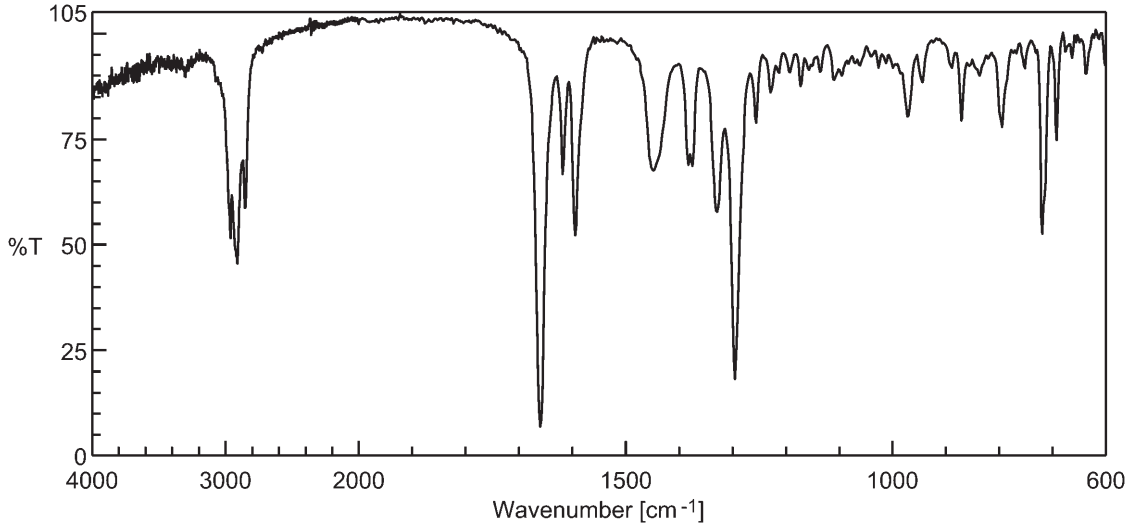
Liquid Paraffin



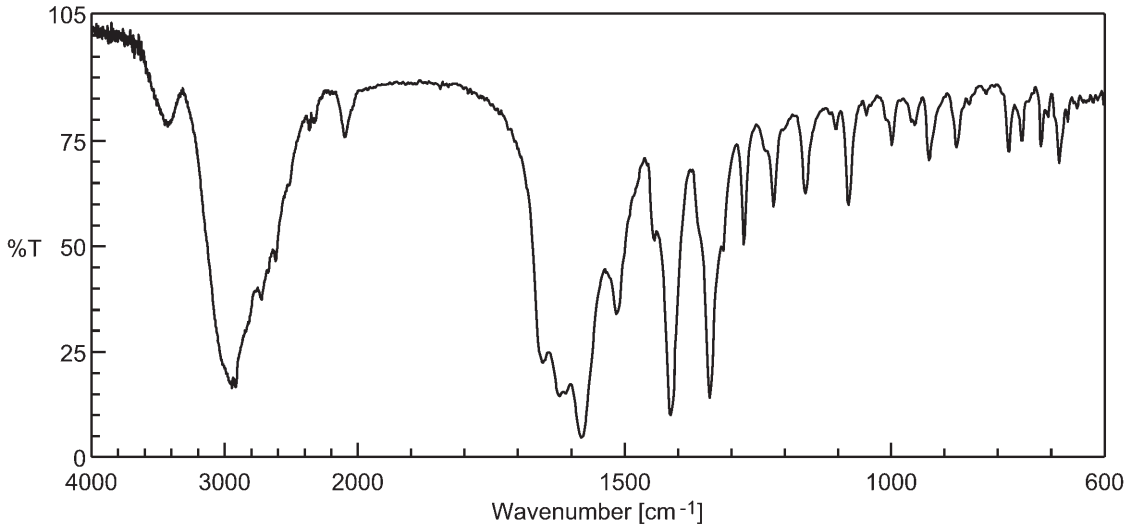
Maltol



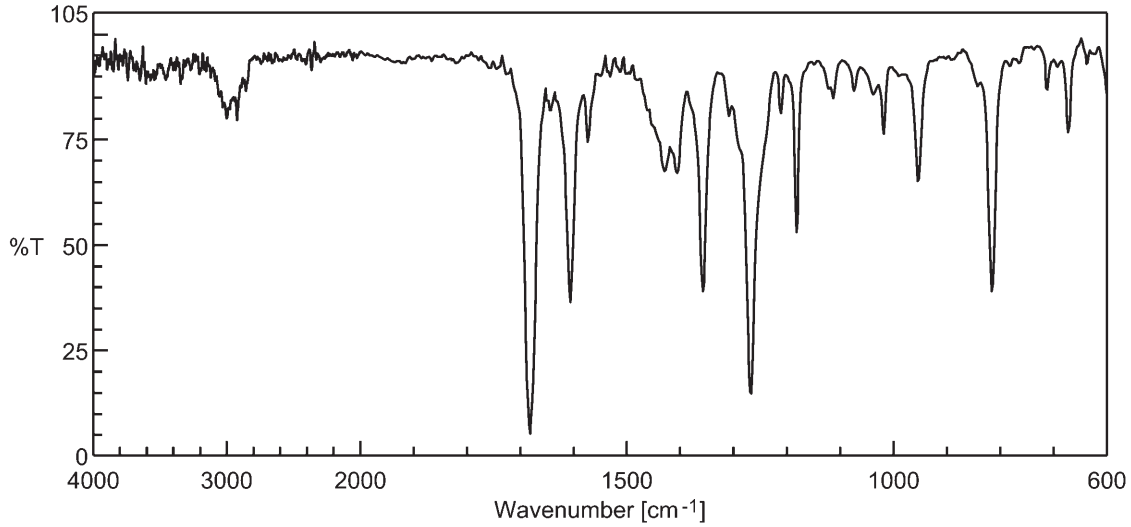
Menaquinone



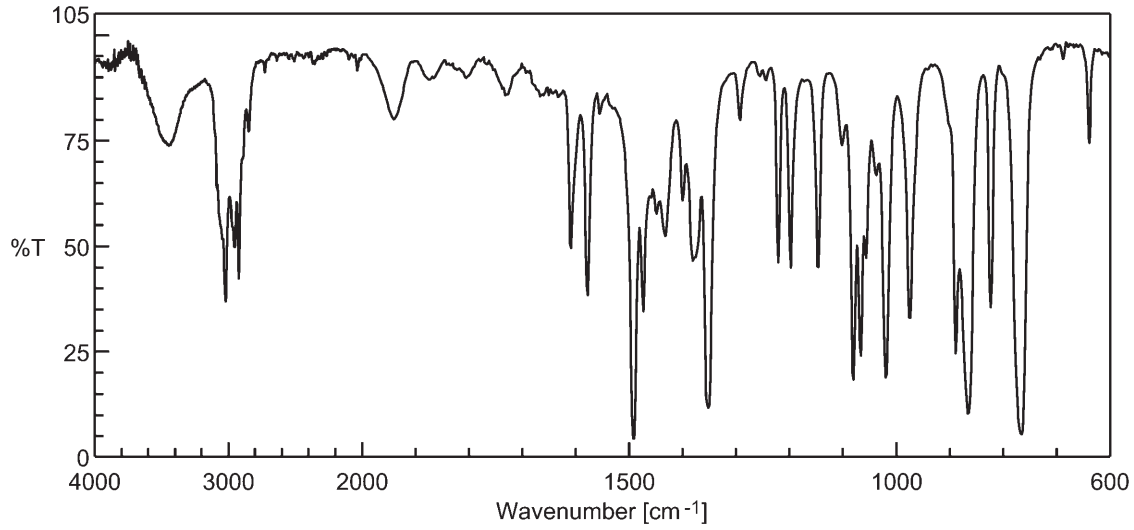
DL-Methionine



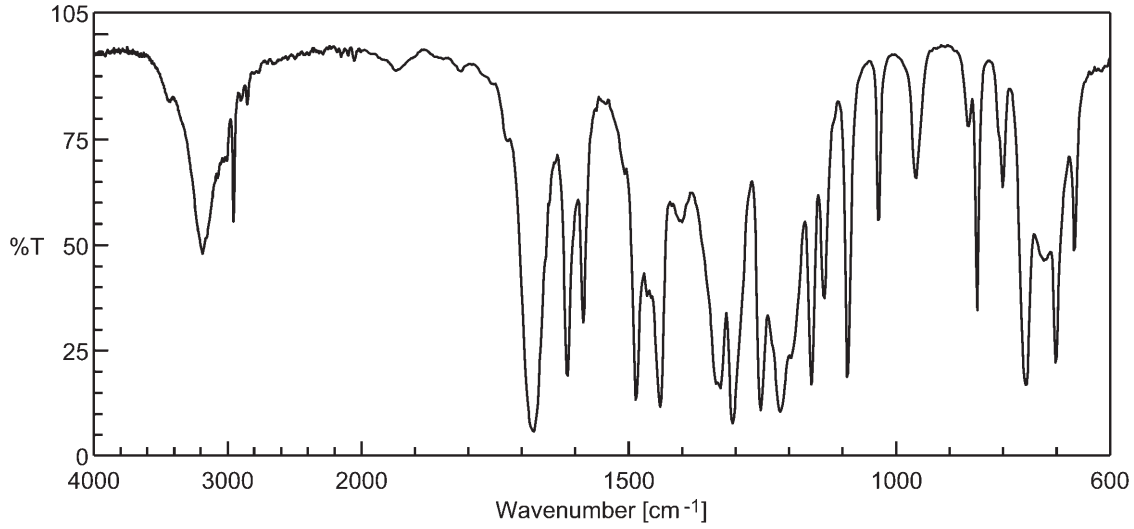
p-Methylacetophenone



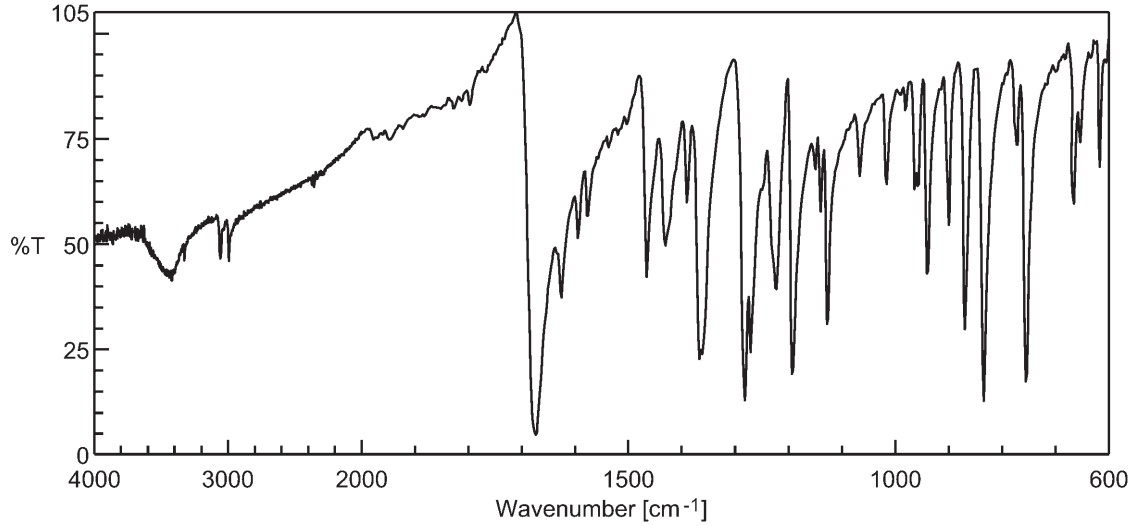
5-Methylquinoxaline



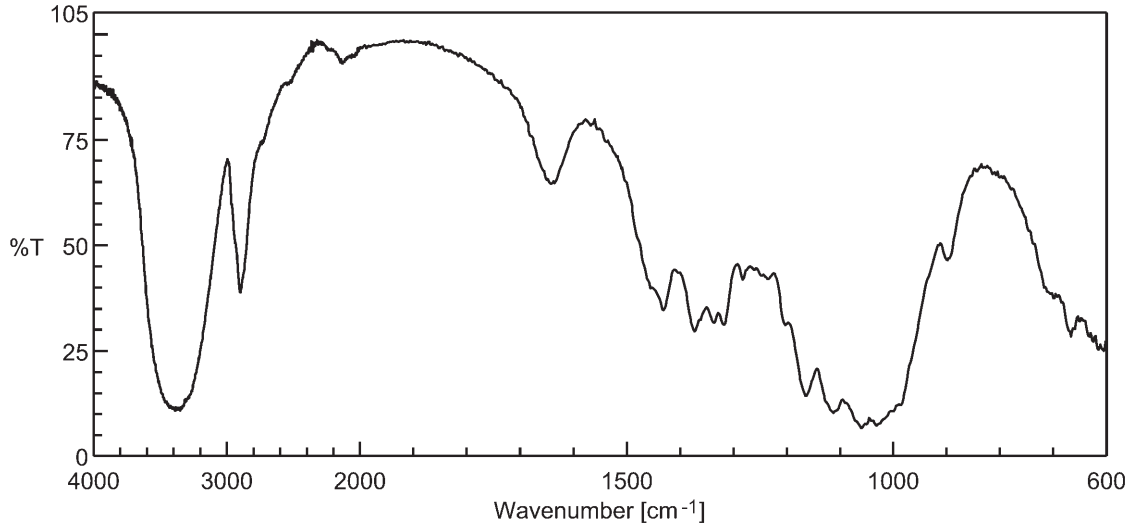
Methyl Salicylate



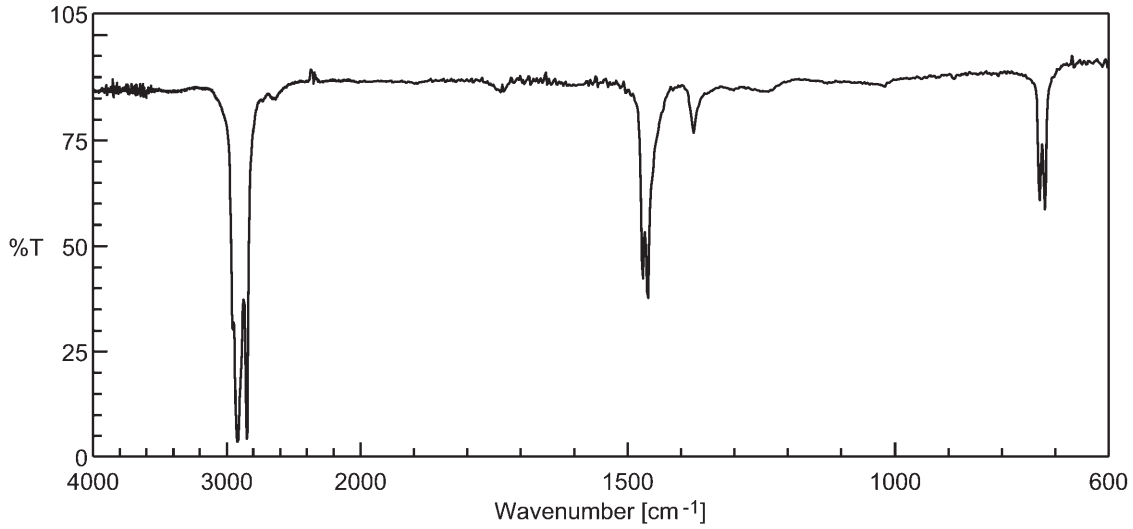
Methyl β-Naphthyl Ketone



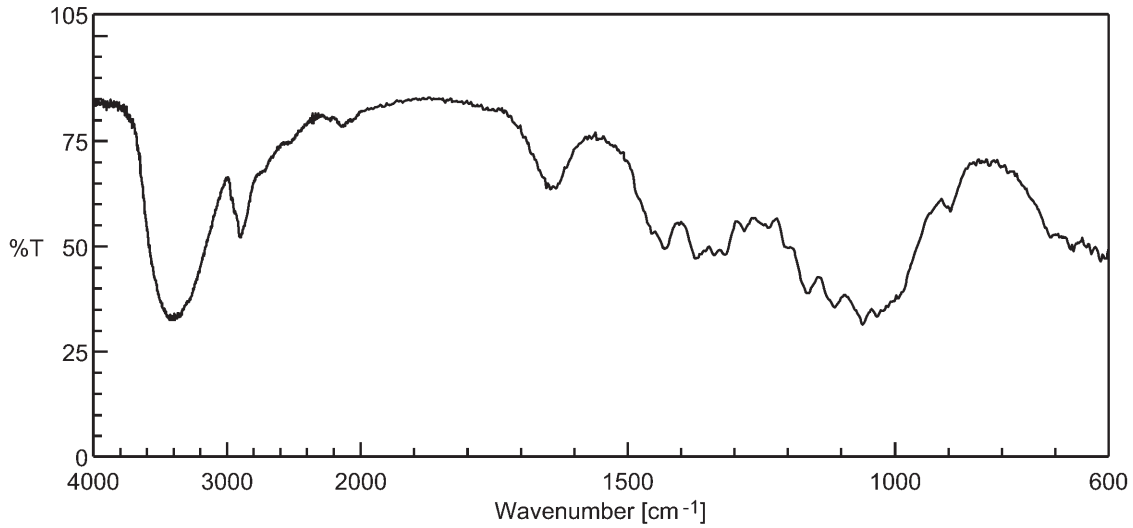
Microcrystalline Cellulose



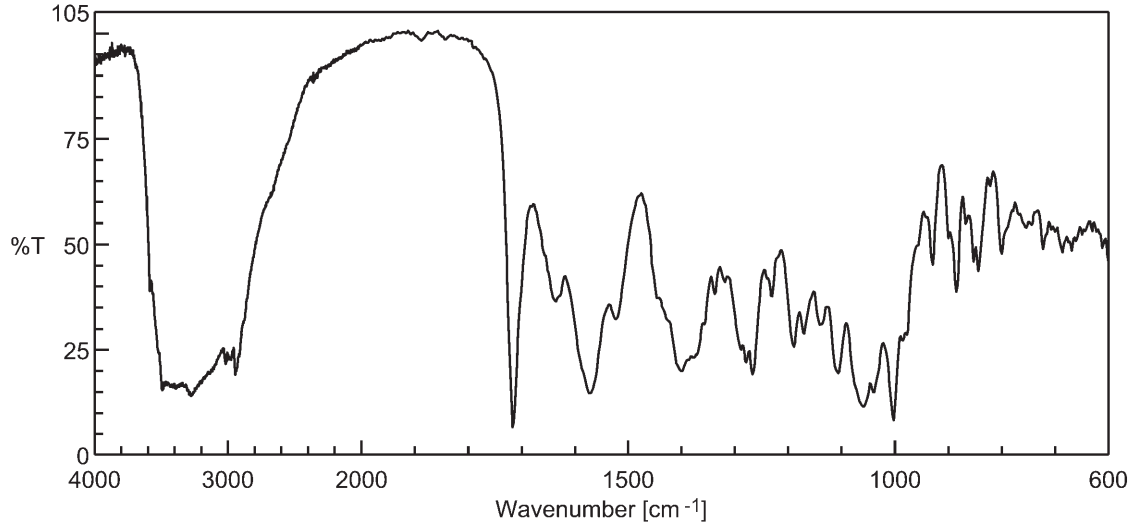
Microcrystalline Wax



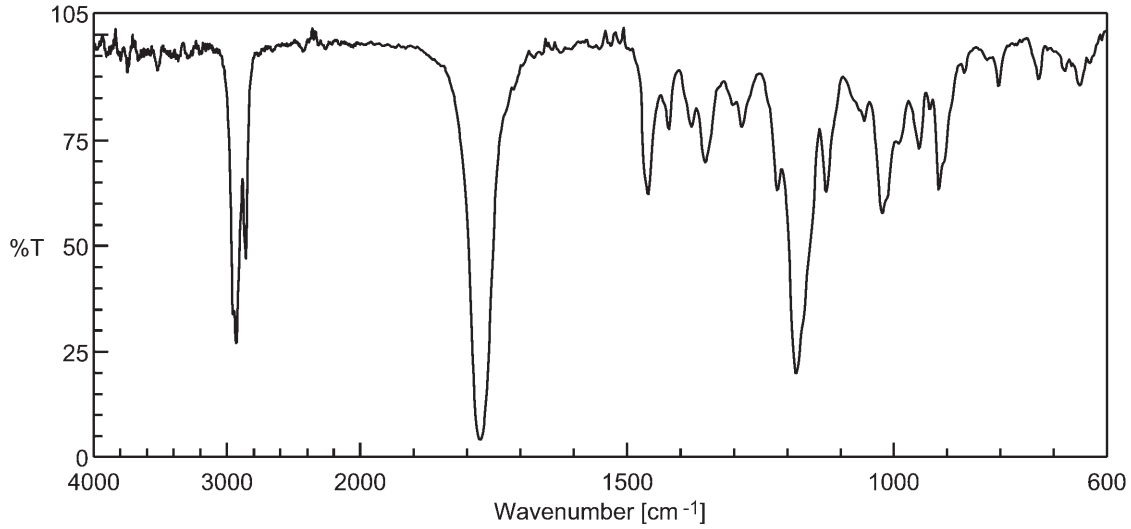
Microfibrillated Cellulose



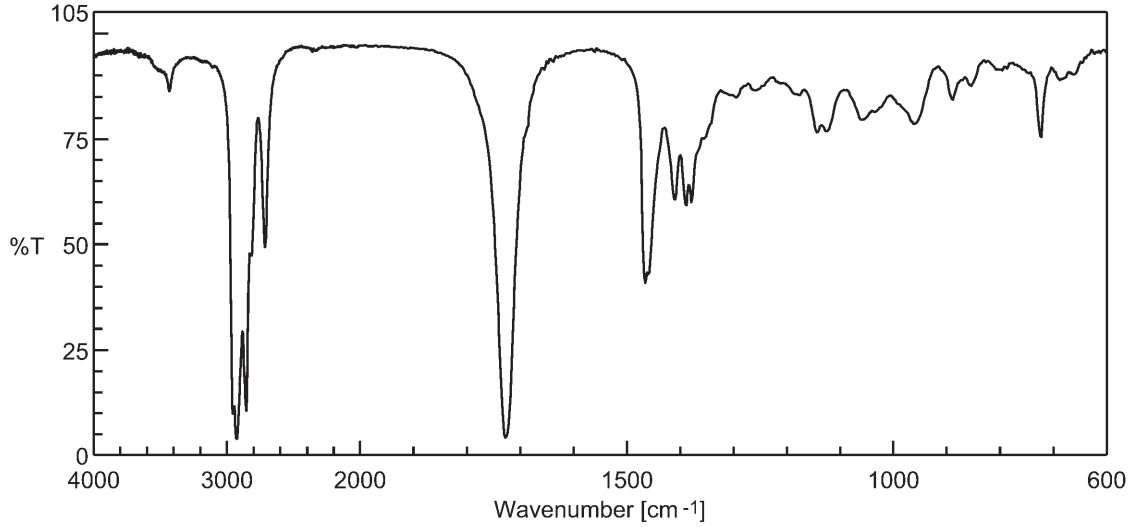
Natamycin



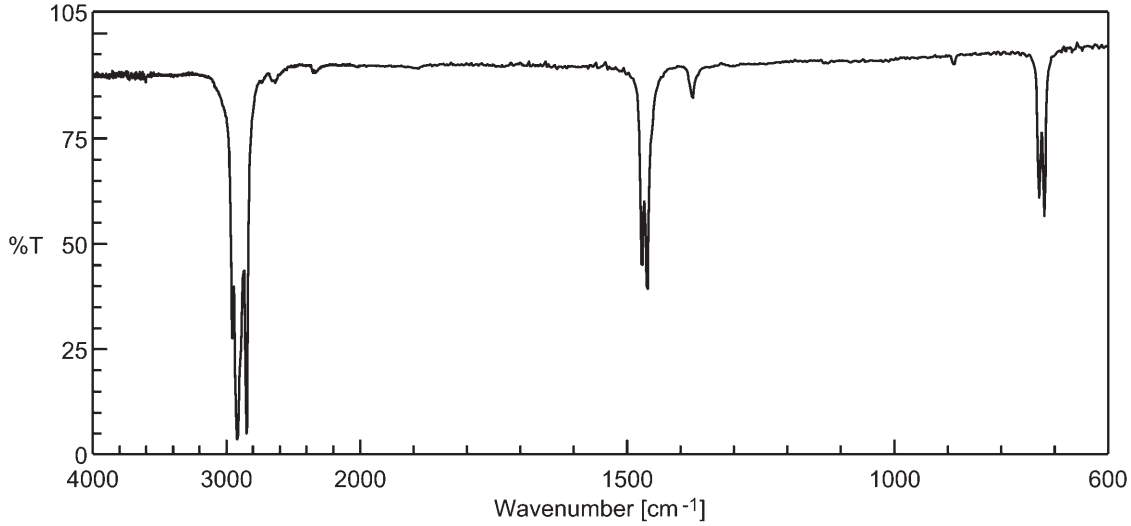
γ-Nonalactone



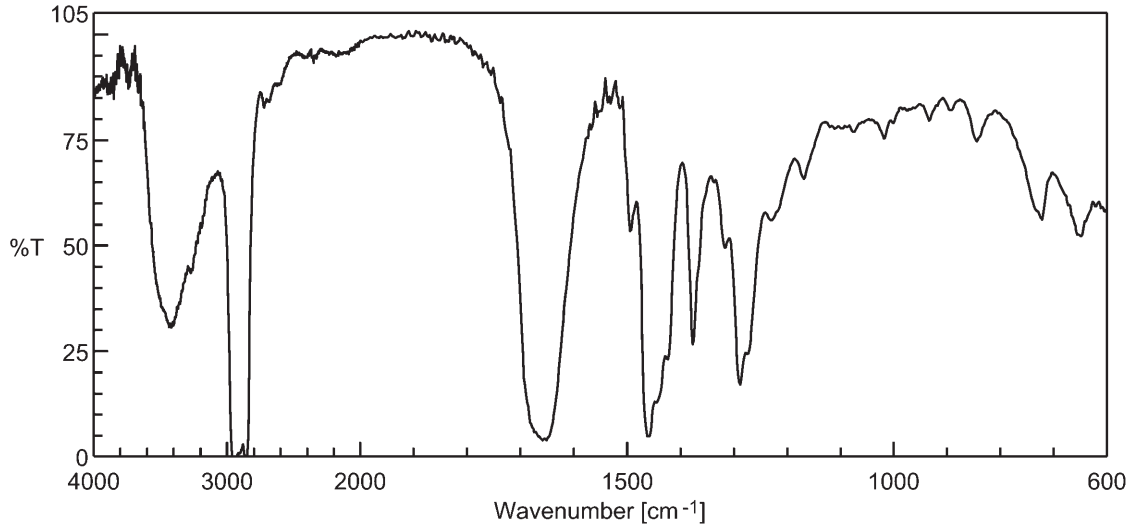
Octanal



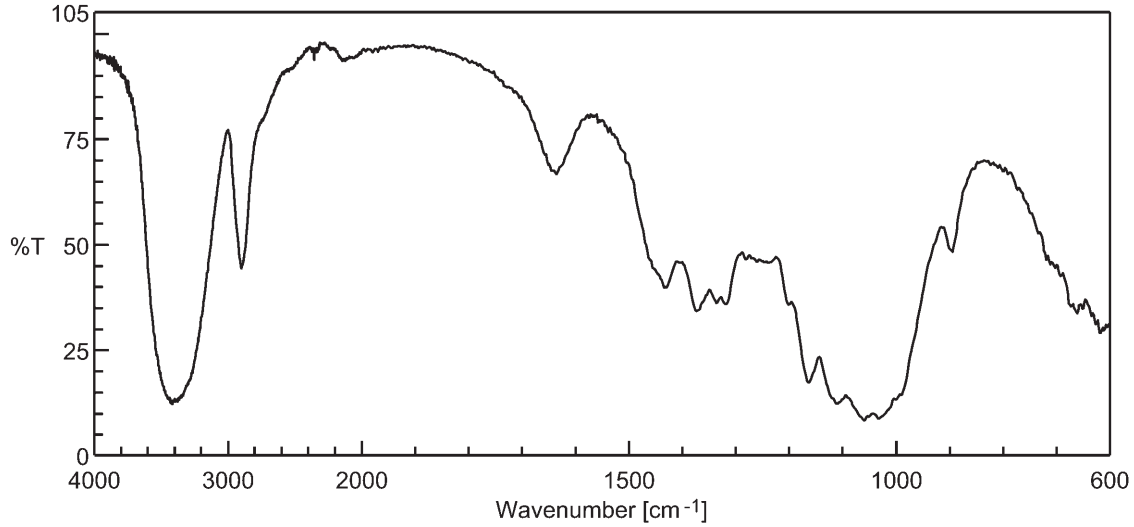
Paraffin Wax



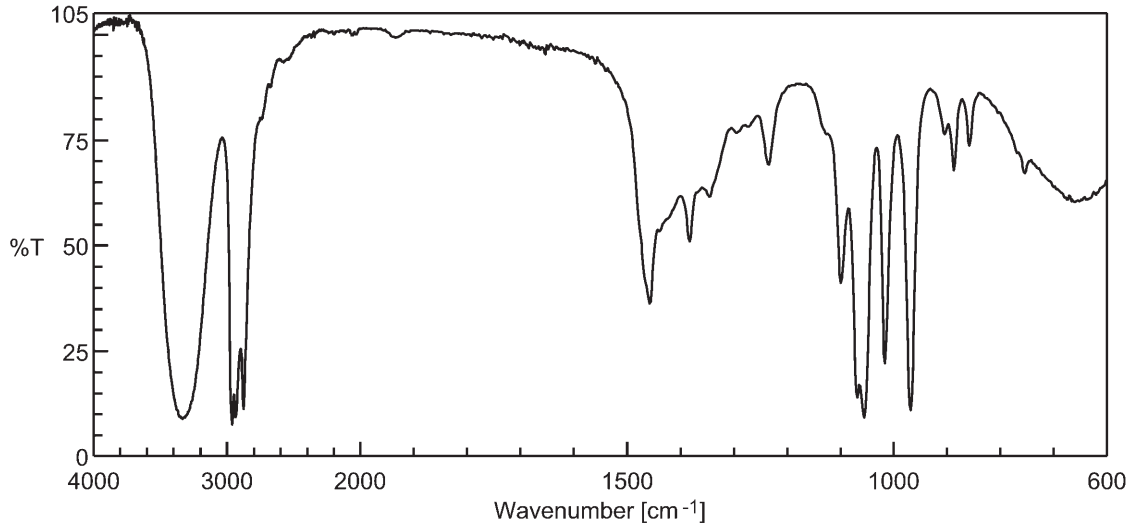
Polyvinylpolypyrrolidone



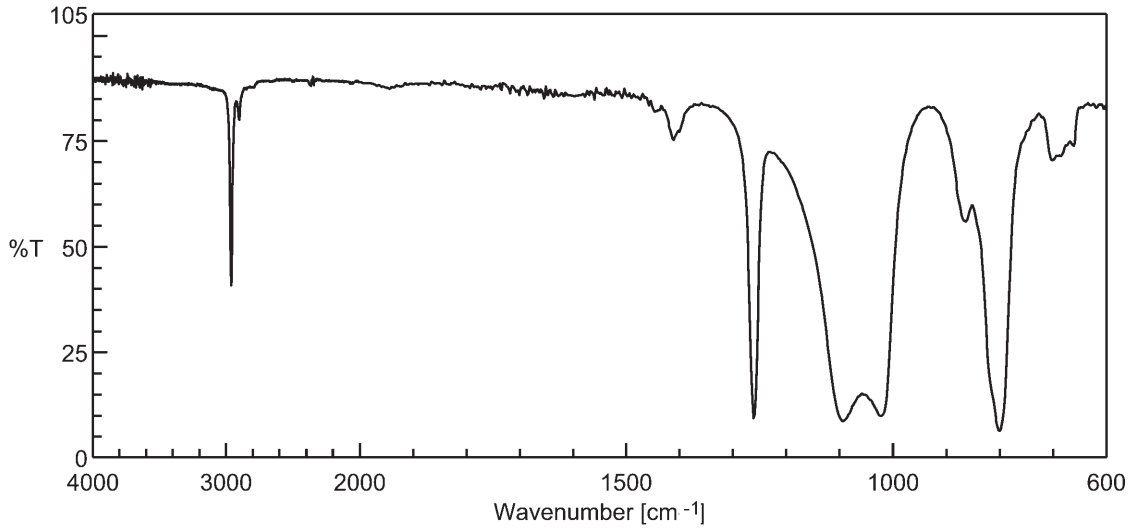
Powdered Cellulose



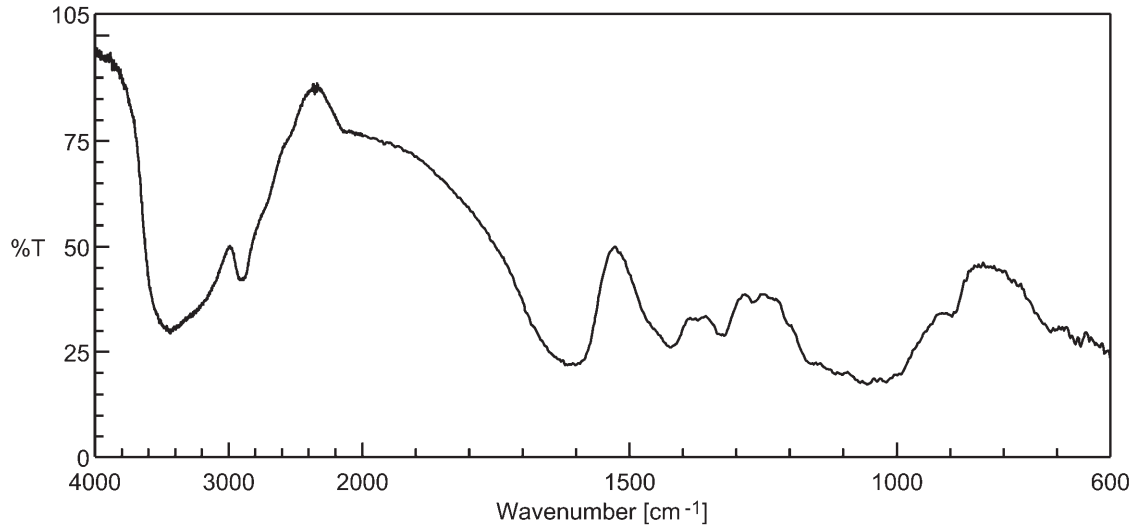
Propanol



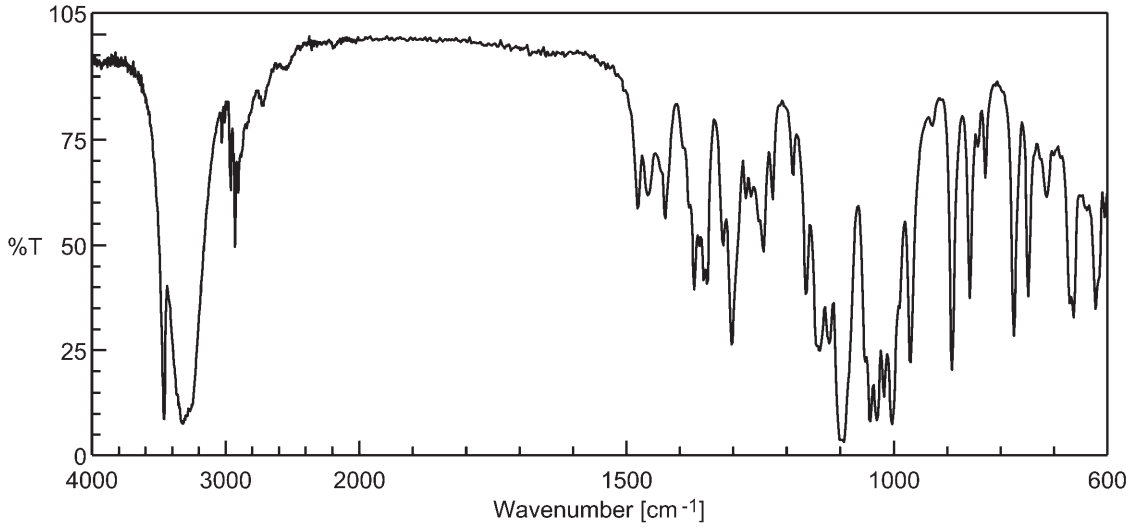
Silicone Resin



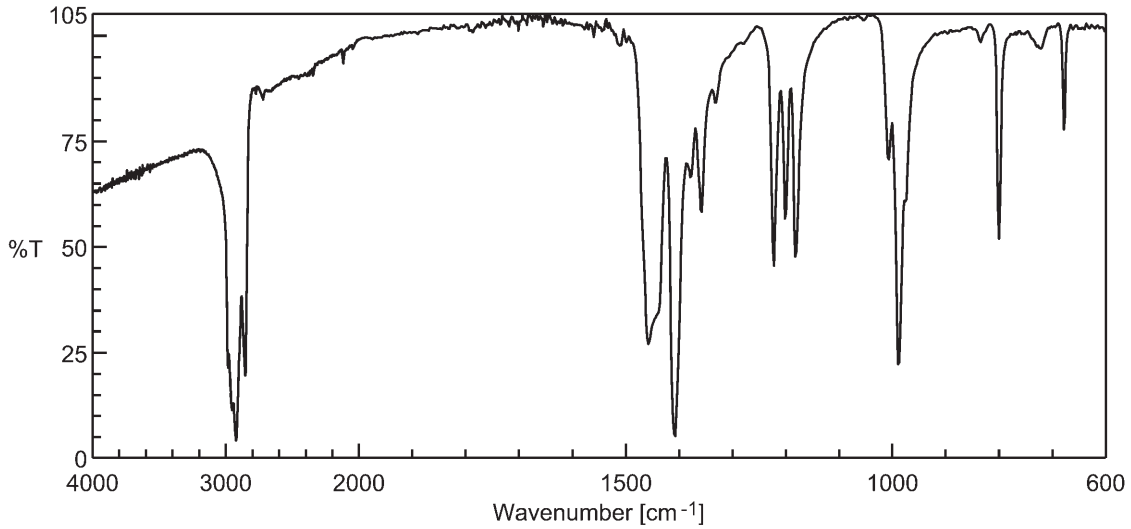
Sodium Carboxymethylcellulose



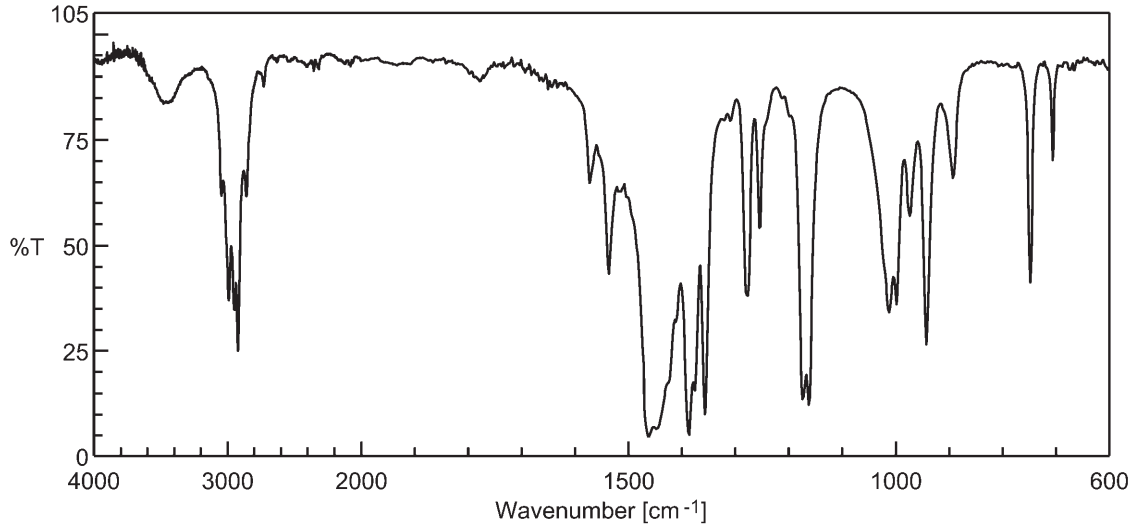
Sucralose



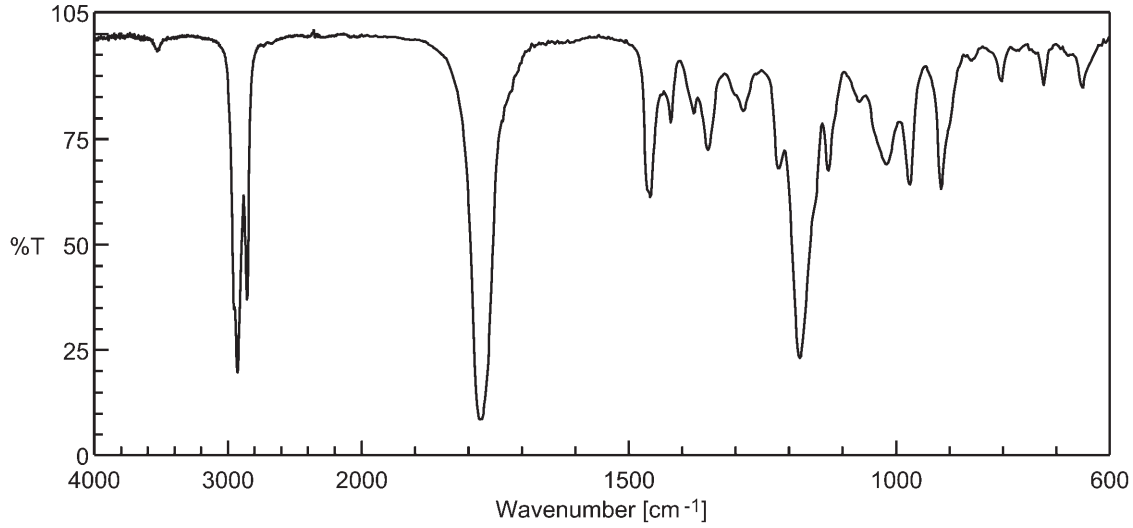
2,3,5,6-Tetramethylpyrazine



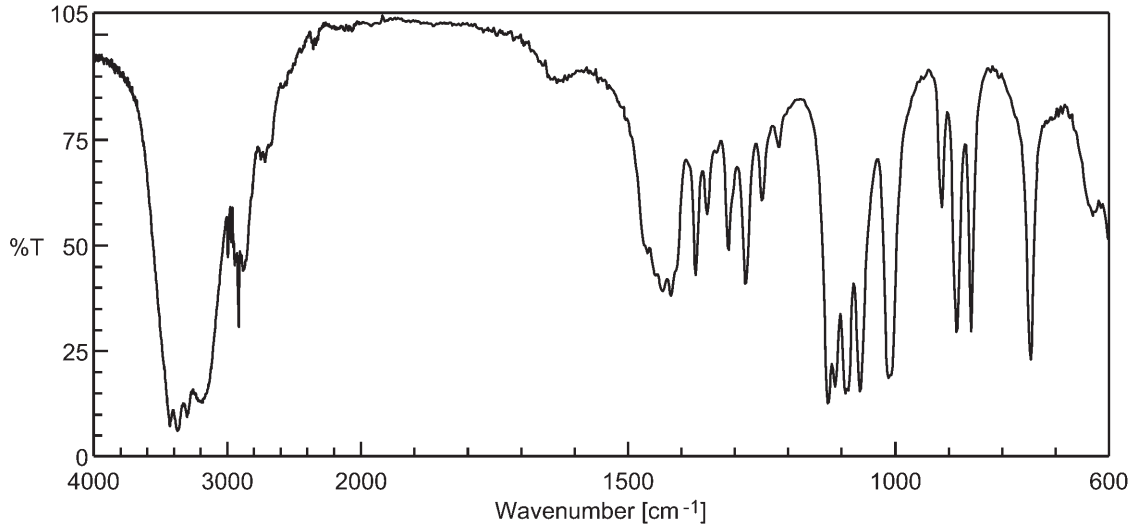
2,3,5-Trimethylpyrazine



γ-Undecalactone



Xylitol



MONOGRAPHS

D. MONOGRAPHS

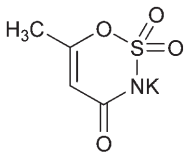
General requirements

Food additives appearing in the Monographs shall meet the specifications and standards specified in the corresponding individual monographs. Notwithstanding compliance with specifications and standards provided in the Monographs, food additives that have been produced with the use of organisms obtained with recombinant DNA technology shall not be distributed or used in Japan unless they have been proven safe through assessment by the Minister of Health, Labour and Welfare and listed as safe. This requirement is not limited to the food additives specified in the document. All substances intended to be distributed or used as food additives in Japan are subject to this requirement.

Acesulfame Potassium

Acesulfame K

アセスルファムカリウム



$C_4H_4KNO_4S$ Mol. Wt. 201.24

Potassium 6-methyl-4-oxo-4*H*-1,2,3-oxathiazin-3-ide 2,2-dioxide [55589-62-3]

Content Acesulfame Potassium, when dried, contains 99.0-101.0% of acesulfame potassium ($C_4H_4KNO_4S$).

Description Acesulfame Potassium occurs as a white crystalline powder. It is odorless and has a strong sweet taste.

Identification

(1) Dissolve 0.010 g of Acesulfame Potassium in 1,000 ml of water. The solution exhibits an absorption maximum at a wavelength of 225–229 nm.

(2) Acesulfame Potassium responds to all tests for Potassium Salt in the Qualitative Tests.

(3) To 0.2 g of Acesulfame Potassium, add 2 ml of diluted acetic acid (3 in 10) and 2 ml of water to dissolve. Add a few drops of sodium cobaltinitrite TS to the solution. A yellow precipitate is formed.

Purity

(1) Clarity and color of solution Colorless and clear (1.0 g, water 5.0 ml).

(2) pH 5.5–7.5 (1.0 g, water 100 ml).

(3) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Lead Not more than 1.0 μg/g as Pb (10.0 g, Method 1).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

(6) Fluoride Not more than 3.0 μg/g as F.

Test Solution Weigh exactly 2.00 g of Acesulfame Potassium, transfer into a beaker, add 10 ml of water, and mix for a while. Add 20 ml of diluted hydrochloric acid (1 in 20) gradually, and dissolve. Heat the solution, boil for 1 minute, transfer into a polyethylene beaker, and immediately cool with ice. Add 10 ml of a solution of disodium ethylenediaminetetraacetate (1 in 40) and 15 ml of sodium citrate solution (1 in 4), and mix. Adjust the pH of the solution to 5.4–5.6 with diluted hydrochloric acid (1 in 10) or sodium hydroxide solution (2 in 5). Transfer the solution into a 100-ml volumetric flask, and dilute with water to volume. Place about 50 ml of the solution in a polyethylene beaker, and use as the test solution.

Control Stock Solution Weigh accurately 2.210 g of sodium fluoride, previously dried at 110˚C for 2 hours, transfer into a polyethylene beaker, add 200 ml of water, and dissolve while stirring. Transfer the solution into a 1,000-ml volumetric flask, add water to make 1,000 ml, and then transfer into a polyethylene bottle.

Control Solution Prepare fresh before use. Transfer 3.0 ml of the stock solution into a 1,000-ml volumetric flask, and add water to make 1,000 ml. Transfer 2.0 ml of the solution into a polyethylene beaker, add 10 ml of a solution of disodium ethylenediaminetetraacetate (1 in 40) and 15 ml of sodium citrate solution (1 in 4), and mix. Adjust the pH of the solution to 5.4–5.6 with diluted hydrochloric acid (1 in 10) or sodium hydroxide solution (2 in 5). Transfer the solution into a 100-ml volumetric flask, and dilute with water to volume. Place about 50 ml of the solution into a polyethylene beaker, and use as the control solution.

Procedure Measure the electric potentials of both solutions, using a potentiometer connected to a reference electrode and a fluorine ion electrode. The electric potential of the test solution is not lower than that of the control solution.

(7) UV active components (Organic impurities) Not more than 20 μg/g as acesulfame potassium.

Test solution Weigh accurately about 1 g of Acesulfame Potassium, and dissolve it in water to make exactly 100 ml.

Control Solution Dilute the test solution with water by 50,000-fold.

Procedure Analyze 20 μl portions of the test solution and the control solution by liquid chromatography under the conditions given below. Continue the chromatography for 3 times the retention time of the main peak of the test solution. The total area of all the peaks, other than the main peak, of the test solution does not exceed the area of the main peak of the control solution.

Operating Conditions

Detector: Ultraviolet spectrophotometer (determination wavelength: 227 nm).

Column: A stainless steel tube of 4.6 mm internal diameter and 25 cm length.

Column packing material: 3- to 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 40°C.

Mobile phase: A 3:2 mixture of 0.01 mol/L tetrabutyl ammonium hydrogen sulfate/acetonitrile.

Flow rate: 1 ml/min.

The column should be capable of separating the peaks of acesulfame potassium and ethyl *p*-hydroxybenzoate when

the following test is conducted: Weigh separately 0.010 g of Acesulfame Potassium and "Ethyl *p*-Hydroxybenzoate," dissolve together in water to make a mixture, and add water to make 1,000 ml. Analyze a 20 µl portion of this solution by liquid chromatography under the above conditions.

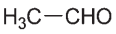
Loss on Drying Not more than 1.0% (105°C, 2 hours).

Assay Weigh accurately about 0.15g of Acesulfame Potassium, previously dried, dissolve it in 50 ml of acetic acid, and titrate with 0.1 mol/L perchloric acid solution. The endpoint is usually confirmed by a potentiometer. When crystal violet–acetic acid TS is used as the indicator (2 drops), the endpoint is when the color of the solution changes from dark blue to green and then the color becomes green that persists for at least 30 seconds. Separately, perform a blank test.

Each ml of 0.1 mol/L perchloric acid solution = 20.12 mg of $C_4H_4KNO_4S$

Acetaldehyde

アセトアルデヒド



C_2H_4O Mol. Wt. 44.05

Acetaldehyde [75-07-0]

Content Acetaldehyde contains not less than 99.0% of acetaldehyde (C_2H_4O).

Description Acetaldehyde is a colorless, clear liquid having a characteristic odor.

Identification Determine the infrared absorption spectrum of Acetaldehyde as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit absorptions having about the same intensity at the same wavenumbers.

Purity

(1) Refractive index n_D^{20}: 1.330–1.334.

(2) Acid value Not more than 5.0 (Flavoring Substances Tests).

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavor Substance Tests. Use operating conditions (2). For sample injection, use a microsyringe that is previously cooled at 5°C at least for 30 minutes.

Storing standard Store in a well-filled, hermetic container under inert gas at 5°C or lower.

Acetic Acid

酢酸

Content Acetic Acid contains 29.0–31.0% of acetic acid ($C_2H_4O_2$ = 60.05).

Description Acetic Acid is a colorless, clear liquid having a characteristic pungent odor.

Identification

(1) Acetic Acid is acidic.

(2) Acetic Acid responds to all tests for Acetate in the Qualitative Tests.

Purity

(1) Heavy metals Not more than 10 µg/g as Pb (3.0 g, Method 1, Control solution Lead Standard Solution 3.0 ml).

(2) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50g, Method 1, Apparatus B).

(3) Readily oxidizable substances Measure 20 ml of Acetic Acid, and add 0.30 ml of 0.02 mol/L potassium permanganate. The pink color of the solution does not disappear within 30 minutes.

(4) Residue on evaporation Not more than 0.010%. Weigh 20.0 g of Acetic Acid, evaporate, and dry at 100°C for 2 hours, and weigh the residue.

Assay Weigh accurately about 3 g of Acetic Acid, and add 15 ml of water. Titrate with 1 mol/L sodium hydroxide (indicator: 2 drops of phenolphthalein TS).

Each ml of 1 mol/L sodium hydroxide = 60.05 mg of $C_2H_4O_2$

Glacial Acetic Acid

Acetic Acid, Glacial

氷酢酸

C_2H_4O Mol. Wt. 60.05

Acetic acid [64-19-7]

Content Glacial Acetic Acid contains not less than 99.0% of acetic acid ($C_2H_4O_2$).

Description Glacial Acetic Acid occurs as colorless or white crystalline lumps or as a colorless, clear liquid. It has a characteristic pungent odor.

Identification

(1) A solution of Glacial Acetic Acid (1 in 4) is acidic.

(2) A solution of Glacial Acetic Acid (1 in 4) responds to all tests for Acetate in the Qualitative Tests.

Purity

(1) Congealing point Not less than 14.5°C.

(2) Heavy metals Not more than 10 µg/g as Pb (2.0 g, Method 1, control solution Lead Standard Solution 2.0 ml).

(3) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.5 0g, Method 1, Apparatus B).

(4) Readily oxidizable substances Weigh 2.0 g of Glacial Acetic Acid, dissolve it in 10 ml of water, and add 0.10 ml of 0.02 mol/L potassium permanganate. The pink color of the solution does not disappear within 30 minutes.

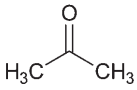
(5) Residue on evaporation Not more than 0.010%. Weigh 20.0 g of Glacial Acetic Acid, evaporate, dry at 100°C for 2 hours, and weigh the residue.

Assay Weigh accurately about 1 g of Glacial Acetic Acid, and add 40 ml of water. Titrate with 1 mol/L sodium hydroxide (indicator: 2 drops of phenolphthalein TS).

Each ml of 1 mol/L sodium hydroxide = 60.05 mg of $C_2H_4O_2$

Acetone

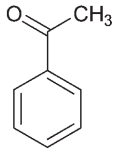
アセトン



C_3H_6O Mol. Wt. 58.08
Propan-2-one [67-64-1]
Content Acetone contains not less than 99.0% of acetone (C_3H_6O).
Description Acetone is a colorless, clear, and volatile liquid having a characteristic odor.
Identification To 1 ml of a solution of Acetone (1 in 200), add 1 ml of sodium hydroxide solution (1 in 25), warm in hot water, and add 3 drops of iodine TS. A yellow precipitate is immediately formed.
Purity
(1) Specific gravity 0.790–0.795.
(2) Boiling point 55.5–57.0°C (Method 1).
(3) Readily oxidizable substances Measure 30 ml of Acetone, and add 0.10 ml of 0.02 mol/L potassium permanganate. The pink color does not disappear within 15 minutes.
(4) Phenol Measure 3.0 ml of Acetone, transfer into a crucible, and evaporate to dryness at about 60°C. Add 3 drops of a solution of sodium nitrite in sulfuric acid (1 in 50), allow to stand for 2–3 minutes, and carefully add 3 ml of sodium hydroxide solution (2 in 25). No color develops.
(5) Residue on evaporation Not more than 0.0016% (w/v).
Measure 125 ml of Acetone, evaporate carefully, dry at 105°C for 2 hours, and weigh the residue.
Assay Weigh accurately about 1 g of Acetone, transfer into a flask containing 20 ml of water, and add water to make exactly 1,000 ml. Measure exactly 10 ml of this solution, transfer into a flask with a ground-glass stopper, add 25 ml of sodium hydroxide solution (1 in 25), and allow to stand for 5 minutes. Add exactly 25 ml of 0.05 mol/L iodine, stopper, allow to stand in a cool, dark place for 10 minutes, and add 30 ml of diluted sulfuric acid (3 in 100). Titrate with 0.1 mol/L sodium thiosulfate (indicator: starch TS). Perform a blank test in the same manner.
Each ml of 0.05 mol/L iodine = 0.9680 mg of C_3H_6O

Acetophenone

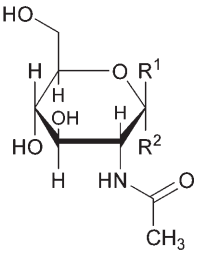
アセトフェノン



C_8H_8O Mol. Wt. 120.15
1-Phenylethanone [98-86-2]
Content Acetophenone contains not less than 98.0% of acetophenone (C_8H_8O).
Description Acetophenone occurs as white crystalline lumps or as a colorless or slightly yellowish, transparent liquid. It has a characteristic odor.
Identification Determine the absorption spectrum of Acetophenone as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.
Purity
(1) Refractive index n_D^{20}: 1.532–1.534.
(2) Congealing point 18–20°C.
(3) Clarity of solution Clear (1.0 ml, 60% (vol) ethanol 4.0 ml).
(4) Halogenated compounds Proceed as directed for Halogenated Compounds in the Flavoring Substances Tests.
Assay Weigh accurately about 1 g of Acetophenone, and proceed as directed in Method 2 in the Aldehyde and Ketone Content Test under the Flavoring Substances Tests. In the test, heat the mixture for 1 hour before titrating.
Each ml of 0.5 mol/L hydrochloric acid = 60.08 mg of C_8H_8O

N-Acetylglucosamine

N-アセチルグルコサミン



N-Acetyl-α-D-glucosamine: R^1=H, R^2=OH
N-Acetyl-β-D-glucosamine: R^1=OH, R^2=H

$C_8H_{15}NO_6$ Mol. Wt. 221.21
2-Acetamido-2-deoxy-D-glucopyranose [7512-17-6]
Definition *N*-Acetylglucosamine is obtained from chitin by enzymatic hydrolysis with hydrochloric acid, followed by isolation. It consists of *N*-acetyl-D-glucosamine.
Content *N*-Acetylglucosamine, when dried, contains 95.0–101.5% of *N*-acetyl-D-glucosamine ($C_8H_{15}NO_6$).
Description *N*-Acetylglucosamine occurs as white to whitish crystals or powder. It is odorless and has a characteristic sweet taste.
Identification To 0.5 ml of a solution (1 in 100) of *N*-Acetylglucosamine, add 0.1 ml of borate buffer (pH 9.1), and heat at 90–100°C for 3 minutes. After quick cooling, add 3.0 ml of *p*-dimethylaminobenzaldehyde TS, and warm at 37°C for 20 minutes. A red-purple color develops.
Purity
(1) Clarity and color of solution Colorless and clear (1.0 g, water 20 ml).
(2) Chloride Not more than 0.30% as Cl (0.10 g, Control solution 0.01 mol/L hydrochloric acid 0.85 ml).

(3) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Lead Not more than 10 μg/g (1.0 g , Method 1).

(5) Arsenic Not more than 2.0 μg/g as As_2O_3 (1.0 g, Method 3, Apparatus B).

Loss on Drying Not more than 1.0% (105°C, 3 hours).

Residue on Ignition Not more than 0.30% (2 g, 600°C, 8 hours).

Assay

Test Solution Weigh accurately about 0.5 g of *N*-Acetylglucosamine, previously dried, and dissolve it in water to make exactly 50 ml. Remove the insoluble matter by filtration or centrifugation.

Standard Solution Weigh accurately about 0.2 g of *N*-acetylglucosamine for assay, previously dried, and dissolve it in water to make exactly 20 ml.

Procedure Analyze 10 μl portions of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Measure the peak areas (A_T and A_S) for the test solution and the standard solution. Determine the content using the formula:

$$\text{Content (\%) of } N\text{-acetyl-D-glucosamine } (C_8H_{15}NO_6) = \frac{\text{Weight (g) of } N\text{-acetylglucosamine for assay}}{\text{Weight of the sample (g)}} \times \frac{A_T}{A_S} \times 100$$

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube of 4.6 mm internal diameter and 25 cm length.

Column packing material: 5-μm amino-bonded silica gel for liquid chromatography.

Column temperature: Room temperature.

Mobile phase: A 3:1 mixture of acetonitrile/water.

Flow rate: Adjust the flow rate so that the retention time of *N*-acetyl-D-glucosamine is about 10 minutes.

Acid Clay

酸性白土

Definition Acid Clay is obtained by purifying montmorillonite clay. It consists mainly of hydrous aluminum silicate.

Description Acid Clay occurs as a grayish white to yellow-brown powder or granules.

Identification

(1) Mix 1.0 g of Acid Clay with 3.0 g of anhydrous sodium carbonate and 0.4 g of boric acid, and heat the mixture in a platinum or nickel crucible until it melts completely. Cool, and then add hydrochloric acid until no effervescence is observed. Add an additional 10 ml of hydrochloric acid, heat on a water bath until the mixture becomes gelatinous, cool, and then filter. The filtrate obtained responds to all the tests for Aluminum Salt as directed in the Qualitative Tests.

(2) To a 100-ml measuring cylinder containing 100 ml of water, add 2.0 g of Acid Clay in small portions, and allow to stand for 24 hours. The precipitate formed is not more than 15 ml.

Purity

(1) pH 4.0–10.0.

Test Solution To 10.0 g of Acid Clay, add 100 ml of water, and heat for 2 hours on a water bath with occasional shaking while replenishing the evaporated water. After cooling, filter by suction through a 47-mm diameter membrane filter (0.45-μm pore size). If the filtrate is turbid, filter repeatedly through the same filter. Wash the residues in the beaker and on the filter with water, combine the washings with the filtrate, and add water to make 100 ml.

(2) Water-soluble substances Not more than 0.50%.

Evaporate 50 ml of the test solution prepared in Purity (1) to dryness. Dry the residue at 110°C for 2 hours, and weigh.

(3) Lead Not more than 40 μg/g as Pb.

Test Solution Weigh 1.0 g of Acid Clay, add 20 ml of diluted hydrochloric acid (1 in 25) and 50 ml of water, shake well, and boil gently for 30 minutes. Allow it to cool and filter. Wash the residue with water, combine the washings with the filtrate, and again add water to make 100 ml. Refer to the solution obtained as solution A. Measure 25 ml of solution A, and evaporate on a water bath to dryness. Dissolve the residue in diluted hydrochloric acid (1 in 10) to make 20 ml.

Control Solution To 1.0 ml of Lead standard Solution, add diluted hydrochloric acid (1 in 10) to make 20 ml.

Procedure Proceed as directed in Method 1 in the Lead Limit Test.

(4) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Evaporate 50 ml of solution A, prepared in Purity (3), to 5 ml on a water bath.

Apparatus Use apparatus B.

Loss on Ignition Not more than 35.0% (at 110°C for 3 hours, then at 550°C for 3 hours).

Activated Acid Clay

活性白土

Definition Activated Acid Clay is obtained by treating acid clay with sulfuric acid. Its principal constituent is hydrous aluminum silicate.

Description Activated Acid Clay occurs as a whitish to gray powder or granules.

Identification Mix 1.0 g of Acid Clay with 3.0 g of anhydrous sodium carbonate and 0.4 g of boric acid, and heat the mixture in a platinum or nickel crucible until it melts completely. Cool, and add hydrochloric acid until no effervescence is observed. Add an additional 10 ml of hydrochloric acid, and heat on a water bath until the mixture becomes gelatinous, cool, and filter. The filtrate obtained responds to all the tests for Aluminum Salt as directed in the Qualitative Tests.

Purity

(1) pH 2.0–6.0.

Test Solution To 10.0 g of Activated Acid Clay, add 100 ml of water, and heat for 2 hours on a water bath with occasional shaking while replenishing the evaporated water. After cooling, filter by suction through a 47-mm diameter membrane filter (0.45-μm pore size). If the filtrate is turbid,

filter repeatedly through the same filter. Wash the residues in the beaker and on the filter with water, combine the washings with the filtrate, and add water to make 100 ml. Use about 20 ml of this solution for testing.

(2) Water-soluble substances Not more than 1.6%.

Evaporate 50 ml of the test solution prepared in Purity (1) to dryness. Dry the residue at 110°C for 2 hours, and weigh.

(3) Lead Not more than 40 μg/g as Pb.

Test Solution Weigh 1.0 g of Activated Acid Clay, add 20 ml of diluted hydrochloric acid (1 in 25) and 50 ml of water, shake well, and boil gently for 30 minutes. Allow it to cool and filter. Wash the residue with water, combine the washings with the filtrate, and again add water to make 100 ml. Refer to the solution obtained as solution A. Measure 25 ml of solution A, and evaporate on a water bath to dryness. To the residue, add diluted hydrochloric acid (1 in 10) to dissolve, and make 20 ml.

Control Solution To 1.0 ml of Lead standard Solution, add diluted hydrochloric acid (1 in 10) to make 20 ml.

Procedure Proceed as directed in Method 1 in the Lead Limit Test.

(4) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Evaporate 50 ml of solution A, prepared in Purity (3), to 5 ml on a water bath.

Procedure Use apparatus B.

Loss on Ignition Not more than 35.0% (at 110°C for 3 hours, then at 550°C for 3 hours).

Active Carbon

活性炭

Description Active Carbon occurs as a black powder, granules, or fibrous substances. It is odorless and tasteless.

Identification If the sample is a powder, use it as is. If it is in a granular or fibrous form, completely grind into a powder before the tests.

(1) Weigh about 0.1 g of Active Carbon, add 10 ml of diluted methylene blue TS and 2 drops of diluted hydrochloric acid (1 in 4), shake well, and filter through a dry filter paper for quantitative analysis (5C). The solution is colorless.

(2) Weigh about 0.5 g of powdered Active Carbon into a test tube. When heated over a direct flame while supplying air to the test tube mouth, it burns without flames. When the gas evolved is passed through calcium hydroxide TS, white turbidity is formed.

Purity If the sample is a powder, use it as is. If it is in a granular or fibrous form, completely grind into a powder before the tests. Weigh 4.0 g of Active Carbon, previously dried at 110–120°C for 3 hours, add 180 ml of water containing 0.1 ml of diluted nitric acid (1 in 100), and heat for about 10 minutes, keeping boiling slowly. Cool, add water to make 200 ml, and filter through a dry filter paper for quantitative analysis (5C). Discard about 30 ml of the initial filtrate, and use the subsequent filtrate (solution A) for tests (1) through (5) below.

(1) Chloride Not more than 0.53% as Cl.

Test Solution 1.0 ml of solution A.

Control Solution 0.30 ml of 0.01 mol/L hydrochloric acid.

(2) Sulfate Not more than 0.48% as SO_4.

Test Solution 2.5 ml of solution A.

Control Solution 0.50 ml of 0.005 mol/L sulfuric acid.

(3) Zinc Not more than 0.10% as Zn.

Test Solution Measure 2 ml of the solution A, and add water containing 0.1 ml of nitric acid (1 in 100) to make 200 ml.

Control Solution Measure 4 ml of Zinc Standard Solution, and add water containing 0.1 ml of nitric acid (1 in 100) to make 200 ml.

Procedure Proceed with the test solution and control solution under the conditions below, as directed under Atomic Absorption Spectrophotometry. The absorbance of the sample solution does not exceed that of the control solution.

Operating Conditions

Light source: Zinc hollow cathode lamp.

Wavelength: 213.9 nm.

Supporting gas: Air.

Combustible gas: Acetylene or hydrogen.

(4) Lead Not more than 10 μg/g as Pb.

Test Solution Measure 50 ml of the solution A, evaporate to dryness on a water bath, dissolve the residue with 10 ml of diluted nitric acid (1 in 150).

Control Solution To 1.0 ml of Lead Standard Solution, add 2 ml of diluted nitric acid (1 in 150) to make 10 ml.

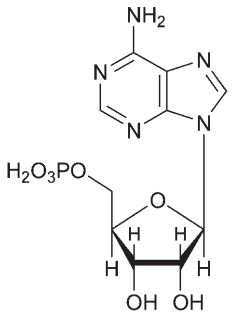
Procedure Determine the absorbances of the test solution and the control solution as directed in Method 1 in the Lead Limit Test. The absorbance of the test solution does not exceed that of the control solution

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (Method 2, Apparatus B).

Measure 25 ml of the solution A, and evaporate on a water bath to dryness. Use the residue as the test sample.

5′-Adenylic Acid

5′-アデニル酸



$C_{10}H_{14}N_5O_7P$ Mol. Wt. 347.22

Adenosine 5′-monophosphoric acid [61-19-8]

Definition 5′-Adenylic Acid is obtained by enzymatic hydrolysis of nucleic acids that are water-extracted from the yeast *Candida utilis*, followed by isolation. It consists of 5′-adenylic acid.

Content 5′-Adenylic Acid, when calculated on the dried basis, contains 98.0–102.0% of 5′-adenylic acid ($C_{10}H_{14}N_5O_7P$).

Description 5′-Adenylic Acid occurs as colorless or white

crystals or as a white crystalline powder.

Identification

(1) Dissolve 0.010 g of 5′-Adenylic Acid in 1,000 ml of diluted hydrochloric acid (1 in 1,000). The solution obtained exhibits an absorption maximum at a wavelength of 255–259 nm.

(2) Dissolve 0.25 g of 5′-Adenylic Acid in 1 ml of sodium hydroxide TS, and add 5 ml of water. To the resulting solution, add 2 ml of magnesia TS. No precipitate is formed. Add 7 ml of nitric acid, and boil for 10 minutes. It responds to test (2) for Phosphate in the Quantitative Tests.

Purity

(1) Clarity and color of solution Colorless and almost clear.

Test Solution Weigh 0.50 g of 5′-Adenylic Acid, dissolve it in 2 ml of sodium hydroxide TS, and add water to make 10 ml.

(2) Heavy metals Not more than 10 μg/g as Pb.

Test Solution Weigh 2.0 g of 5′-Adenylic Acid, and add 8 ml of sodium hydroxide TS and 30 ml of water to dissolve. Neutralize the solution with diluted acetic acid (1 in 20) or ammonia TS, and then add 2 ml of the acetic acid (1 in 20) and water to make 50 ml.

Control Solution To 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(3) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.50 g of 5′-Adenylic Acid, and dissolve it in 5 ml of diluted hydrochloric acid (1 in 4).

Apparatus Use apparatus B.

(4) Absorbance ratio Weigh 0.010 g of 5′-Adenylic Acid, and dissolve it in diluted hydrochloric acid (1 in 1,000) to make 1,000 ml. When the absorbances of the solution at 250 nm, 260 nm, and 280 nm are expressed as A_1, A_2, and A_3, respectively, A_1/A_2 is 0.82–0.88, and A_3/A_2 is 0.19–0.23.

(5) Other nucleic acid degradation products

Test Solution Weigh 0.10 g of 5′-Adenylic Acid, dissolve it in 0.5 ml of sodium hydroxide TS, and add water to make 20 ml.

Procedure Analyze a 1-μl portion of the test solution by thin-layer chromatography using a 6:5:2 mixture of 1-propanol/ammonia TS/acetone as the developing solvent. No control solution is used. Use a thin-layer plate coated with fluorescent silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the original line, and air-dry the plate. Examine under ultraviolet light (around 250 nm) in a dark place. Only one spot is observed.

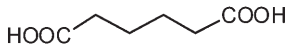
Loss on Drying Not less than 6.0% (120°C, 4 hours).

Assay Weigh accurately about 0.2 g of 5′-Adenylic Acid, dissolve it in 1 ml of sodium hydroxide TS, and add water to make exactly 200 ml. To exactly 2 ml of this solution, add diluted hydrochloric acid (1 in 1,000) to make exactly 200 ml. Measure the absorbance (A) of the solution at 257 nm, and determine the content using the formula:

$$\text{Content (\%) of 5′-adenylic acid } (C_{10}H_{14}N_5O_7P) = \frac{0.2 \times 2.315 \times A}{\text{Dry basis weight (g) of the sample}} \times 100$$

Adipic Acid

アジピン酸



$C_6H_{10}O_4$ Mol. Wt. 146.14

Hexanedioic acid [124-04-9]

Content Adipic Acid contains 99.6–101.0% of adipic acid ($C_6H_{10}O_4$).

Description Adipic Acid occurs as white crystals or crystalline powder. It is odorless and has an acid taste.

Identification

(1) To 5 ml of a solution of Adipic Acid (1 in 20), add ammonia TS to adjust the pH to about 7, and add 2–3 drops of iron(III) chloride solution (1 in 10). A brown precipitate is formed.

(2) Transfer 0.05 g of Adipic Acid into a test tube, add 0.05 g of resorcinol and 1 ml of sulfuric acid, and shake. Heat at 130°C for 10 minutes. Add, dropwise, sodium hydroxide solution (3 in 10) while cooling to make alkaline. Add water to make 10 ml. A red-purple color develops.

Purity

(1) Melting point 151.5–154°C.

(2) Heavy metals Not more than 10 μg/g as Pb.

Test Solution To 2.0 g of Adipic Acid, add 2 ml of hydrochloric acid and 0.4 ml of nitric acid. Evaporate to dryness on a water bath, add 1 ml of diluted hydrochloric acid (1 in 4) and 15 ml of water to the residue, and dissolve by heating. Cool, add 1 drop of phenolphthalein TS, and then add ammonia TS dropwise until a slightly pink color develops. Add 2 ml of diluted acetic acid (1 in 20), filter if necessary, and add water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

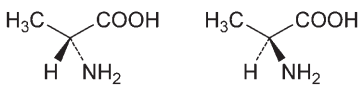
Water Content Not more than 0.20% (1 g, Direct Titration).

Assay Weigh accurately about 1.5 g of Adipic Acid, and dissolve it in 75 ml of freshly boiled and cooled water. Titrate with 0.5 mol/L sodium hydroxide (indicator: 2 drops of phenolphthalein TS).

Each ml of 0.5 mol/L sodium hydroxide = 36.54 mg of $C_6H_{10}O_4$

DL-Alanine

DL-アラニン



$C_3H_7NO_2$ Mol. Wt. 89.09

(2*RS*)-2-Aminopropanoic acid [302-72-7]

Content DL-Alanine, when calculated on the dried basis,

contains 98.5–102.0% of DL-alanine ($C_3H_7NO_2$).

Description DL-Alanine occurs as a colorless to white crystalline powder having a sweet taste.

Identification Determine the absorption spectrum of DL-Alanine as directed in the Potassium Bromide Disk Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Clarity and color of solution Colorless and clear (1.0 g, Water 10 ml).

(2) pH 5.5–7.0 (1.0 g, Water 20 ml).

(3) Chloride Not more than 0.021% as Cl (0.50 g, Control solution 0.01 mol/L hydrochloric acid 0.30 ml.)

(4) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50g, Method 1, Apparatus B).

Loss on Drying Not more than 0.30% (105°C, 3 hours).

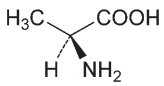
Residue on Ignition Not more than 0.20%.

Assay Weigh accurately about 0.2 g of DL-Alanine, dissolve it in 3 ml of formic acid, add 50 ml of acetic acid, and titrate with 0.1 mol/L perchloric acid. The endpoint is usually confirmed potentiometrically. When crystal violet–acetic acid TS (1 ml) is used as the indicator, the endpoint is when the color of the solution changes from purple through blue to green. Perform a blank test in the same manner, make any necessary correction, and calculate on the dried basis.

Each ml of 0.1 mol/L perchloric acid = 8.909 mg of $C_3H_7NO_2$

L-Alanine

L-アラニン



$C_3H_7NO_2$ Mol. Wt. 89.09

(2*S*)-2-Aminopropanoic acid [56-41-7]

Content L-Alanine, when calculated on the dried basis, contains 98.0–102.0% of L-alanine ($C_3H_7NO_2$).

Description L-Alanine occurs as white crystals or crystalline powder. It is odorless, and has a sweetish taste.

Identification

(1) To 5 ml of a solution of L-Alanine (1 in 1,000), add 1 ml of ninhydrin solution (1 in 50), and heat for 3 minutes in a water bath. A bluish purple color develops.

(2) Dissolve 0.2 g of L-Alanine in 10 ml of diluted sulfuric acid (1 in 20), add 0.1 g of potassium permanganate, and heat to boil. The odor of acetaldehyde develops.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +13.5 to +15.5°.

Weigh accurately about 10 g of L-Alanine, and dissolve it in 6 mol/L hydrochloric acid to make exactly 100 ml. Measure the angular rotation of this solution, and calculate on the dried basis.

(2) Clarity and color of solution Colorless and clear (1.0 g, water 10 ml).

(3) pH 5.7–6.7 (1.0 g, water 20 ml).

(4) Chloride Not more than 0.10% as Cl (0.07 g, Control solution 0.01 mol/L hydrochloric acid 0.20 ml).

(5) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method1, Control solution Lead Standard Solution 2.0 ml).

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Drying Not more than 0.30% (105°C, 3 hours).

Residue on Ignition Not more than 0.20%.

Assay Weigh accurately about 0.2 g of L-Alanine, and proceed as directed in the Assay for L-Asparagine.

Each ml of 0.1 mol/L perchloric acid = 8.909 mg of $C_3H_7NO_2$

L-Alanine Solution

L-アラニン液

Content L-Alanine Solution contains not more than 15% of L-alanine ($C_3H_7NO_2$ = 89.09) and 95.0–110.0% of the labeled content.

Description L-Alanine Solution is a colorless liquid. It is odorless or has a very slight characteristic odor. It has a sweetish taste.

Identification

(1) To 5 ml of diluted L-Alanine Solution (1 in 200), add 1 ml of ninhydrin solution (1 in 50), and heat for 3 minutes in a water bath. A bluish purple color develops.

(2) To 5 g of L-Alanine Solution, add 50 ml of diluted hydrochloric acid (1 in 2), and mix. It shows dextrorotatory.

Purity

(1) Heavy metals Not more than 20 μg/g of L-alanine ($C_3H_7NO_2$) as Pb.

Test Solution Weigh an amount of L-Alanine Solution equivalent to 1.0 g of L-alanine ($C_3H_7NO_2$), add about 40 ml of water, then 2 ml of acetic acid (1 in 20), and add water to make 50 ml.

Control Solution To 2.0 ml of Lead Standard Solution, add 2 ml of acetic acid (1 in 20), and add water to make 50 ml.

(2) Arsenic Not more than 4.0 μg/g of L-alanine ($C_3H_7NO_2$) as As_2O_3.

Test Solution Weigh an amount of L-Alanine Solution equivalent to 0.5 g of L-alanine ($C_3H_7NO_2$), dissolve it in 5 ml of water, and heat if necessary.

Apparatus Apparatus B

Residue on Ignition Not more than 0.20% of the amount of L-alanine ($C_3H_7NO_2$).

Assay Weigh accurately an amount of L-Alanine Solution equivalent to about 0.2 g of L-alanine ($C_3H_7NO_2$), and proceed as directed in the Assay for L-Asparagine.

Each ml of 0.1 mol/L perchloric acid = 8.909 mg of $C_3H_7NO_2$

Alginic Acid

アルギン酸

[9005-32-7]

Content Alginic Acid, when dried, contains 91.0–104.5% of alginic acid.

Description Alginic Acid occurs in white to light yellow filamentous, granular, or powdered form. It has a slight characteristic odor and taste.

Identification Prepare a test solution by dissolving 0.25 g of Alginic Acid in 50 ml of sodium hydroxide TS. To 10 ml of the test solution, add 2 ml of calcium chloride solution (2.5 in 100). A gelatinous precipitate is formed. To 10 ml of the test solution, add 5 ml of ammonium sulfate saturated solution. No precipitate is formed.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: −80 to −180° (0.50 g, sodium hydroxide TS, 100 ml, on the dried basis).

(2) pH 2.0–3.4 (3% suspension).

(3) Sulfate Not more than 0.96% as SO_4.

Test Solution Weigh about 0.10 g of Alginic Acid accurately in a flask, dissolve it in 20 ml of sodium hydroxide TS, and neutralize with diluted hydrochloric acid (1 in 4). Next, add 1 ml of hydrochloric acid, shake well, heat in a water bath for several minutes, cool, and filter. Wash the flask three times with 10 ml of water each time, filter the washings through the same filter paper, combine the filtrates, and add water to make 50 ml. Measure 10 ml of this solution, and add water to make 50 ml.

Control Solution To 0.40 ml of 0.005 mol/L sulfuric acid, add 1 ml of diluted hydrochloric acid (1 in 4) and water to make 50 ml.

(4) Phosphate Weigh 0.10 g of Sodium Alginate accurately, dissolve it 20 ml of sodium hydroxide solution TS, and neutralize with diluted nitric acid (1 in 4), and make it uniformly. Cool, add 5 ml of diluted nitric acid (1 in 4) and 20 ml of Ammonium Molybdate TS, and warm. No yellow precipitate is formed.

(5) Heavy metals Not more than 40 μg/g as Pb (0.50 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(6) Lead Not more than 10 μg/g as Pb (1.0 g, Method 1).

(7) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Loss on Drying Not more than 15.0% (105°C, 4 hours).

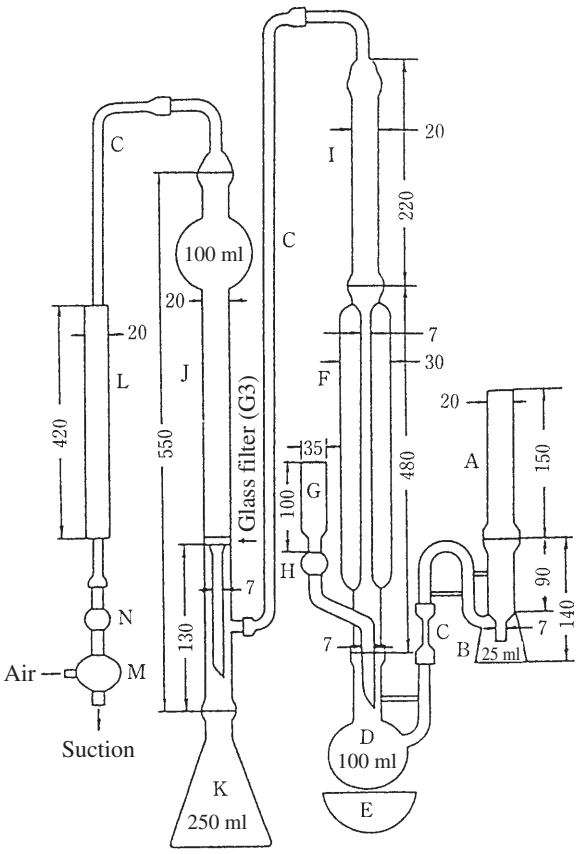
Residue on Ignition Not more than 10.0% (calculated on the dried basis).

Microbial Limits Proceed as directed in the Microbial Limit Tests. The total bacterial count is not more than 5,000/g and *Escherichia coli* is negative.

Assay

(1) Apparatus The apparatus is outlined in the figure given in the right column. Use 35/25-spherical ground glass for joints.

(2) Procedure Weigh accurately about 0.25 g of Alginic Acid, previously dried, into reaction flask D, add 25 ml of diluted hydrochloric acid (1 in 120) and several pieces of zeolite, and connect the flask to reflux condenser F. Moisten the spherical ground-glass joint with a small amount of phosphoric acid. Raise the mercury in mercury valve B about 5 cm in its tube by compressed air from three-way stop cock M, shut the cock, and confirm for a few minutes that the top of the mercury raised does not go back down. Flow carbon dioxide-free air into the apparatus with suction at a flow rate of about 3–6 L per hour, boil gently for 2 minutes by heating on mantle heater E, and allow to cool for 15 minutes. Place 23 ml of hydrochloric acid in drop tube G, partially disconnect the top of absorption tube J, immediately add exactly 25 ml of 0.25 mol/L sodium hydroxide, and then add 5 drops of *n*-butanol. Connect absorption tube J again. Flow carbon dioxide-free air into the apparatus with suction at a flow rate of about 2 L per hour, add hydrochloric acid into reaction flask D through drop tube G, and boil the sample in the flask for 3 hours by heating on mantle heater E. Next, stop the heating and suction, and slowly transfer the 0.25 mol/L sodium hydroxide solution placed in absorption tube J into conical flask K by compressed air from three-way stop cock M. Rinse the inside of absorption tube J three



(Unit: mm)

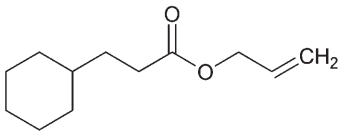
A: Soda lime tube
B: Mercury bulb
C: Rubber joint tube
D: Reaction flask
E: Mantle heater
F: Reflux condenser
G: Dropping tube
H: Stopcock
I: Trap (packed with 25 g of zinc powder with a size of not more than 860 μm)
J: Absorption tube
K: Conical flask
L: Soda lime tube
M: Three-way stopcock
N: Flow control valve

times with 15 ml of water each time, and transfer the rinse into flask K after each rinse, using compressed air. Remove flask K from the apparatus, add 10 ml of barium chloride solution (1 in 10), stopper, and shake gently for about 2 minutes. Add 2 drops of phenolphthalein TS, and titrate with 0.1 mol/L hydrochloric acid. Perform a blank test in the same manner.

Each ml of 0.1 mol/L hydrochloric acid = 25.00 mg of Alginic Acid

Allyl Cyclohexylpropionate

シクロヘキシルプロピオン酸アリル



$C_{12}H_{20}O_2$ Mol. Wt. 196.29

Allyl 3-cyclohexylpropionate [2705-87-5]

Content Allyl Cyclohexylpropionate contains not less than 98.0% of allyl cyclohexylpropionate ($C_{12}H_{20}O_2$).

Description Allyl Cyclohexylpropionate is a colorless to light yellow, transparent liquid having a characteristic odor.

Identification Determine the absorption spectrum of Allyl Cyclohexylpropionate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Refractive index n_D^{20}: 1.457–1.462.

(2) Specific gravity 0.948–0.953.

(3) Clarity of solution Clear (1.0 ml, 80% (vol) ethanol 4.0 ml).

(4) Acid value Not more than 1.0 (Flavoring Substances Tests).

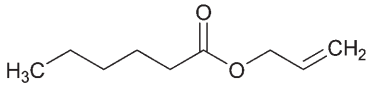
Assay Weigh accurately about 1.5 g of Allyl Cyclohexylpropionate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 98.14 mg of $C_{12}H_{20}O_2$

Allyl Hexanoate

Allyl Caproate

ヘキサン酸アリル



$C_9H_{16}O_2$ Mol. Wt. 156.22

Prop-2-en-1-yl hexanoate [123-68-2]

Content Allyl Hexanoate contains not less than 98.0% of allyl hexanoate ($C_9H_{16}O_2$).

Description Allyl Hexanoate is a colorless to light yellow, transparent liquid having a pineapple-like odor.

Identification Determine the absorption spectrum of Allyl Hexanoate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Refractive index n_D^{20}: 1.422 –1.426.

(2) Specific gravity 0.887–0.893.

(3) Clarity of solution Clear (1.0 ml, 70% (vol) ethanol 7.0 ml).

(4) Acid value Not more than 1.0. (Flavoring Substances Tests).

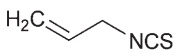
Assay Weigh accurately about 1 g of Allyl Hexanoate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 78.11 mg of $C_9H_{16}O_2$

Allyl Isothiocyanate

Volatile Oil of Mustard

イソチオシアン酸アリル



C_4H_5NS Mol. Wt. 99.16

Allyl isothiocyanate [57-06-7]

Content Allyl Isothiocyanate contains not less than 97.0% of allyl isothiocyanate (C_4H_5NS).

Description Allyl Isothiocyanate is a colorless to light yellow, transparent liquid having a strong and irritating mustard-like odor.

Identification

(1) Measure 3 ml of Allyl Isothiocyanate, add gradually 4 ml of sulfuric acid while cooling, and shake. A gas is evolved. The resulting solution is transparent yellow, and gradually becomes viscous. The strong, irritating mustard-like odor disappears.

(2) To 2 ml of Allyl Isothiocyanate, add 3 ml of ethanol and 4 ml of ammonia TS, warm to about 50°C, and allow

to stand. The solution is transparent at first, but crystals are formed in about 3 hours.

Purity

(1) Refractive index n_D^{20}: 1.528–1.531.

(2) Specific gravity 1.018–1.023.

(3) Phenols and thiocyanate compounds Measure 1.0 ml of Allyl Isothiocyanate, dissolve it in 5 ml of ethanol, and add 1 drop of iron(III) chloride solution (1 in 10). Neither red nor blue color develops.

Assay Weigh accurately about 3 g of Allyl Isothiocyanate, and dissolve it in ethanol to make exactly 100 ml. Measure exactly 5 ml of this solution, add 5 ml of ammonia TS, and add exactly 50 ml of 0.1 mol/L silver nitrate. Heat under a reflux condenser in a water bath for 1 hour. Cool, add water to make exactly 100 ml, and filter through a dry filter paper. Discard about 10 ml of the initial filtrate, then measure exactly 50 ml of the subsequent filtrate, add 5 ml of nitric acid and 2 ml of ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 mol/L ammonium thiocyanate. Perform a blank test in the same manner.

Each ml of 0.1 mol/L silver nitrate = 4.958 mg of C_4H_5NS

Aluminum Ammonium Sulfate

Crystal: Ammonium Alum
Dried: Burnt Ammonium Alum

硫酸アルミニウムアンモニウム

$AlNH_4(SO_4)_2 \cdot nH_2O$ (n = 12, 10, 4, 3, 2, or 0)

Mol. Wt. dodecahydrate 453.33
anhydrous 237.15

Aluminum ammonium sulfate dodecahydrate [7784-26-1]
Aluminum ammonium sulfate decahydrate
Aluminum ammonium sulfate tetrahydrate
Aluminum ammonium sulfate trihydrate
Aluminum ammonium sulfate dihydrate
Aluminum ammonium sulfate [7784-25-0]

Definition Aluminum Ammonium Sulfate occurs in crystalline form, called Aluminum Ammonium Sulfate, and in dried form, called Aluminum Ammonium Sulfate (dried).

Content Aluminum Ammonium Sulfate, when dried at 200°C for 4 hours, contains not less than 96.5% of aluminum ammonium sulfate ($AlNH_4(SO_4)_2$).

Description Aluminum Ammonium Sulfate occurs as colorless to white crystals, powder, flakes, granules, or lumps. It is odorless and has an astringent taste.

Identification A solution of Aluminum Ammonium Sulfate (1 in 20) responds to all tests for Aluminum Salt and for Ammonium Salt, and responds to tests (1) and (3) for Sulfate in the Qualitative Tests.

Purity

(1) Clarity and color of solution or water-insoluble substances

Crystal: Clarity and color of solution Colorless and almost clear (1.0 g, water 10 ml).

Dried: Water-insoluble substances Not more than 2.0%.

Weigh 2.0 g of Aluminum Ammonium Sulfate, and add 200 ml of water of about 80°C. Heat in a water bath for 10 minutes while stirring. Cool, and filter through a glass filter (1G4), previously dried at 105°C for 30 minutes, cooled, and accurately weighed. Wash the insoluble residue with 100 ml of water, and dry at 105°C for 2 hours together with the glass filter, weigh, and obtain the insoluble substances.

(2) Heavy metals Not more than 40 μg/g as Pb (0.50 g as powder, previously dried at 200°C for 4 hours, Method 1, Control solution Lead Standard Solution 2.0 ml).

(3) Iron Not more than 0.019% as Fe (0.052 g as powder, previously dried at 200°C for 4 hours, Method 1, Control solution Iron Standard Solution 1.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g as powder, previously dried at 200°C for 4 hours, Method 1, Apparatus B).

Assay Weigh accurately about 0.8 g (as powder) of Aluminum Ammonium Sulfate, previously dried at 200°C for 4 hours, add 100 ml of water, dissolve by heating in a water bath while shaking, and filter. Wash the residue with water, combine the filtrate with the washings, and add water to make exactly 200 ml. Measure exactly 25 ml of this solution, and proceed as directed in the Assay for Aluminum Potassium Sulfate.

Each ml of 0.01 mol/L EDTA = 2.371 mg of $AlNH_4(SO_4)_2$

Aluminum Potassium Sulfate

Crystal: Alum or Potassium Alum
Dried: Burnt Alum

硫酸アルミニウムカリウム

$AlK(SO_4)_2 \cdot nH_2O$ (n = 12, 10, 6, 3, 2, or 0)

Mol. Wt. dodecahydrate 474.39
anhydrous 258.21

Aluminum potassium sulfate dodecahydrate [7784-24-9]
Aluminum potassium sulfate decahydrate
Aluminum potassium sulfate hexahydrate
Aluminum potassium sulfate trihydrate
Aluminum potassium sulfate dihydrate
Aluminum potassium sulfate [10043-67-1]

Definition Aluminum Potassium Sulfate occurs in crystalline form, called Aluminum Potassium Sulfate, and in dried form, called Aluminum Potassium Sulfate (dried).

Content Aluminum Potassium Sulfate, when dried at 200°C for 4 hours, contains not less than 96.5% of aluminum potassium sulfate [$AlK(SO_4)_2$].

Description Aluminum Potassium Sulfate occurs as colorless to white crystals, powder, flakes, granules, or lumps. It is odorless and has an astringent taste.

Identification A solution of Aluminum Potassium Sulfate (1 in 20) responds to all tests for Aluminum Salt, to test (1) for Potassium Salt, and to tests (1) and (3) for Sulfate in the Qualitative Tests.

Purity

(1) Clarity and color of solution or water-insoluble substances

Crystal: Clarity and color of solution Colorless and almost clear.

Proceed as directed in Purity (1) for Aluminum Ammonium Sulfate.

Dried: Water-insoluble substances Not more than 2.0%.

Proceed as directed in Purity (1) for Aluminum Ammonium Sulfate.

(2) Heavy metals Not more than 40 μg/g as Pb (0.50 g as powder, previously dried at 200°C for 4 hours, Method 1, Control solution Lead Standard Solution 2.0 ml).

(3) Iron Not more than 0.019% as Fe (0.054 g as powder, previously dried at 200°C for 4 hours, Method 1, Control solution Iron Standard Solution 1.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g as powder, previously dried at 200°C for 4 hours, Method 1, Apparatus B).

Assay Weigh accurately about 0.8 g (as powder) of Aluminum Potassium Sulfate, previously dried at 200°C for 4 hours, add 100 ml of water, dissolve by heating in a water bath while shaking, and filter. Wash the residue thoroughly with water, combine the filtrate and the washings, and add water to make exactly 200 ml. Measure exactly 25 ml of this solution, add exactly 50 ml of 0.01 mol/L EDTA, and heat to boiling. After cooling, add 7 ml of sodium acetate solution (2 in 15) and 85 ml of absolute ethanol, and titrate the excess EDTA with 0.01 mol/L zinc acetate (indicator: 3 drops of xylenol orange TS) until the yellow color of the solution changes to red.

Each ml of 0.01 mol/L EDTA = 2.582 mg of $AlK(SO_4)_2$

Ammonia

アンモニア

NH_3 Mol. Wt. 17.03

Ammonia [7664-41-7]

Description Ammonia is a colorless gas having a characteristic odor.

Identification

(1) Bring a glass rod wetted with hydrochloric acid close to Ammonia. White fumes are evolved.

(2) Ammonia changes the color of a red litmus paper wetted with water to blue.

Purity Perform the following tests, using a test solution prepared by saturating water at 20°C with Ammonia.

(1) Sulfur compounds Add 5 ml of silver nitrate–ammonia TS to 5 ml of the test solution. Heat at 60°C for 5 minutes while shaking well in a dark place. No brown color develops.

(2) Readily oxidizable substances Add 7 ml of water to 3.0 ml of the test solution. Pour gradually 30 ml of diluted sulfuric acid (1 in 20), shake, and add 0.10 ml of 0.02 mol/L potassium permanganate. The pink color of the solution does not disappear.

Ammonium Alginate

アルギン酸アンモニウム

Ammonium Alginate [9005-34-9]

Content Ammonium Alginate, when dried, contains 88.7–103.6% of ammonium alginate.

Description Ammonium Alginate occurs in white to light yellowish brown, filamentous, granular, or powdered form.

Identification

(1) Prepare a test solution as follows: To 0.5 g of Ammonium Alginate, add 50 ml of water while stirring, warm the mixture at 60–70°C for 20 minutes with occasional shaking to make it homogenous, and cool.

(i) To 5 ml of the test solution, add 1 ml of calcium chloride solution (3 in 40). A gelatinous precipitate is formed immediately.

(ii) To 1 ml of the test solution, add 1 ml of a saturated solution of ammonium sulfate. No precipitate is formed.

(2) Ammonium Alginate responds to the test for Ammonium Salt in the Qualitative Tests.

Purity

(1) Water-insoluble substances Not more than 2.0% (on the dried basis).

Weigh accurately about 2 g of Ammonium Alginate in a 2,000-ml Erlenmeyer flask, add 800 ml of water, neutralize with sodium hydroxide TS, and then add an additional 3 ml of sodium hydroxide TS. Add 40 ml of hydrogen peroxide, cover the flask, and boil for 1 hour with frequent stirring. Filter while hot through a glass filter with a glass fiber filter paper with suction. The filter and filter paper should be previously dried at 105°C for about 1 hour, cooled in a desiccator, and accurately weighed. If filtration is slow due to the high viscosity of the sample solution, boil again until the viscosity is sufficiently reduced to permit filtration. Wash the filter with the filter paper thoroughly with hot water, dry them at 105°C for 1 hour, cool, and weigh accurately. Calculate as the percentage of the dry weight.

(2) Lead Not more than 5.0 μg/g as Pb (2.0 g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Loss on Drying Not more than 15.0% (105°C, 4 hour).

Residue on Ignition Not more than 7.0% (3 g, 800°C, 15 minutes, on the dried basis).

Microbial Limit When the tests are conducted as directed in the Microbial Limit Tests in the General Tests, the total bacterial count is not more than 5,000/g, and the fungi (yeasts and molds) count is not more than 500/g. Coliforms are negative.

Coliform test To 1 g of Ammonium Alginate, add fluid lactose broth medium or BGLB medium to make 100 ml. Depending on the sample characteristics, the sample may be dispersed in more than the specified volume of liquid medium. If necessary, adjust its pH to 6–8, and incubate at 30–35°C for 24–72 hours. Examine the medium for growth, and shake gently if growth is present. Using a platinum loop, place a streak on the surface of MacConkey agar medium. Incubate at 30–35°C for 18–24 hours. If colonies of pink to red Gram-negative rod-shaped bacteria with a reddish precipitation zone around the periphery are not found, the sample is determined to be negative for coliforms.

If colonies matching the above description are found, transfer each of the colonies separately to the surface of EMB agar

medium, and incubate at 30–35°C for 18–24 hours. If typical colonies with a metallic sheen or dark purple-red color are not found, the sample is determined to be negative for coliforms.

If typical colonies with a metallic sheen or dark purple-red color are found, transfer fermentation vials of lactose broth medium, and incubate at 30–35°C for 18–48 hours. Gas forming, Gram-negative, non-spore-forming, rod-shaped bacteria in the vial are assumed to be coliforms. Rapid detection kits for coliforms may be used.

Effectiveness of culture media and confirmation of antimicrobial substances Use an appropriate *Escherichia coli* strain from among NBRC 3972, ATCC 8739, NCIMB 8545 or their respective equivalents. Incubate in lactose broth medium, fluid soybean-casein digest medium, or soybean-casein digest agar medium at 30–35°C for 18–24 hours. Prepare a suspension containing about 1,000 viable bacteria per ml by diluting the incubated culture with sodium chloride–peptone buffer solution, phosphate buffer, or lactose broth agar medium. Mix 0.1 ml of this suspension with the medium to be used for the test, and examine the effectiveness of the medium and the presence of anti-microbial substances both in the presence and absence of the sample.

Assay Proceed as directed in the Assay for Alginic Acid.

Each ml of 0.25 mol/L sodium hydroxide = 27.12 mg of ammonium alginate

Ammonium Bicarbonate

Ammonium Hydrogen Carbonate

炭酸水素アンモニウム

NH_4HCO_3 Mol. Wt. 79.06

Ammonium hydrogencarbonate [1066-33-7]

Content Ammonium Bicarbonate contains 20.0–30.0% of ammonia (NH_3 = 17.03).

Description Ammonium Bicarbonate occurs as white or translucent crystals, crystalline powder, or lumps having an odor of ammonia.

Identification Ammonium Bicarbonate responds to all tests for Ammonium Salt and for Bicarbonate in the Qualitative Tests.

Purity

(1) Clarity of solution Almost clear.

Proceed as directed in Purity (1) for Ammonium Carbonate.

(2) Chloride Not more than 0.004% as Cl.

Proceed as directed in Purity (2) for Ammonium Carbonate.

(3) Heavy metals Not more than 10 μg/g as Pb.

Proceed as directed in Purity (3) for Ammonium Carbonate.

(4) Arsenic Not more than 4.0 μg/g as As_2O_3.

Proceed as directed in Purity (4) for Ammonium Carbonate.

Residue on Ignition Not more than 0.010% (10 g).

Assay Proceed as directed in the Assay for Ammonium Carbonate.

Each ml of 0.1 mol/L hydrochloric acid = 1.703 mg of NH_3

Ammonium Carbonate

炭酸アンモニウム

Content Ammonium Carbonate contains not less than 30.0% of ammonia (NH_3 = 17.03).

Description Ammonium Carbonate occurs as white or translucent crystals, crystalline powder, or lumps having an odor of ammonia.

Identification Ammonium Carbonate responds to the test for Ammonium Salt and test (1) for Carbonate in the Qualitative Tests. Add magnesium sulfate to a solution of Ammonium Carbonate (1 in 20), and heat. A precipitate is produced.

Purity

(1) Clarity of Solution Almost clear (2.0 g, water 20 ml).

(2) Chloride Not more than 0.004% as Cl (2.0 g, Control solution 0.01 mol/L hydrochloric acid 0.20 ml).

(3) Heavy metals Not more than 10 μg/g as Pb.

Test Solution Weigh 2.0 g of Ammonium Carbonate, sublimate on a water bath, add 1 ml of diluted acetic acid (1 in 20) to the residue, and evaporate to dryness on a water bath. Dissolve the residue in 2 ml of diluted acetic acid (1 in 20), and add water to make 50 ml.

Control Solution To 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Residue on Ignition Not more than 0.01% (10 g).

Assay Weigh accurately a flask with a ground-glass stopper containing about 30 ml of water, add about 2.5 g of Ammonium Carbonate, and weigh the flask with sample accurately. Transfer into a 250-ml volumetric flask, and add water to make exactly 250 ml. Measure exactly 25 ml of this solution, and gradually add exactly 50 ml of 0.1 mol/L hydrochloric acid. Titrate the excess hydrochloric acid with 0.1 mol/L sodium hydroxide (indicator: 4–5 drops of bromophenol blue TS).

Each ml of 0.1 mol/L hydrochloric acid = 1.703 mg of NH_3

Ammonium Chloride

塩化アンモニウム

NH_4Cl Mol. Wt. 53.49

Ammonium chloride [12125-02-9]

Content Ammonium Chloride, when dried, contains not less than 99.0% of ammonium chloride (NH_4Cl).

Description Ammonium Chloride occurs as a white crystalline powder or crystalline lumps having a salty and cool taste.

Identification Ammonium Chloride responds to all tests for Ammonium Salt and for Chloride in the Qualitative Tests.

Purity

(1) Clarity of solution Almost clear (2.0 g, water 20 ml).

(2) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g,

Method 1, Apparatus B).
Loss on Drying Not more than 2.0% (4 hours).
Residue on Ignition Not more than 0.5%.
Assay Weigh accurately about 3 g of Ammonium Chloride, previously dried, and dissolve it in water to make exactly 250 ml. Measure exactly 25 ml of this solution into a flask, and add 10 ml of sodium hydroxide solution (2 in 5). Immediately fit the flask to a distillation apparatus connected to a receiver containing exactly 40 ml of 0.1 mol/L sulfuric acid. Heat to distill the ammonia into sulfuric acid. Titrate the excess sulfuric acid in the receiver with 0.2 mol/L sodium hydroxide (indicator: 3 drops of methyl red TS).

Each ml of 0.1mol/L sulfuric acid = 10.70 mg of NH_4Cl

Ammonium Dihydrogen Phosphate

Ammonium Phosphate, Monobasic
Primary Ammonium Phosphate

リン酸二水素アンモニウム

$NH_4H_2PO_4$ Mol. Wt. 115.03
Ammonium dihydrogenphosphate [7722-76-1]
Content Ammonium Dihydrogen Phosphate contains 96.0–102.0% of ammonium dihydrogen phosphate ($NH_4H_2PO_4$).
Description Ammonium Dihydrogen Phosphate occurs as colorless to white crystals or as a white crystalline powder.
Identification Ammonium Dihydrogen Phosphate responds to all tests for Ammonium Salt and for Phosphate in the Qualitative Tests.
Purity

(1) Clarity and color of solution Colorless and almost clear (1.0 g, water 20 ml).

(2) pH 4.1–5.0 (1.0 g, water 100 ml).

(3) Chloride Not more than 0.035% as Cl (0.50 g, Control solution 0.01 mol/L hydrochloric acid 0.50 ml).

(4) Sulfate Not more than 0.038% as SO_4 (0.50 g, Control solution 0.005 mol/L sulfuric acid 0.40 ml).

(5) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of Ammonium Dihydrogen Phosphate, dissolve by adding 2 ml of diluted acetic acid (1 in 20) and 30 ml of water, and add water to make 50 ml.

Control Solution To 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Assay Weigh accurately about 3 g of Ammonium Dihydrogen Phosphate, dissolve it in 30 ml of water, add 5 g of sodium chloride, and shake well. Keep the solution at about 15°C, and titrate with 1 mol/L sodium hydroxide (indicator: 2 drops of phenolphthalein TS).

Each ml of 1 mol/L sodium hydroxide = 115.0 mg of $NH_4H_2PO_4$

Ammonium Persulfate

過硫酸アンモニウム

$(NH_4)_2S_2O_8$ Mol. Wt. 228.20
Diammonium peroxodisulfate [7727-54-0]
Content Ammonium Persulfate contains not less than 95.0% of ammonium persulfate ($(NH_4)_2S_2O_8$).
Description Ammonium Persulfate occurs as colorless crystals or as a white crystalline powder.
Identification

(1) To 0.5 g of Ammonium Persulfate, add 5 ml of sodium hydroxide solution (1 in 25), and heat. A gas with an odor of ammonia is evolved. This gas changes the color of a red litmus paper wetted with water to blue.

(2) To 5 ml of diluted sulfuric acid (1 in 20), add 2–3 drops of manganese sulfate solution (1 in 100), then add 1 drop of silver nitrate solution (1 in 50) and 0.2 g of Ammonium Persulfate, and warm. A pink color develops.

Purity

(1) Clarity and color of solution Colorless and almost clear (1.0 g, water 10 ml).

(2) Heavy metals Not more than 30 μg/g as Pb.

Test Solution Weigh 1.0 g of Ammonium Persulfate, heat gradually first, and slightly ignite until white fumes are no longer evolved. Add 1 ml of hydrochloric acid and 5 drops of nitric acid to the residue, and evaporate to dryness on a water bath. Add 5 ml of diluted hydrochloric acid (1 in 4) to the residue, and evaporate to dryness again on a water bath. Dissolve the residue in 2 ml of diluted acetic acid (1 in 20) and about 20 ml of water, and add water to make 50 ml.

Control Solution To 3.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(3) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Dissolve 1.0 g of Ammonium Persulfate in 10 ml of water, add 1 ml of sulfuric acid and 10 ml of sulfurous acid, evaporate to about 2 ml, and add water to make 10 ml. Use 5 ml of this solution as the test solution.

Apparatus Apparatus B.

Residue on Ignition Not more than 0.20%.
Assay Weigh accurately about 1.5 g of Ammonium Persulfate, dissolve it in water to make exactly 250 ml. Measure exactly 50 ml of this solution, and add exactly 40 ml of 0.05 mol/L ferrous ammonium sulfate. Add 5 ml of phosphoric acid, and titrate the excess ferrous ammonium sulfate with 0.02 mol/L potassium permanganate. Perform a blank test in the same manner.

Each ml of 0.05 mol/L ferrous ammonium sulfate = 11.41 mg of $(NH_4)_2S_2O_8$

Ammonium Sulfate

硫酸アンモニウム

$(NH_4)_2SO_4$ Mol. Wt. 132.14
Ammonium sulfate [7783-20-2]
Content Ammonium Sulfate contains not less than 99.0% of ammonium sulfate [$(NH_4)_2SO_4$].
Description Ammonium Sulfate occurs as colorless crystals

or white lumps.

Identification Ammonium Sulfate responds to all tests for Ammonium Salt and for Sulfate in the Qualitative Tests.

Purity

(1) Clarity and color of solution Colorless and almost clear (1.0 g, water 20 ml).

(2) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Residue on Ignition Not more than 0.25%.

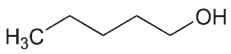
Assay Weigh accurately about 3 g of Ammonium Sulfate, and dissolve it in water to make exactly 250 ml. Measure exactly 25 ml of this solution, add 10 ml of sodium hydroxide solution (2 in 5), and immediately connect to a distilling apparatus equippted with a receiver, connected to a spray trap and a condenser, containing exactly 40 ml of 0.1 mol/L sulfuric acid. Heat to distill ammonia into the sulfuric acid, and titrate the excess sulfuric acid with 0.2 mol/L sodium hydroxide (indicator: 3 drops of methyl red TS).

Each ml of 0.1 mol/L sulfuric acid = 13.21 mg of $(NH_4)_2SO_4$

Amyl Alcohol

1-Amyl Alcohol
Pentan-1-ol

アミルアルコール



$C_5H_{12}O$ Mol. Wt. 88.15

Pentan-1-ol [71-41-0]

Content Amyl Alcohol contains not less than 98.0% of amyl alcohol ($C_5H_{12}O$).

Description Amyl Alcohol is a colorless to light yellow, transparent liquid having a characteristic odor.

Identification Determine the absorption spectrum of Amyl Alcohol as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Refractive index n_D^{20}: 1.407–1.412.

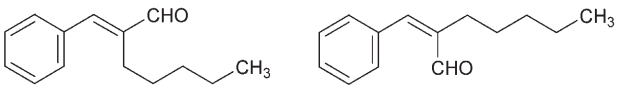
(2) Specific gravity d_{25}^{25}: 0.810–0.816.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavor Substance Tests. Use operating conditions (2).

α-Amylcinnamaldehyde

α-Amylcinnamic Aldehyde
Alpha-Pentylcinnamaldehyde

α-アミルシンナムアルデヒド



$C_{14}H_{18}O$ Mol. Wt. 202.29

2-(Phenylmethylene)heptanal [122-40-7]

Content α-Amylcinnamaldehyde contains not less than 98.0% of α-amylcinnamaldehyde ($C_{14}H_{18}O$).

Description α-Amylcinnamaldehyde is a light yellow to yellow, transparent liquid having a characteristic odor.

Identification Determine the absorption spectrum of α-Amylcinnamaldehyde as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Refractive index n_D^{20}: 1.554–1.560.

(2) Specific gravity 0.967–0.972.

(3) Clarity of solution Clear (1.0 ml, 80% (vol) ethanol 5.0 ml).

(4) Acid value Not more than 1.0 (Flavoring Substances Tests).

Residue on Ignition Not more than 0.05%.

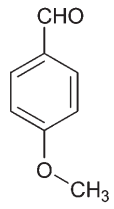
Assay Weigh accurately about 1.5 g of α-Amylcinnamaldehyde, and proceed as directed in Method 2 in the Aldehyde and Ketone Content Test under the Flavoring Substances Tests. Heat the mixture for 30 minutes before titrating.

Each ml of 0.5 mol/L hydrochloric acid = 101.1 mg of $C_{14}H_{18}O$

Anisaldehyde

***p*-Methoxybenzaldehyde**
4-Methoxybenzaldehyde

アニスアルデヒド



$C_8H_8O_2$ Mol. Wt. 136.15

4-Methoxybenzaldehyde [123-11-5]

Content Anisaldehyde contains not less than 97.0% of anisaldehyde ($C_8H_8O_2$).

Description Anisaldehyde is a colorless to light yellow,

transparent liquid having a characteristic odor.

Identification To 5 drops of Anisaldehyde, add 1 ml of sodium hydrogen sulfite TS, and shake. The mixture forms crystalline lumps. When shaken with 7 ml of water, the crystalline lumps dissolve almost clearly.

Purity

(1) Refractive index n_D^{20}: 1.570–1.574.

(2) Specific gravity 1.122–1.127.

(3) Clarity of solution Clear (1.0 ml, 60% (vol) ethanol 5 ml).

(4) Acid value Not more than 6.0 (Flavoring Substances Tests).

Assay Weigh accurately about 0.8 g of Anisaldehyde, and proceed as directed in Method 2 in the Aldehyde and Ketone Content Test under the Flavoring Substances Tests. In the test, allow the mixture to stand for 15 minutes before titrating.

Each ml of 0.5 mol/L hydrochloric acid = 68.07 mg of $C_8H_8O_2$

Annatto, Water-soluble

水溶性アナトー

Definition Water-soluble Annatto is prepared from the red pericarp of seeds of the annatto tree *Bixa orellana* Linné by hydrolysis. The coloring principle is the potassium or sodium salt of norbixin.

Content Water-soluble Annatto contains the equivalent of 100–125% of the labeled content of norbixin ($C_{24}H_{28}O_4$=380.48).

Description Water-soluble Annatto occurs as a red-brown to brown powder, lumps, liquid, or pasty substances having a slight characteristic odor.

Identification

(1) Dissolve 0.5 g of Water-soluble Annatto in 20 ml of water, add 2 ml of diluted sulfuric acid (1 in 20), shake, and filter. Wash the residue on the filter paper three times with 20 ml of water each time.

(i) Dissolve a little portion of the residue in sodium hydroxide solution (1 in 2,500). The solution exhibits absorption maxima at wavelengths of approximately 452–456 nm and 480–484 nm.

(ii) Dissolve a little portion of the residue in 10 ml of ethanol. Apply one drop of the solution on a filter paper, and air-dry. Drip 2–3 drops of 5% sodium nitrite and then 2–3 drops of 0.5 mol/L sulfuric acid on it. The yellow color on the filter paper disappears.

(2) To 1 g of Water-soluble Annatto, add 50 ml of water, shake, and filter. Add 2 ml of diluted hydrochloric acid (1 in 4) to the filtrate. A red-brown to yellow-brown precipitate is formed.

Purity

(1) Free alkali Dissolve 10 g of Water-soluble Annatto in 100 ml of water, shake, add 8 ml of 1 mol/L hydrochloric acid, stir thoroughly, and allow to stand for 30 minutes. The pH of the filtrate is not more than 7.0.

(2) Heavy metals Not more than 10 μg/g as Pb.

Test Solution Weigh 2.0 g of Water-soluble Annatto, and evaporate to dryness if necessary on a water bath.

Control Solution Use 2.0 ml of Lead Standard Solution.

Procedure Proceed as directed in Method 2.

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (Coloring Matter Tests).

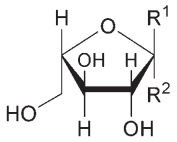
(4) Absorbance ratio Proceed as directed under Identification (1)(i). When the absorbances at the maximum absorption wavelengths around 480–484 nm and 452–456 nm are A_1 and A_2, respectively, A_2/A_1 is 1.11–1.25.

Assay Weigh accurately 0.1 to 1 g of Water-soluble Annatto, add 0.01 mol/L sodium hydroxide solution to make exactly 100 ml, and mix thoroughly. Measure exactly 1 ml of the solution, and add 0.01 mol/L sodium hydroxide solution to make exactly 100 ml. Measure the absorbance (A) of this solution at the maximum absorption wavelength of approximately 454 nm, and calculate the content of norbixin by the formula:

$$\text{Content (\%) of norbixin } (C_{24}H_{28}O_4) = \frac{A}{3{,}473} \times \frac{100}{\text{Weight (g) of the sample}} \times 100$$

L-Arabinose

L-アラビノース



β-L-Arabinose: R^1=H, R^2=OH
α-L-Arabinose: R^1=OH, R^2=H

$C_5H_{10}O_5$ Mol. Wt. 150.13

L-Arabinofuranose [87-72-9]

Definition L-Arabinose is obtained by hydrolysis and isolation from the polysaccharides (mainly arabinan) of gum Arabic, gum ghatti, corn fibers, or sugar beet pulps. It consists of L-arabinose ($C_5H_{10}O_5$).

Content L-Arabinose, when dried, contains 95.0–101.0% of L-arabinose ($C_5H_{10}O_5$).

Description L-Arabinose occurs as colorless or white crystals or as a white to light yellowish white crystalline powder. It is odorless and has a sweet taste.

Identification

(1) Add 2–3 drops of a solution of L-Arabinose (1 in 20) to 5 ml of boiled Fehling's TS. A red precipitate is formed.

(2) Dissolve 1 g of L-Arabinose in 3 ml of water, add 3 ml of a 5:2 mixture of diluted hydrochloric acid (1 in 4)/diphenylamine solution in ethanol (1 in 40), and heat on a water bath for 5 minutes. A yellow to light orange color is produced.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: Not less than + 95°.

Weigh accurately about 2 g of L-Arabinose, add water to make exactly 50 ml, and allow to stand at room temperature for 24 hours. Measure the angular rotation, and calculate on the dried basis.

(2) Clarity and color of solution Colorless and almost clear (4.0 g, water 20 ml).

(3) Free acids Dissolve 1.0 g of L-Arabinose in 10 ml of freshly boiled and cooled water. Add 1 drop of phenolphthalein TS, and then add 1 drop of 0.2 mol/L sodium hydroxide solution. A red color develops.

(4) Sulfate Not more than 0.005% as SO_4 (1.0 g, Control solution 0.005 mol/L sulfuric acid 0.10 ml).

(5) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(6) Lead Not more than 10 μg/g as Pb (1.0 g, Method 1).

(7) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50g, Method 3, Apparatus B).

Loss of Drying Not more than 1.0% (105°C, 3 hours).

Residue on Ignition Not more than 0.20% (5 g, 600°C, 8 hours).

Assay

Test Solution and Standard Solution Weigh accurately about 2 g each of the test sample and L-arabinose for assay, previously dried. To each, add exactly 10 ml of a 4:1 mixture of water/propylene glycol, and then add water to make exactly 50 ml.

Procedure Analyze 10 μl each of the test solution and standard solution by liquid chromatography according to the operating conditions given below. Measure the peak areas of L-arabinose and propylene glycol for the test solution and the standards solution, and determine the peak area ratios (Q_T and Q_S) of L-arabinose to propylene glycol for each solution. Calculate the content by the formula:

$$\text{Content (\%) of L-arabinose } (C_5H_{10}O_5) = \frac{\text{Weight (g) of L-arabinose for assay}}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 100$$

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube of 4–8 mm internal diameter and 20–35 cm length.

Column packing material: 7- to 11-μm strongly acidic cation-exchange resin for liquid chromatography.

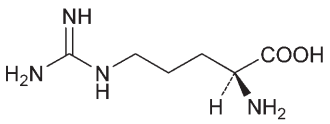
Column temperature: A constant temperature of 60–70°C.

Mobile phase: Water.

Flow rate: Adjust so that the retention time of L-arabinose is 10–15 minuets.

L-Arginine

L-アルギニン



$C_6H_{14}N_4O_2$ Mol. Wt. 174.20

(2*S*)-2-Amino-5-guanidinopentanoic acid [74-79-3]

Content L-Arginine, when calculated on the dried basis, contains 98.0–120.0% of L-arginine($C_6H_{14}N_4O_2$).

Description L-Arginine occurs as white crystals or crystalline powder. It has a characteristic odor and has a characteristic taste.

Identification

(1) To 5 ml of a solution of L-Arginine (1 in 1,000), add 1 ml of ninhydrin solution (1 in 50), and heat for 3 minutes in a water bath. A bluish purple color develops.

(2) A solution of L-Arginine is alkaline.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +25.0 to +27.9°.

Weigh accurately about 8 g of L-Arginine, and dissolve it in 6 mol/L hydrochloric acid to make exactly 100 ml. Measure the angular rotation of this solution, and calculate on the dried basis.

(2) Clarity and color of solution Colorless and clear (1.0 g, water 20 ml).

(3) pH 10.5–12.5 (1.0 g, water 20 ml).

(4) Chloride Not more than 0.10% as Cl (0.07 g, Control solution 0.01 mol/L hydrochloric acid 0.02 ml).

(5) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of L-Arginine, dissolve it in about 30 ml of water, add 1 drop of Phenolphthalein TS, and neutralize with diluted hydrochloride (1 in 4). Add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Use 2.0 ml of Lead Standard Solution.

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.5 g, Method 1, Apparatus B).

Loss on Drying Not more than 1.0% (105°C, 3 hours).

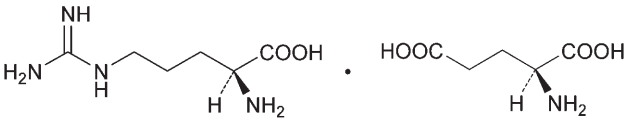
Residue on Ignition Not more than 0.20%.

Assay Weigh accurately about 0.2 g of L-Arginine, and proceed as directed in the Assay for L-Asparagine.

Each ml of 0.1 mol/L perchloric acid = 8.710 mg of $C_6H_{14}N_4O_2$

L-Arginine L-Glutamate

L-アルギニン L-グルタミン酸塩



$C_{11}H_{23}N_5O_6$ Mol. Wt. 321.33

(2*S*)-2-Amino-5-guanidinopentanoic acid
mono[(2*S*)-2-Aminopentanedioate] [4320-30-3]

Content L-Arginine L-Glutamate, when calculated on the anhydrous basis, contains 98.0–102.0% of L-arginine L-glutamate ($C_{11}H_{23}N_5O_6$).

Description L-Arginine L-Glutamate occurs as a white powder. It is odorless or has a slight odor and has a characteristic taste.

Identification

(1) To 5 ml of a solution of L-Arginine L-Glutamate (1 in 1,000), add 1 ml of ninhydrin solution (1 in 1,000), and heat for 3 minutes. A purple color develops.

(2) Use a solution of L-Arginine L-Glutamate (1 in 500)

as the test solution. Add water to 0.1 g of L-arginine hydrochloride and 0.1 g of sodium L-glutamate to make 100 ml, and use this solution as the control solution. Analyze 5 µl portions of the test solution and the control solution by paper chromatography using a 5:2:1 mixture of 1-butanol/water/acetic acid as the developing solvent. Use a No. 2 filter paper for chromatography, and stop the development when the developing solvent ascends to a point about 30 cm above the original line. Air-dry the filter paper, dry at 100°C for 20 minutes, and spray with a solution of ninhydrin in acetone (1 in 50). Heat it at 100°C for 5 minutes to develop a color, and observe in daylight. Two spots corresponding to the spots from the control solution are observed.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +28.0 to +30.0° (4 g, hydrochloric acid (1 in 2), 50 ml, on the anhydrous basis).

(2) Clarity and color of solution Colorless and almost clear (1.0 g, water 20 ml).

(3) pH 6.0–7.5 (1.0 g, water 20 ml).

(4) Chloride Not more than 0.041% as Cl (0.30 g, Control solution 0.01 mol/L hydrochloric acid 0.35 ml).

(5) Heavy metals Not more than 20 µg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(6) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Water Content Not more than 15.4% (0.3 g, Back Titration).

Residue on Ignition Not more than 0.30%.

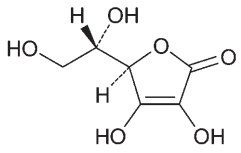
Assay Proceed as directed in the Assay for DL-Alanine, and calculate on the anhydrous base.

Each ml of 0.1 mol/L perchloric acid = 10.71 mg of $C_{11}H_{23}N_5O_6$

L-Ascorbic Acid

Vitamin C

L-アスコルビン酸



$C_6H_8O_6$ Mol. Wt. 176.12

(5*R*)-5-[(1*S*)-1,2-Dihydroxyethyl]-3,4-dihydroxyfuran-2(5*H*)-one [50-81-7]

Content L-Ascorbic Acid, when dried, contains not less than 99.0% of L-ascorbic acid ($C_6H_8O_6$).

Description L-Ascorbic Acid occurs as white to yellowish white crystals or crystalline powder. It is odorless and has an acid taste.

Identification

(1) Dissolve 0.1 g of L-Ascorbic Acid in 100 ml of metaphosphoric acid solution (1 in 50), and add iodine TS to 5 ml of the solution dropwise until a slightly yellowish color develops. Add 1 drop each of cupric sulfate solution (1 in 1,000) and pyrrole, and warm in a water bath at 50–60°C for 5 minutes. A blue to blue-green color develops.

(2) To 10 ml of a solution of L-Ascorbic Acid (1 in 100), add 1–2 drops of 2,6-dichlorophenolindophenol sodium salt TS. A blue color develops and disappears immediately.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +20.5 to +21.5° (1 g, freshly boiled and cooled water, 10 ml, on the dried basis).

(2) Melting point 187–192°C.

(3) Heavy metals Not more than 20 µg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Drying Not more than 0.40% (reduced pressure, 3 hours).

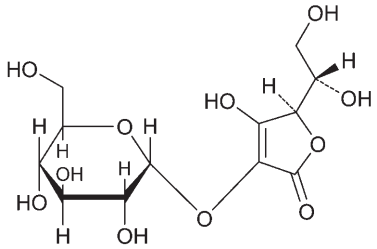
Residue on Ignition Not more than 0.10% .

Assay Weigh accurately about 0.2 g of L-Ascorbic Acid, previously dried, and dissolve it in 50 ml of metaphosphoric acid solution (1 in 50). Titrate with 0.05 mol/L iodine (indicator: starch TS).

Each ml of 0.05 mol/L iodine = 8.806 mg of $C_6H_8O_6$

L-Ascorbic Acid 2-Glucoside

L-アスコルビン酸 2-グルコシド



$C_{12}H_{18}O_{11}$ Mol. Wt. 338.26

(5*R*)-5-[(1*S*)-1,2-Dihydroxyethyl]-4-hydroxy-2-oxo-2,5-dihydrofuran-3-yl α-D-glucopyranoside [129499-78-1]

Content L-Ascorbic Acid 2-Glucoside contains not less than 98.0% of L-ascorbic acid 2-glucoside ($C_{12}H_{18}O_{11}$) when calculated on the dried basis.

Description L-Ascorbic Acid 2-Glucoside occurs as a while to yellowish white powder or crystallized powder. It is odorless and has an acid taste.

Identification Determine the absorption spectrum of L-Ascorbic Acid 2-Glucoside as directed in the Potassium Bromide Disk Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +186.0 to +188.0° (5 g, water, 100 ml, on the dried basis).

(2) Melting point 158–163ºC.

(3) Heavy metals Not more than 10 µg/g as Pb (2.0g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 1.0 μg/g as As_2O_3 (2.0g, Method 3, Apparatus B).

Loss on Drying Not more than 1.0% (105℃, 2 hours).

Residue on Ignition Not more than 0.10%.

Assay Weigh accurately 0.5 g each of the sample and L-ascorbic acid 2-glucoside for assay, and separately dissolve them in water. To each solution, add exactly 10 ml of 5% (w/v) glycerol solution, as the internal standard solution, and water to make exactly 50 ml. Use these solutions as the test solution and the standard solution, respectively. Analyze 20 μl portions of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Calculate the peak area ratio of L-ascorbic acid 2-glucoside to glycerol for each solution, and express as Q_T for the test solution and Q_S for the standard solution. Calculate the content by the formula:

$$\text{Content (\%) of L-ascorbic acid 2-glucoside } (C_{12}H_{18}O_{11}) = \frac{\left(\begin{array}{c}\text{Dried basis weight (g) of}\\ \text{L-ascorbic acid 2-glucoside for assay}\end{array}\right)}{\text{Dried basis weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 100$$

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube of 4–8 mm internal diameter and 20–50 cm length

Column packing material: Strongly acidic cation-exchange resin for liquid chromatography.

Column temperature: 35°C.

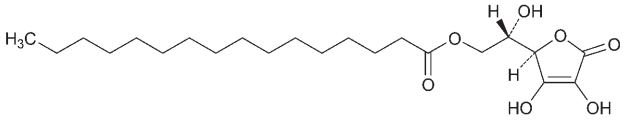
Mobile phase: Nitric acid (1 in 10,000).

Flow rate: Adjust so that the retention time of L-ascorbic acid 2-glucoside is about 10 minutes.

L-Ascorbyl Palmitate

Vitamin C Palmitate

L-アスコルビン酸パルミチン酸エステル



$C_{22}H_{38}O_7$ Mol. Wt. 414.53

(2*S*)-2[(5*R*)-3,4-Dihydroxy-5-oxo-2,5-dihydrofuran-2-yl]-2-hydroxyethyl hexadecanoate [137-66-6]

Content L-Ascorbyl Palmitate contains not less than 95.0% of L-ascorbyl palmitate ($C_{22}H_{38}O_7$).

Description L-Ascorbyl Palmitate occurs as a white or yellowish white powder.

Identification

(1) To 0.1 g of L-Ascorbyl Palmitate, add 100 ml of sodium lauryl sulfate–propylene glycol TS, and dissolve by warming. Cool, and add iodine TS dropwise to 5 ml of this solution until a slight yellow color develops. Add 1 drop each of cupric sulfate solution (1 in 1,000) and pyrrole, and warm at 50–60°C for 5 minutes. A blue to blue-green color develops.

(2) To 10 ml of a solution of L-Ascorbyl Palmitate in ethanol (1 in 100), add 1 or 2 drops of 2,6-dichlorophenolindophenol sodium salt TS. The color of the solution changes to blue and disappears immediately.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +21 to +24° (10 g, methanol, 100 ml).

(2) Melting point 107–117°C.

(3) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Residue on Ignition Not more than 0.10% (2 g).

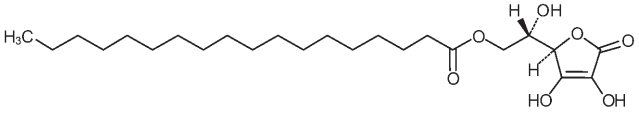
Assay Weigh accurately about 0.2 g of L-Ascorbyl Palmitate, add 30 ml of ethanol, dissolve by warming if necessary. Add 15 ml of metaphosphoric acid solution (1 in 5) and 10 ml of diluted sulfuric acid (1 in 2). Add exactly 10 ml of potassium iodate TS, shake well, and allow to stand for 10 minutes in a dark place. Add 10 ml of potassium iodide TS and 100 ml of water, and allow to stand for 5 minutes in a dark place. Titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: 10 ml of starch TS). Perform a blank test in the same manner.

Each ml of 0.1 mol/L sodium thiosulfate = 20.73 mg of $C_{22}H_{38}O_7$

L-Ascorbyl Stearate

Vitamin C Stearate

L-アスコルビン酸ステアリン酸エステル



$C_{24}H_{42}O_7$ Mol. Wt. 442.59

(2*S*)-2[(5*R*)-3,4-Dihydroxy-5-oxo-2,5-dihydrofuran-2-yl]-2-hydroxyethyl octadecanoate [25395-66-8]

Content L-Ascorbyl Stearate contains not less than 95.0% of L-ascorbyl stearate ($C_{24}H_{42}O_7$).

Description L-Ascorbyl Stearate occurs as a white to yellowish white powder.

Identification

(1) To 0.1 g of L-Ascorbyl Stearate, add 100 ml of sodium lauryl sulfate–propylene glycol TS, and dissolve by warming. Cool, and add iodine TS dropwise to 5 ml of this solution until a slightly yellow color develops. Add 1 drop each of cupric sulfate solution (1 in 1,000) and pyrrole, and warm at 50–60°C for 5 minutes. A blue to blue-green color develops.

(2) To 10 ml of a solution of L-Ascorbyl Stearate in ethanol (1 in 100), add 1–2 drops of 2,6-dichlorophenolindophenol sodium salt TS. A blue color develops, and then disap-

pears immediately.

Purity

(1) Melting point 114–119°C.

(2) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0ml).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

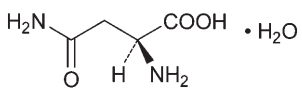
Residue on Ignition Not more than 0.10%.

Assay Weigh accurately about 0.2 g of L-Ascorbyl Stearate, add 30 ml of ethanol, and dissolve by warming if necessary. Add 15 ml of metaphosphoric acid solution (1 in 5) and 10 ml of diluted sulfuric acid (1 in 2). Add exactly 10 ml of potassium iodate TS, shake well, and allow to stand for 10 minutes in a dark place. Add 10 ml of potassium iodide TS and 100 ml of water, and allow to stand for 5 minutes in a dark place. Titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: 10 ml of starch TS). Perform a blank test in the same manner.

Each ml of 0.1 mol/L sodium thiosulfate = 22.13 mg of $C_{24}H_{42}O_7$

L-Asparagine

L-アスパラギン



$C_4H_8N_2O_3 \cdot H_2O$ Mol. Wt. 150.13

(2*S*)-2-Amino-3-carbamoylpropanoic acid monohydrate [anhydrous 70-47-3]

Content L-Asparagine, when calculated on the dried basis, contains 98.0–102.0% of L-asparagine ($C_4H_8N_2O_3$=132.12).

Description L-Asparagine occurs as white crystals or crystalline powder. It is odorless, and has a sweetish taste.

Identification

(1) To 5 ml of a solution of L-Asparagine (1 in 1,000), add 1 ml of ninhydrin solution (1 in 50), and heat for 3 minutes in a water bath. A purple color develops.

(2) To 0.1g of L-Asparagine, add 5 ml of sodium hydroxide solution (1 in 10), and heat in a water bath. The generated gas (NH_3) changes red litmus paper moistened with water to blue.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +33.0 to +36.5°.

Weigh accurately about 10 g of L-Asparagine, and dissolve it in 6 mol/L hydrochloric acid to make exactly 100 ml. Measure the angular rotation of this solution and calculate on the dried basis.

(2) Clarity and color of solution Colorless and clear (1.0 g, water 50 ml).

(3) pH 3.5–5.5 (1.0 g, water 100 ml).

(4) Chloride Not more than 0.10% as Cl (0.07 g, Control solution 0.01 mol/L hydrochloric acid 0.20 ml).

(5) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Loss on Drying 11.5–12.5% (130°C, 3 hours).

Residue on Ignition Not more than 0.10%

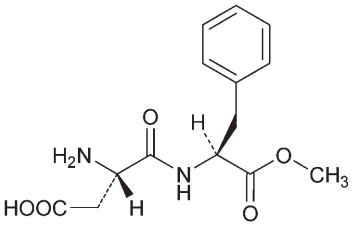
Assay Weigh accurately about 0.3 g of L-Asparagine, dissolve it in 3 ml of formic acid, add 50 ml of acetic acid, and titrate with 0.1 mol/L perchloric acid. The endpoint is usually confirmed by a potentiometer. When an indicator (1 ml of crystal violet–acetic acid TS) is used, titrate until the color of the solution changes from purple through blue to green. Perform a blank test in the same manner, make any necessary correction, and calculate on the dried basis.

Each ml of 0.1 mol/L perchloric acid = 13.21 mg of $C_4H_8N_2O_3$

Aspartame

L-α-Aspartyl-L-phenylalanine Methyl Ester

アスパルテーム



$C_{14}H_{18}N_2O_5$ Mol. Wt. 294.30

Methyl L-α-aspartyl-L-phenylalaninate [22839-47-0]

Content Aspartame, when calculated on the dried basis, contains 98.0–102.0% of aspartame ($C_{14}H_{18}N_2O_5$).

Description Aspartame occurs as a white crystalline powder or granules. It is odorless and has a strong sweet taste.

Identification

(1) Determine the absorption spectrum of Aspartame as directed in the Paste Method under the Infrared Spectrophotometry. It exhibits absorption bands at about 3300 cm^{-1}, 1737 cm^{-1}, 1666 cm^{-1}, 1379 cm^{-1}, 1227 cm^{-1}, and 699 cm^{-1}.

(2) To 5 ml of a solution of Aspartame (1 in 1,000), add 1 ml of ninhydrin solution (1 in 50), and heat on a water bath for 3 minutes. A blue-purple color develops.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +14.5 to +16.5° (2 g, 15 mol/L formic acid, 50 ml, on the dried basis). Measure within 30 minutes.

(2) Clarity and color of solution Colorless and clear (0.20 g, hydrochloric acid (1 in 60) 20 ml).

(3) pH 4.5–6.0.

Test Solution Weigh 1.0 g of Aspartame, and dissolve it in water to make 125 ml.

(4) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

(6) 5-Benzyl-3,6-dioxo-2-piperazineacetic acid Not more than 1.5% as 5-benzyl-3,6-dioxo-2-piperazineacetic acid.

Test Solution Weigh 0.010 g of Aspartame, place into a

test tube with a stopper, add 1.0 ml of silylation TS, stopper, and shake. Heat at 80°C for 30 minutes, shake for 15 seconds, and allow to cool.

Control Solution Measure 3.0 ml of a solution of 5-benzyl-3,6-dioxo-2-piperazineacetic acid in methanol (1 in 20,000) into a test tube with a stopper, evaporate to dryness on a water bath, add 1.0 ml of silylation TS to the residue, and then proceed in the same manner as for the test solution.

Analyze 3.0 µl portions of the test solution and the control solution by gas chromatography using the operating conditions given below. The peak height of the 5-benzyl-3,6-dioxo-2-piperazineacetic acid for the test solution does not exceed the peak height of the 5-benzyl-3,6-dioxo-2-piperazineacetic acid for the control solution.

Operating Conditions

Detector: Hydrogen flame ionization detector.

Column: A glass or stainless steel tube of 3–4 mm internal diameter and 2 m length.

Column packing material

Liquid phase: 3% Methyl silicon polymer of the amount of support.

Support: 149- to 177-µm diatomaceous earth for gas chromatography.

Column temperature: A constant temperature of 195–205°C.

Carrier gas: Use helium or nitrogen.

Flow rate: Adjust the flow rate so that 5-benzyl-3,6-dioxo-2-piperazineacetic acid appears about 7–9 minutes after injection.

(7) Other optical isomers Not more than 0.04% as L-α-aspartyl-D-phenylalanine methyl ester.

Test Solution Weigh 0.50 g of Aspartame, and dissolve it in citrate buffer (pH 2.2) to make 100 ml.

Control Solution Measure 10 ml of a solution of L-α-aspartyl-D-phenylalanine methyl ester (1 in 50,000), and add citrate buffer (pH 2.2) to make 100 ml.

Procedure Analyze equal volumes of the test solution and the control solution by liquid chromatography using the operating conditions given below. The peak height of the L-α-aspartyl-D-phenylalanine methyl ester of the test solution does not exceed the peak height of the L-α-aspartyl-D-phenylalanine methyl ester of the control solution.

Operating Conditions

Detector: Visible spectrophotometer (measurement wavelength: 570 nm).

Column: A glass tube of 9 mm internal diameter and 55 cm length.

Column packing material: 17-µm strongly acidic cation-exchange resin for gas chromatography.

Column temperature: 55°C.

Mobile phase: Citrate buffer (pH 5.28).

Flow rate: 1 ml/min.

Reaction coil: Teflon tube 0.5 mm in internal diameter and 29 m in length.

Reaction chamber temperature: 100°C.

Flow rate of ninhydrin–ethylene glycol monomethyl ether TS: 0.5 ml/min.

Injection amount of the test solution and control solution: A constant amount of 50–500 µl.

Loss on Drying Not more than 4.5% (105°C, 4 hours).

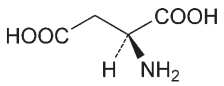
Residue on Ignition Not more than 0.20%.

Assay Weigh accurately 0.3 g of Aspartame, dissolve it in 3 ml of formic acid, add 50 ml of acetic acid, and immediately titrate with 0.1 mol/L perchloric acid. For confirmation of endpoint, use potentiometer. When 0.5 ml of α-naphtholbenzein TS is used as the indicator, the endpoint is when the brown color of the solution changes to green. Perform a blank test in the same manner to make any necessary correction. Calculate on the dried basis.

Each ml of 0.1 mol/L perchloric acid = 29.43 mg of $C_{14}H_{18}N_2O_5$

L-Aspartic Acid

L-アスパラギン酸



$C_4H_7NO_4$ Mol. Wt. 133.10

(2*S*)-2-Aminobutanedioic acid [56-84-8]

Content L-Aspartic Acid, when calculated on the dried basis, contains 98.0–102.0% of L-aspartic acid ($C_4H_7NO_4$).

Description L-Aspartic Acid occurs as white crystals or crystalline powder. It is odorless, and has a sour taste.

Identification

(1) To 5 ml of a solution of L-Aspartic Acid (1 in 1,000), add 1 ml of ninhydrin solution (1 in 50), and heat for 3 minutes in a water bath. A bluish purple color develops.

(2) To 5 ml of a solution of L-Aspartic Acid in 1 mol/L hydrochloric acid (1 in 25), add 1 ml of sodium nitrite solution (1 in 10). The solution effervesces, evolving a colorless gas.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +24.0 to +26.0°.

Weigh accurately about 8 g of L-Aspartic Acid, and dissolve it in 6 mol/L hydrochloric acid to make exactly 100 ml. Measure the angular rotation of this solution and calculate on the dried basis.

(2) Clarity and color of solution Colorless and clear (1.0 g, 1 mol/L hydrochloric acid 20 ml).

(3) pH 2.5–3.5 (saturated solution).

(4) Chloride Not more than 0.1% as Cl (0.07 g, Control solution 0.01 mol/L hydrochloric acid 0.20ml).

(5) Heavy metals Not more than 20 µg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(6) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Loss on Drying Not more than 0.30% (105°C, 3 hours).

Residue on Ignition Not more than 0.10%.

Assay Weigh accurately about 0.3 g of L-Aspartic Acid, dissolve it in 6 ml of formic acid, and proceed as directed in the Assay for L-Asparagine.

Each ml of 0.1 mol/L perchloric acid = 13.31 mg of $C_4H_7NO_4$

Bacillus natto Gum

納豆菌ガム

Definition Bacillus natto Gum is obtained from the culture fluid of *Bacillus subtilis* and consists mainly of polyglutamic acid.

Content Bacillus natto Gum, when dried, contains not less than 70.0% of polyglutamic acid.

Description Bacillus natto Gum occurs as a white to light brown, hygroscopic, powder, lumps, or granules. It has little or no odor.

Identification

(1) Place 5 ml of a solution (1 in 200) of Bacillus natto Gum in a test tube with a stopper, add 5 ml of hydrochloric acid, and stopper tightly. Hydrolyze the mixture at 110°C for 24 hours. Cool, and add sodium hydroxide solution (6 in 25) to make it weakly acidic. To 5 ml of this solution, add 1 ml of ninhydrin TS, and heat in a water bath for 5 minutes. A purple color develops.

(2) Add 1 g of Bacillus natto Gum to 50 ml of water, and stir for 30 minutes. The solution is clear.

(3) Add 1 g of Bacillus natto Gum to 10 ml of hydrochloric acid, and stir for 30 minutes. A turbidity or precipitate is produced.

Purity

(1) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 4, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 10 μg/g as Pb (1.0 g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50g, Method 1, Apparatus B).

Loss on Drying Not more than 15.0% (Reduced pressure, 40°C, 24 hours).

Residue on Ignition Not more than 43.0%.

Microbial Limits Proceed as directed in the Microbial Limit Tests. The total bacterial count is not more than 10,000/g, and *Escherichia coli* is negative.

Assay

Test Solution Weigh accurately about 0.1 g, previously dried, and dissolve it in water to make 10 ml. Place exactly 5 ml of this solution in a hydrolysis test tube, add exactly 5 ml of hydrochloric acid, seal tightly, and hydrolyze at 110°C for 24 hours. After cooling, measure exactly 1 ml of the resulting solution, and add water to make exactly 200 ml.

Standard Solution Weigh accurately about 0.1 g L-glutamic acid for assay, previously dried, add 1 ml of diluted hydrochloric acid (1 in 6) and 20 ml of water to dissolve, and add water to make exactly 100 ml. Measure exactly 5 ml of this solution, and add water to make exactly 200 ml.

Procedure Analyze 20 μl portions of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Measure the peak areas (A_T and A_S) for the test solution and the standard solution, and calculate the content by the formula:

$$\text{Content (\%) of polyglutamic acid} = \frac{\text{Weight (g) of L-glutamic acid for assay}}{\text{Weight (g) of the sample}} \times \frac{A_T}{A_S} \times 0.8775 \times 100$$

Operating Conditions

Detector: Visible-range spectrophotometer (Determination wavelength: 570 nm).

Column: A stainless steel tube of 4.6 mm internal diameter and 6 cm length.

Column packing material: Strongly acidic cation exchange resin for liquid chromatography.

Column temperature: A constant temperature at about 55°C.

Chemical reaction bath temperature: A constant temperature at about 135°C.

Mobile phase: Buffer for bacillus natto gum (pH 3.3).

Reaction reagent: Ninhydrin TS for bacillus natto gum assay.

Mobile phase flow rate: Adjust so that the retention time of glutamic acid is about 7 minutes.

Reaction reagent flow rate: 0.35 ml/minute.

Baking Powder

合成膨脹剤

Single Baking Powder

一剤式合成膨脹剤

Description Single Baking Powder occurs as a white to gray-white powder or brittle lumps of clustered powder.

Purity

(1) Nitric acid-insoluble substances Not more than 2.0%.

Weigh 5.0 g of Single Baking Powder, add 30 ml of water, and shake for 3 minutes. Filter with a filter paper to separate the insoluble substances, and wash the residue on the filter paper thoroughly with water filled with carbon dioxide. Bore a small hole at the bottom of the filter paper, wash the residue into a beaker with 40 ml of diluted nitric acid (1 in 10), and boil for 1 minute. Cool, filter through a filter paper for quantitative analysis (5B), and wash it with water until the washings are no loner acidic. Place the residue with the filter paper in a ceramic crucible, previously weighed. Then ignite the crucible at about 550°C to constant weight, and weigh the mass.

(2) pH 5.0–8.5.

Test Solution Weigh 1.0 g of Single Baking Powder, add 50 ml of water, heat in a water bath until effervescence ceases, and cool.

(3) Heavy metals Measure a small amount of Single Baking Powder, and heat. If the sample is carbonized, proceed as directed in (3)(i); if it is not carbonized, proceed as directed in (3)(ii).

(i) Not more than 40 μg/g as Pb (0.50 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(ii) Not more than 40 μg/g as Pb.

Test solution Weigh 2.0 g of Single Baking Powder, add 5 ml of nitric acid, and heat on a water bath for 15 minutes. Cool, add 5 ml of water, and filter. Wash the residue on the filter paper with 5 ml of water, combine the filtrate and the washings, add 2 drops of phenolphthalein TS, and then add

sodium hydroxide solution (1 in 10) until the color of the solution changes to a slightly pink color. Add 5 ml of diluted hydrochloric acid (1 in 4). Adjust the pH to 2.5–3.5 with ammonia TS, add 8 ml of diluted acetic acid (1 in 20) and water to make 100 ml, measure 25 ml of this solution, add water to make 50 ml.

Control Solution To 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(4) Arsenic Take a small amount of Single Baking Powder and heat it. If the sample is carbonized, proceed as directed in (4)(i); if it is not carbonized, proceed as directed in (4)(ii).

(i) Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(ii) Not more than 4.0 μg/g as As_2O_3

Test Solution Weigh 5.0 g of Single Baking Powder, transfer into a 100-ml flask, add 10 ml of water, heat until effervescence ceases, and neutralize with diluted hydrochloric acid (1 in 4) or sodium hydroxide solution (1 in 25). When ammonia solution or ammonia TS is used to neutralize the solution, adjust the pH to 2.5–3.5. Add 5 ml of hydrochloric acid, and heat in a water bath for 30 minutes. Cool, and add water to make 25 ml. Measure 5 ml of this solution, add 10 ml of sulfurous acid, and evaporate to about 2 ml, and add water to make 10 ml. Use 5 ml of the second solution as the test solution.

Apparatus Use Apparatus B.

(5) Gas evolution Measure the volume of the gas evolved. The volume is not less than 70 ml.

Duplex Baking Powder

二剤式合成膨脹剤

Prepare a sample by mixing the two substances in the proportion indicated, and proceed as directed under Single Baking Powder.

Ammonia Type Baking Powder

アンモニア系合成膨脹剤

Proceed as directed under Single Baking Powder. The pH specified in Purity (2) is 6.0–9.0. Use water as the replacement solution for the determination of the gas volume specified in Purity (5).

Bees Wax

ミツロウ

Definition Bees Wax is obtained from the honeycombs of bees *Apis* spp. and consists mainly of myricyl palmitate.

Description Bees Wax occurs as a white to yellowish white or yellow to light brown solids having characteristic odor of honey .

Identification To 1 g of Bees Wax, add 50 ml of 2-propanol, and dissolve by warming to 65°C in a water bath. Add 5 ml of lukewarm water while stirring. White flocculent substances are formed.

Purity

(1) Melting point 60–67°C.

(2) Acid value 5–24.

Proceed as directed in Purity (2) for Candelilla Wax.

(3) Peroxide value Not more than 5.

Weigh accurately about 5 g of Bees Wax, transfer into a 200 ml Erlenmeyer flask with a ground-glass stopper. Add 30 ml of a 3:2 mixture of acetic acid/chloroform, stopper, heat in warm water, and dissolve with gentle shaking. Cool, replace the air in the flask with nitrogen. While passing nitrogen through, add exactly 1 ml of Potassium Iodide TS into the flask. Then stop the passage of the nitrogen, stopper immediately, shake for 1 minute, and allow to stand for 5 minutes in a dark place. Add 30 ml of water to this solution, stopper again, and shake vigorously. Titrate with 0.01 mol/L Sodium Thiosulfate. Calculate the content by the following formula. Perform the blank test in the same manner, and make any necessary correction.

$$\text{Peroxide value} = \frac{\left(\begin{array}{c}\text{Consumption (ml) of}\\ \text{0.01mol/L sodium thiosulfate}\end{array}\right)}{\text{Weight (g) of the sample}} \times 10$$

(4) Saponification value 77–103 (Fats and Related Substances Tests).

(5) Heavy metals Not more than 40 μg/g as Pb (0.50 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(6) Lead Not more than 10 μg/g (1.0 g, Method 1).

(7) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(8) Fats, Japan wax, rosin and soap To 1 g of Bees Wax, add 35 ml of sodium hydroxide solution (1 in 7). Heat for 30 minutes on a water bath with occasional shaking while replenishing the evaporated water. Cool, and filter the solution. Acidify the filtrate with hydrochloric acid. No precipitate is formed.

Residue on Ignition Not more than 0.1%.

Beet Red

ビートレッド

Definition Beet Red is obtained from the roots of the beet *Beta vulgaris* Linné and consists mainly of isobetanine and betanine. It may contain dextrin or lactose.

Color Value The Color Value ($E^{10\%}_{1cm}$) of Beet Red is not less than 15 and is in the range of 90–110% of the labeled value.

Description Beet Red occurs as a red-purple to dark purple powder, lumps, paste, or liquid, having a slightly characteristic odor.

Identification

(1) Weigh the equivalent of 1 g of Beet Red with a Color Value 15, and dissolve it in 50 ml of acetate buffer (pH 5.4).

A red-purple color develops.

(2) To 5 ml of the solution obtained in Identification (1), add 1 ml of sodium hydroxide solution. The color changes to yellow.

(3) A solution of Beet Red in acetate buffer (pH 5.4) exhibits an absorption maximum at a wavelength of 525–540 nm.

(4) Weigh the equivalent of 1 g of Beet Red with Color Value 15, dissolve it in 5 ml of water, add 20 ml of methanol, and mix. Centrifuge the solution at 3,000 rpm for 10 minutes, and use the supernatant as the test solution. Analyze an 8 µl portion of the test solution by thin-layer chromatography using a 4:3:2 mixture of 1-butanol/water/acetic acid as the developing solvent. No control solution is used. Use a thin-layer plate coated with microcrystalline cellulose for thin-layer chromatography as the solid support and then dried at 60–80°C for 20 minutes. Stop the development when the solvent front has ascended to a point about 10 cm above the original line, and air-dry the plate. A purple spot is observed at an R_f value of about 0.3–0.5. Place the plate in a container filled with ammonia vapor, and allow to stand for at least 30 minutes. The purple color of the spot changes to light gray to dark brown.

Purity

(1) Heavy metals Not more than 40 µg/g as Pb (0.50 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 10 µg/g as Pb (1.0 g, Method 1).

(3) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(4) Nitrate Not more than 0.27% as NO_3 per Color Value 15.

Test Solution Weigh accurately about 0.1 g of Beet Red, and dissolve it in water to make exactly 100 ml.

Standard Solution Measure exactly 0.2 ml, 1 ml, 10 ml, and 50 ml of Nitrate Ion Standard Stock Solution, and add water to each to make exactly 100 ml.

Analyze 20 µl portions of the test solution, the standard solutions, and the Standard Stock Solution by ion chromatography. Measure the peak height or peak area of nitrate ion for each of the standard solutions and the Standard Stock Solution, and prepare a calibration curve. Measure the peak height or peak area of nitrate ion for the test solution, and obtain the amount using the calibration curve.

Operating Conditions

Detector: Electric conductivity detector.

Column: A stainless steel tube of 4.6–6.0 mm internal diameter and 5–10 cm length.

Column packing material: Porous anion exchange resin.

Guard column: Used a column with the same internal diameter that is packed with the same packing material as for the column above.

Temperature: 40°C.

Eluant: Aqueous solution (pH 4.0) containing 2.5 mmol/L phthalic acid and 2.4 mmol/L tris(hydroxymethyl)aminomethane.

Flow rate: 1.5 ml/min.

Color Value Test Proceed as directed in the Color Value Test, using the conditions below.

Operating Conditions

Solvent: Acetate buffer (pH 5.4).

Wavelength: Maximum absorption wavelength of 525–540 nm.

Bentonite

ベントナイト

Definition Bentonite is obtained by drying bentonite mined from mineral deposits. It consists mainly of hydrous aluminum silicate.

Description Bentonite occurs as a white to light yellow-brown powder or flakes. When moistened, it produces an odor like soil or clay.

Identification

(1) To 0.5 g of Bentonite, add 3 ml of diluted sulfuric acid (1 in 3), and heat until white fumes are evolved. After cooling, add 20 ml of water, and filter. To 5 ml of the filtrate, add 3 ml of ammonia TS. A white gelatinous precipitate is formed, and when alizarin S solution (1 in 1,000) is added, the precipitate turns to red.

(2) Wash the residue obtained by filtration in Identification (1) with water, add 2 ml of methylene blue solution (1 in 10,000), and again wash with water. The residue turns blue.

(3) Mix 6.0 g of Bentonite with 0.3 g of magnesium oxide. Add the mixture in small portions to a 500-ml measuring cylinder with a stopper containing 200 ml of water, and shake for 1 hour. Transfer 100 ml of the suspension to a 100-ml measuring cylinder, and allow to stand for 24 hours. The solution separats into two layers. The upper clear solution is not more than 2 ml.

Purity

(1) pH 8.5–10.5 (2% suspension).

(2) Lead Not more than 40 µg/g as Pb.

Test Solution Weigh 2.0 g of Bentonite, add 12 ml of diluted hydrochloric acid (1 in 10) and 8 ml of water, boil for 30 minutes while replenishing the evaporated water, and then evaporate to dryness. Dry at 100°C for an additional 1 hour. To the residue, add 20 ml of diluted hydrochloric acid (1 in 10), boil gently for 5 minutes, and filter the supernatant through a filter paper. To the residue, again add 10 ml of diluted hydrochloric acid (1 in 10), boil gently for 5 minutes, filter through the same filter paper, and combine both filtrates. To the combined filtrate, add water to 100 ml. Refer to the resulting solution as solution A. Evaporate 25 ml of solution A to dryness on a water bath, and then dissolve the residue in diluted hydrochloric acid (1 in 10) to make 20 ml of a solution.

Control Solution Dilute 1.0 ml of Lead Standard Solution with hydrochloric acid (1 in 10) up to 10 ml.

Procedure Proceed as directed in Method 1 in the Lead Limit Test.

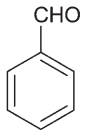
(3) Arsenic Not more than 4.0 µg/g as As_2O_3.

Use 25 ml of solution A obtained in Purity (2) as the test solution. Use Apparatus B for the test.

Loss of Drying Not more than 12.0% (105°C, 2 hours).

Benzaldehyde

ベンズアルデヒド



C_7H_6O Mol. Wt. 106.12

Benzaldehyde [100-52-7]

Content Benzaldehyde contains not less than 97.0% of benzaldehyde (C_7H_6O).

Description Benzaldehyde is a colorless liquid having an almond-like odor.

Identification Determine the absorption spectrum of Benzaldehyde as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Refractive index n_D^{20}: 1.544–1.547.

(2) Specific gravity 1.044–1.049.

(3) Acid value Not more than 5.0 (Flavoring Substances Tests).

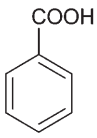
(4) Halogenated compounds Proceed as directed for Halogenated Compounds in the Flavoring Substances Tests.

Assay Weigh accurately about 0.8 g of Benzaldehyde, and proceed as directed in Method 2 in the Aldehyde and Ketone Content Test under the Flavoring Substances Tests. In the test, allow the mixture to stand for 10 minutes before titrating.

Each ml of 0.5 mol/L hydrochloric acid = 53.06 mg of C_7H_6O

Benzoic Acid

安息香酸



$C_7H_6O_2$ Mol. Wt. 122.12

Benzenecarboxylic acid [65-85-0]

Content Benzoic Acid, when dried, contains not less than 99.5% of benzoic acid ($C_7H_6O_2$).

Description Benzoic Acid occurs as white laminar crystals or needles. It is odorless or has a slight odor of benzaldehyde.

Identification Dissolve 1 g of Benzoic Acid in 20 ml of sodium hydroxide solution (1 in 25). The solution responds to test (2) for Benzoate in the Qualitative Tests.

Purity

(1) Melting point 121–123°C.

(2) Heavy metals Not more than 10 μg/g as Pb.

Test Solution Weigh 2.0 g of Benzoic Acid, dissolve it in 25 ml of acetone, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution To 2.0 ml of Lead Standard Solution, add 25 ml of acetone, 2 ml of diluted acetic acid (1 in 20), and water to make 50 ml.

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(4) Readily oxidizable substances Add 1.5 ml of sulfuric acid to 100 ml of water, and then add 0.02 mol/L potassium permanganate dropwise while boiling until the pink color persists for 30 seconds. Weigh 1.0 g of Benzoic Acid, and dissolve it in the solution. Titrate with 0.02 mol/L potassium permanganate at about 70°C until the pink color persists for 15 seconds. The amount is not more than 0.5 ml.

(5) Chlorinated compounds Not more than 0.014% as Cl.

Test Solution Weigh 0.50 g of Benzoic Acid and 0.7 g of calcium carbonate, and transfer together in a porcelain crucible. Add a small amount of water, mix, dry at 100°C, and heat at about 600°C for 10 minutes. Cool, add 20 ml of diluted nitric acid (1 in 10) to dissolve the residue, and filter. Wash the insoluble substances with about 15 ml of water, combine the washings and the filtrate, and add water to make 50 ml.

Control Solution Weigh 0.7 g of calcium carbonate, dissolve it in 20 ml of diluted nitric acid (1 in 10), filter if necessary, and add 0.20 ml of 0.01 mol/L hydrochloric acid and water to make 50 ml.

Procedure Add 0.5 ml of silver nitrate solution (1 in 50) to both solutions, shake well, and allow to stand for 5 minutes. The test solution is not more turbid than the control solution.

(6) Phthalic acid Not more than 50 μg/g.

Test Solution Weigh 1.0 g of Benzoic Acid, dissolve it in 20 ml of methanol, and add diluted acetic acid (1 in 100) to make exactly 50 ml.

Control Solution Weigh 0.0100 g of phthalic acid, dissolve it in 30 ml of methanol, and add diluted acetic acid (1 in 100) to make exactly 100 ml. Measure 1.0 ml of this solution, and add a 3:2 mixture of diluted acetic acid (1 in 100)/methanol to make exactly 100 ml.

Procedure Measure 20 μl each of the test solution and the control solution, and proceed with the operating conditions given below, as directed under Liquid Chromatography. The peak height of the phthalic acid of the test solution does not exceed that of the control solution.

Operating Conditions

Detector: Ultraviolet spectrophotometer (determination wavelength: 228 nm).

Column: A stainless steel tube of 4.6 mm internal diameter and 25 cm length.

Column packing material: 7-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 40°C.

Mobile phase: A 7:3 mixture of diluted acetic acid (1 in 100)/methanol.

Flow rate: 1 ml/minute.

Loss on Drying Not more than 0.50% (3 hours).

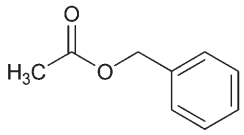
Assay Weigh accurately about 0.25 g of Benzoic Acid, previously dried, dissolve it in 25 ml of 50% (vol) ethanol neu-

tralized with 0.1 mol/L sodium hydroxide, and titrate with 0.1 mol/L sodium hydroxide (indicator: 3 drops of phenol red TS).

Each ml of 0.1 mol/L sodium hydroxide = 12.21 mg of $C_7H_6O_2$

Benzyl Acetate

酢酸ベンジル



$C_9H_{10}O_2$ Mol. Wt. 150.17

Phenylmethyl acetate [140-11-4]

Content Benzyl Acetate contains not less than 98.0% of benzyl acetate ($C_9H_{10}O_2$).

Description Benzyl Acetate is a colorless, transparent liquid having a characteristic odor.

Identification Determine the absorption spectrum of Benzyl Acetate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensity of absorption at the same wavenumbers.

Purity

(1) Refractive index n_D^{20}: 1.501–1.504.

(2) Specific gravity 1.055–1.059.

(3) Clarity of solution Clear (2.0 ml, 70% (vol) ethanol 4.0 ml).

(4) Acid value Not more than 1.0 (Flavoring Substances Tests).

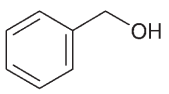
(5) Halogenated compounds Proceed as directed for Halogenated Compounds in the Flavoring Substances Tests.

Assay Weigh accurately about 0.8 g of Benzyl Acetate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 75.09 mg of $C_9H_{10}O_2$

Benzyl Alcohol

ベンジルアルコール



C_7H_8O Mol. Wt. 108.14

Phenylmethanol [100-51-6]

Content Benzyl Alcohol contains not less than 98.0% of benzyl alcohol (C_7H_8O).

Description Benzyl Alcohol is a colorless, transparent liquid having a weak characteristic odor.

Identification Add 2–3 drops of Benzyl Alcohol to 5 ml of potassium permanganate solution (1 in 20), and acidify with diluted sulfuric acid (1 in 20). An odor of benzaldehyde is evolved.

Purity

(1) Refractive index n_D^{20}: 1.538–1.541.

(2) Specific gravity 1.045–1.050.

(3) Clarity of solution Measure 1.0 ml of Benzyl Alcohol, and dissolve it in 35 ml of water. Even though the solution is turbid, the oily layer does not separate immediately.

(4) Free acid and free alkali Measure 10 ml of Benzyl Alcohol, dissolve it in 10 ml of neutralized ethanol, and add 2 drops of phenolphthalein TS. No pink color develops. To this solution, add 0.20 ml of 0.1 mol/L sodium hydroxide, and shake. A pink color develops.

(5) Aldehyde Weigh exactly 5 g of Benzyl Alcohol, and proceed as directed in Method 2 under Aldehyde and Ketone Content in the Flavoring Substances Tests. The volume of consumed 0.5 mo/l hydrochloric acid is not more than 0.20 ml.

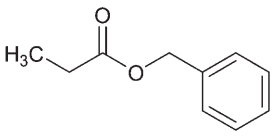
(6) Halogenated compounds Proceed as directed for Halogenated Compounds in the Flavoring Substances Tests.

Assay Weigh accurately about 0.5 g of Benzyl Alcohol, and proceed as directed in Method 2 under the Alcohol Content Test in the Flavoring Substances Tests.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 54.07 mg of C_7H_8O

Benzyl Propionate

プロピオン酸ベンジル



$C_{10}H_{12}O_2$ Mol. Wt. 164.20

Phenylmethyl propanoate [122-63-4]

Content Benzyl Propionate contains not less than 98.0% of benzyl propionate ($C_{10}H_{12}O_2$).

Description Benzyl Propionate is a colorless, transparent liquid having a characteristic odor.

Identification (1) To 1 ml of Benzyl Propionate, add 5 ml of ethanolic 10% potassium hydroxide TS. Warm for 20 minutes in warm water. The characteristic odor disappears. Cool, and acidify with diluted sulfuric acid (1 in 20). An odor of propionic acid is evolved.

Purity

(1) Refractive index n_D^{20}: 1.496–1.500.

(2) Specific gravity 1.032–1.036.

(3) Clarity of solution Clear (1.0 ml, 70% (vol) ethanol 5.0 ml).

(4) Acid value Not more than 1.0. (Flavoring Substances Tests).

(5) Halogenated compounds Proceed as directed for the

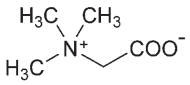
Halogenated Compounds Test in the Flavoring Substances Tests.

Assay Weigh accurately about 1 g of Benzyl Propionate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 82.10 mg of $C_{10}H_{12}O_2$

Betaine

ベタイン



$C_5H_{11}NO_2$ Mol. Wt. 117.15

2-(*N,N,N*-Trimethylammonio)acetate [107-43-7]

Definition Betaine is obtained by isolation from molasses from the sugar beet *Beta vulgaris* Linné. It consists mainly of betaine ($C_5H_{11}NO_2$).

Content Betaine, when dried, contains 98.0–102.0% of betaine ($C_5H_{11}NO_2$).

Description Betaine occurs as white, hygroscopic and deliquescent crystals having a slight odor. It has sweet and slightly bitter tastes.

Identification Determine the absorption spectrum of Betaine, previously dried, as directed in the Paste Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Clarity and color of solution Colorless and clear (1.0 g, water 10 ml).

(2) pH 5.0–7.0 (1.0 g, water 20 ml).

(3) Chloride Not more than 0.005% as Cl (1.0 g, Control solution 0.01 mol/L hydrochloric acid 0.15 ml).

(4) Sulfate Not more than 0.01% as SO_4 (1.0 g, Control solution 0.005 mol/L sulfuric acid 0.20 ml).

(5) Heavy metals Not more than 5.0 μg/g as Pb (4.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss of Drying Not more than 3.0% (105°C, 3 hours).

Residue on Ignition Not more than 0.10% (500°C, 3 hours).

Assay *Test Solution* Weigh accurately about 1 g of Betaine, previously dried, and dissolve it in water to make exactly 100 ml.

Standard Solutions Weigh exactly 0.5 g and 1.0 g portions, respectively, of betaine for assay, previously dried, and separately dissolve them in water to make exactly 100 ml of each.

Procedure Analyze 10 μl portions of the test solution and the standard solutions by liquid chromatography using the operating conditions given below, and measure the peak areas. Prepare a calibration curve from the peak areas for the standard solutions. Determine the betaine content, using the following formula, from the calibration curve and the peak area for the test solution.

$$\text{Content (\%) of betaine } (C_5H_{11}NO_2) = \frac{\text{Amount (g) of betaine in the test solution}}{\text{Weight (g) of the sample}} \times 100$$

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube of 4 mm internal diameter and 25 cm length.

Column packing material: Strongly acidic cation exchange resin.

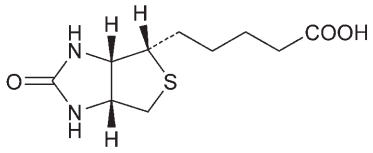
Column temperature: 70°C.

Mobile phase: Water.

Flow rate: Adjust so that the retention time of betaine is about 9 minuets.

Biotin

ビオチン



$C_{10}H_{16}N_2O_3S$ Mol. Wt. 244.31

5-[(3a*S*,4*S*,6a*R*)-2-Oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanoic acid [58-85-5]

Content Biotin contains not less than 98.0% biotin ($C_{10}H_{16}N_2O_3S$) when dried.

Description Biotin occurs as white crystals or crystalline powder. It is odorless and tasteless.

Identification

(1) To 5 ml of a solution (1 in 10,000) of Biotin in ethanol, add 1 ml of *p*-dimethylaminocinnamaldehyde TS and 3 drops of sulfuric acid, and shake. An orange to red color develops.

(2) Determine the absorption spectrum of Biotin, previously dried, as directed in the Potassium Bromide Disk Method under Infrared Spectrophotometry. The spectrum exhibits absorption bands at about 3315 cm^{-1}, 1708 cm^{-1}, 1687 cm^{-1}, 1481 cm^{-1}, 1320 cm^{-1}, and 1274 cm^{-1}.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +89 to +93° (0.4 g, dilute sodium hydroxide TS, 20 ml, on the dried basis).

(2) Clarity and color of solution Colorless and clear (1.0 g, 0.5 mol/L sodium hydroxide TS 10 ml).

(3) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 2.8 μg/g as As_2O_3.

Test Solution Take 0.70 g of Biotin in a Kjeldahl flask, add 5 ml of nitric acid and 2 ml of sulfuric acid, place a small funnel in the mouth of the flask, and heat carefully until white fumes are evolved. After cooling, add two 2-ml portions of nitric acid, heat, add two to three 2-ml portions of hydrogen peroxide, and heat until the solution is colorless to pale yellow. After cooling, add 2 ml of a saturated ammonium oxalate solution, heat to evaporate until white fumes are evolved

again. After cooling, add water to make 5 ml.

Apparatus Use Apparatus B.

(5) Related substances

Test Solution Dissolve 0.10 g of Biotin, weighed accurately, in diluted ammonium water (7 in 100) and make exactly 10 ml.

Standard Solution Take exactly 1 ml of the test solution, add diluted ammonium water (7 in 100) to make exactly 500 ml.

Procedure Analyze 5 µl portions of the test solution and the standard solution by thin-layer chromatography, using a 5:2:1 mixture of 1-butanol/water/acetic acid as the developing solvent. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. When the solvent front has ascended to a point 10 cm above the original line, stop developing. Air-dry the plate, and dry at 105°C for an additional 30 minutes. Spray evenly with a mixture of equal volumes of a solution of *p*-dimethylaminocinnamaldehyde in ethanol (1 in 500) and a solution of sulfuric acid in ethanol (1 in 50). The main red spot from the test solution is observed, and no other spots from the test solution are darker in color than the main spot from the standard solution.

Loss on Drying Not more than 0.50% (105°C, 4 hour).

Residue on Ignition Not more than 0.10%.

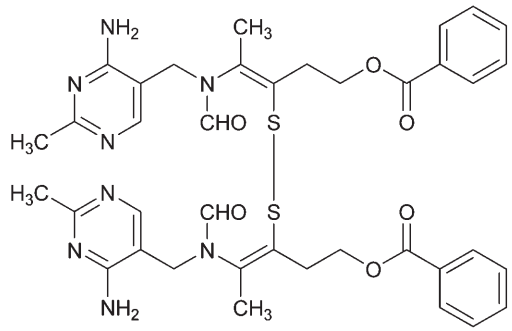
Assay Weigh accurately about 0.25 g of Biotin previously dried, and add exactly 20 ml of 0.1 mol/L sodium hydroxide solution to dissolve. Titrate the excess sodium hydroxide with 0.1 mol/L hydrochloric acid. Use 2 drops of phenolphthalein as the indicator. Separately, perform a blank test to make any necessary correction.

Each ml of 0.1 mol/L sodium hydroxide = 24.43 mg of $C_{10}H_{16}N_2O_3S$

Bisbentiamine

Benzoylthiamine Disulfide

ビスベンチアミン



$C_{38}H_{42}N_8O_6S_2$ Mol. Wt. 770.92

N,N'-(Disulfanediylbis{2-[2-(benzoyloxy)ethyl]-1-methylethene-2,1-diyl})bis{*N*-[(4-amino-2-methylpyrimidin-5-yl)methyl]formamide} [2667-89-2]

Content Bisbentiamine, when dried, contains 98.0–102.0% of bisbentiamine ($C_{38}H_{42}N_8O_6S_2$).

Description Bisbentiamine occurs as white crystals or crystalline powder. It is odorless and has a slightly bitter taste.

Identification

(1) To 0.05 g of Bisbentiamine, add 5 ml of methanol, and dissolve by warming. Add 2 ml of a 1:1 mixture of sodium hydroxide solution (3 in 20)/hydroxylamine hydrochloride solution (3 in 20), and warm in a water bath at 50–60°C for 2 minutes. To the resulting solution, add 0.8 ml of hydrochloric acid and 0.5 ml of iron(III) chloride solution (1 in 10), and 8 ml of water. A red-purple color develops.

(2) To 5 mg of Bisbentiamine, add 1 ml of methanol, and dissolve by warming. Add 2 ml of water, 2 ml of cysteine hydrochloride solution (1 in 100), and 1 ml of sodium hydroxide solution (1 in 25), shake, and allow to stand for 5 minutes. To the resulting solution, add 1 ml of freshly prepared potassium ferricyanide solution (1 in 10) and 5 ml of 2-methyl-1-propanol, shake vigorously for 2 minutes, and allow to stand. Examine under ultraviolet light. The 2-methyl-1-propanol layer emits a blue-purple fluorescence, which disappears when the solution is made acidic, and reappears when it is made alkaline.

Purity

(1) Melting point 140–145°C (decomposition).

(2) Clarity and color of solution Colorless and clear (0.10 g, methanol 20 ml).

(3) Heavy metals Not more than 20 µg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

Loss on Drying Not more than 0.50% (24 hours).

Residue on Ignition Not more than 0.20%.

Assay Weigh accurately about 0.5 g of Bisbentiamine, previously dried, dissolve it in 50 ml of acetic acid, and titrate with 0.1 mol/L perchloric acid (indicator: 1 ml of crystal violet–acetic acid TS) until the color of the solution changes from purple through blue to green. Perform a blank test in the same manner, and make any necessary correction.

Each ml of 0.1 mol/L perchloric acid = 38.55 mg of $C_{38}H_{42}N_8O_6S_2$

Black Currant Color

ブラックカーラント色素

Definition Black Currant Color is obtained from the fruits of the black currant *Ribes nigrum* Linné and consists mainly of delphinidin-3-rutinoside. It may contain dextrin or lactose.

Color Value The Color Value ($E^{10\%}_{1cm}$) of Black Currant Color is not less than 40 and is in the range of 90–110% of the labeled value.

Description Black Currant Color occurs as a dark red powder, viscous paste, or liquid having a slightly characteristic odor.

Identification

(1) Weigh the equivalent of 1 g of Black Currant Color with a Color Value 40, dissolve it in 100 ml of citrate buffer (pH 3.0). A red to red-purple color develops.

(2) Add sodium hydroxide solution (1 in 25) to the solution obtained in Identification (1) to make alkaline. The color changes to dark green.

(3) A solution of Black Currant Color in citrate buffer (pH 3.0) exhibits an absorption maximum at a wavelength of 510–520 nm.

Purity

(1) Heavy metals Not more than 40 µg /g as Pb (0.50g,

Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 10 µg /g as Pb (1.0g, Method 1).

(3) Arsenic Not more than 4.0 µg /g as As_2O_3 (0.50g, Method 3, Apparatus B).

(4) Sulfur dioxide Not more than 0.005% per Color Value.

Proceed as directed in Purity (4) for Grape Skin Extract.

Color Value Test Proceed as directed in the Color Value Test, using the conditions below.

Operating Conditions

Solvent: Citrate buffer (pH 3.0).

Wavelength: Maximum absorption wavelength of 510–520nm.

Bone Charcoal

骨炭

Definition Bone Charcoal is obtained by carbonizing and crushing the bones of cattle, *Bos Taurus* Linné. It consists mainly of calcium phosphate and carbon powder.

Description Bone Charcoal occurs as a black powder or granules. It is odorless and tasteless.

Identification

(1) Weigh about 0.1 g of Bone Charcoal, previously triturated for a granular sample, add 10 ml of dilute methylene blue TS and 2 drops of diluted hydrochloric acid (1 in 4), shake well, and filter through a dry filter paper for quantitative analysis (No. 5C). The solution obtained is colorless.

(2) Transfer about 0.5 g of Bone Charcoal, previously triturated for a granular sample, into a test tube, and heat by direct fire while supplying air from the tube mouth. The sample burns without a flame. When the generated gas is passed through calcium hydroxide TS, a white turbidity is produced.

(3) To 0.1 g of Bone Charcoal, previously incinerated, add 10 ml of diluted hydrochloric acid (1 in 7), and dissolve by warming. Add 2.5 ml of ammonia TS while shaking, and then add 5 ml of ammonium oxalate solution (1 in 30). A white precipitate is formed.

(4) To 0.1 g of Bone Charcoal, previously incinerated, add 5 ml of dilute nitric acid, and dissolve by warming. Add 2 ml of ammonium molybdate TS. A yellow precipitate is formed.

Purity

Sample Preparation If the sample is in granular form, triturate it before weighing. If it is in powder form, use it as is. Weigh 4.0 g of Bone Charcoal, previously dried at 110–120°C for 3 hours, add 180 ml of water containing 0.1 ml of diluted nitric acid (1 in 100), and heat for about 10 minutes to maintain gentle boiling. After cooling, add 200 ml of water, and filter through a dry filter paper for quantitative analysis (No. 5C). Discard the first 30 ml of the filtrate, and use the subsequent filtrate (solution A) for the following tests.

(1) Chloride Not more than 0.53% as Cl.

Test Solution Use 1.0 ml of solution A.

Control Solution Use 0.30 ml of 0.01 mol/L hydrochloric acid.

(2) Sulfate Not more than 0.48% as SO_4.

Test Solution Use 2.5 ml of solution A.

Control Solution Use 0.50 ml of 0.005 mol/L sulfuric acid.

(3) Lead Not more than 10 µg/g as Pb.

Test Solution Evaporate 50 ml of solution A to dryness on a water bath. To the residue, add 10 ml of diluted nitric acid (1 in 150) to dissolve.

Control Solution To 1.0 ml of Lead Standard Solution, add diluted nitric acid (1 in 150) to make 10 ml.

Procedure Proceed as directed in Method 1 in the Lead Limit Test.

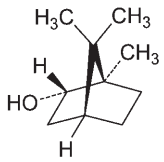
(4) Arsenic Not more than 4.0 µg/g as As_2O_3.

Evaporate 25 ml of solution A to dryness on a water bath. Use the residue as the test sample. Proceed as directed in Method 2 in the Arsenic Limit Test, using Apparatus B.

d-Borneol

Borneol

d-ボルネオール



$C_{10}H_{18}O$ Mol. Wt. 154.25

(*1R,2S,4R*)-1,7,7-Trimethylbicyclo[2.2.1]heptan-2-ol

[464-43-7]

Content *d*-Borneol contains not less than 95.0% of *d*-borneol ($C_{10}H_{18}O$).

Description *d*-Borneol occurs as white crystals, crystalline powder, or lumps, having a Borneo camphor-like odor.

Identification

(1) Grind and mix *d*-Borneol with an equal amount of thymol. It liquefies.

(2) Place about 0.1g of *d*-Borneol in a test tube, heat the bottom of the test tube tilted about 45° in a colorless flame of Bunsen burner for 1 minute. A white sublimate is produced on the upper place of the test tube.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +16.05 to +37.0° (2.5 g, ethanol, 25 ml).

(2) Melting point 205–210°C.

(3) Heavy metals Not more than 10 µg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 4, Apparatus B).

Assay Weigh accurately about 1 g of *d*-Borneol into a 200-ml flask with a stopper, and add exactly 5 ml of acetic anhydride –pyridine TS. Equip the flask with a reflux condenser, moisten the ground-glass joint with 2–3 drops of pyridine, and heat in a water bath for 3 hours. After cooling, Rinse the inside of flask by running 10 ml of water through the reflux condenser, and allow to cool to ordinary temperature. Add 10 ml of water, stopper tightly, and shake well. Rinse the joint part of the stopper and the inside of the flask with 5 ml of neutralized ethanol, and titrate with ethanolic 0.5 mol/L potassium hydroxide (indicator: 10 drops of cresol-red-thymol blue TS). Perform a blank test in the same manner.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 77.12 mg of $C_{10}H_{18}O$

Bromelain

ブロメライン

Definition Bromelain is a proteolytic enzyme obtained from the fruit and rhizoma of the pineapple *Ananas comosus* Merrill. It may contain lactose or dextrin.

Enzyme Activity Bromelain contains the enzyme activity equivalent to not less than 500,000 units per gram.

Description Bromelain occurs as a white to light yellowish brown powder. It is odorless or has a slight characteristic odor.

Identification Proceed as directed in Identification (1) for Papain.

Purity

(1) Lead Not more than 5.0 μg/g as Pb (2.0 g, Method 1).

(2) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(3) Cyanide Weigh 5.0 g of Bromelain, transfer into a distillation flask, add 2 g of tartaric acid and 50 ml of water, and add 1 drop of silicone resin if necessary. Connect the flask with distillation apparatus that is joined to a receiver with a condenser containing 2 ml of 1 mol/L sodium hydroxide solution and 10 ml of water. Distill until 20 ml of distillate is obtained, and add water to the distillate to make 50 ml. To 25 ml of this solution, add 0.5 ml of ferrous sulfate TS, 0.5 ml of iron(III) hydrochloride solution (0.18 in 100), and 1 ml of dilute sulfuric acid. No blue color develops.

Microbial Limits Proceed as directed in the Microbial Limit Tests. The total bacterial count is not more than 50,000/g, and *Escherichia coli* is negative.

Enzyme Activity Determination

(i) Test solution Dissolve 5.27 g of L-cysteine hydrochloride, 2.23 g of disodium ethylenediamine- tetraacetate and 23.4 g of sodium chloride in water. Adjust the pH to 4.5 with 1 mol/L sodium hydroxide TS, and add water to make 1,000 ml. Use this solution as the diluent.

Weigh accurately about 0.1 g of Bromelain, transfer into a mortar, add the diluent, and mix. Add the diluent to make exactly 100 ml. Centrifuge this solution if necessary. Dilute the supernatant liquid with the diluent to prepare a solution containing 30 to 50 units per ml.

(ii) Procedure Measure exactly 1 ml of the test solution, transfer into a test tube, and warm for 5 minutes at 37±0.5°C. Add exactly 5 ml of casein TS (pH 7.0), previously warmed to 37±0.5°C, shake immediately and react for exactly 10 minutes at 37±0.5°C. Add exactly 5 ml of trichloroacetic acid TS, and shake. Allow to stand for 40 minutes at 37±0.5°C, and filter through a filter paper for quantitative analysis (5C). Discard the first 3 ml of the filtrate, and measure the absorbance (A_T) of the subsequent filtrate at 275 nm, using water as the reference.

Separately, measure exactly 1 ml of the test solution, add excatly 5 ml of trichloroacetic acid TS, and shake well. Add exactly 5 ml of casein TS (pH 7.0), shake well, allow to stand for 40 minutes at 37±0.5°C. Measure the absorbance (A_0) of this solution, proceeding in the same manner as for the measurement of absorbance A.

Separately, measure the absorbances (A_S and A_{S0}) of Tyrosine Standard Solution and 0.1 mol/L hydrochloric acid, respectively, at 275 nm, using water as the reference.

Calculate the enzyme activity by the formula below. One unit of the enzyme activity is the quantity of enzyme that produces amino acids equivalent to 1 μg of tyrosine per minute when the test is performed as directed in the Procedure.

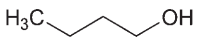
$$\text{The enzyme activity of Bromelain (units/g)} = \frac{(A_T - A_0) \times 50}{A_S - A_{S0}} \times \frac{11}{10} \times \frac{1{,}000}{W}$$

W = weight (mg) of Bromelain in 1 ml of the test solution.

Butanol

Butan-1-ol
Butyl Alcohol

ブタノール



$C_4H_{10}O$ Mol. Wt. 74.12

Butan-1-ol [71-36-3]

Content Butanol contains not less than 99.5% of butanol ($C_4H_{10}O$).

Description Butanol is a colorless, clear liquid having a characteristic odor.

Identification Determine the infrared absorption spectrum of Butanol as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit absorptions having about the similar intensities at the same wavenumbers.

Purity

(1) Refractive index n_D^{20}: 1.393–1.404.

(2) Specific gravity d_{25}^{25}: 0.807–0.809.

(3) Acid value Not more than 2.0 (Flavoring Substances Tests).

(4) Dibutyl ether Not more than 0.15%.

Perform the test by Gas Chromatography, according to the direction in the Assay given below. The peak area of dibutyl ether is not more than 0.15% of the total peak area of all peaks. Use operating conditions that can provide complete resolution of the peaks of butanol and dibutyl ether when 1 μl of a solution of butyl ether in butanol (15 in 10,000) is chromatographed.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavor Substance Tests, using operating conditions (2).

Butyl Acetate

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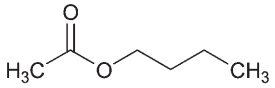

$C_6H_{12}O_2$ Mol. Wt. 116.16

Butyl acetate [123-86-4]

Content Butyl Acetate contains not less than 98.0% of butyl acetate ($C_6H_{12}O_2$).

Description Butyl Acetate is a colorless, transparent liquid having a characteristic odor.

Identification To 1 ml of Butyl Acetate, add 5 ml of ethanolic 10% potassium hydroxide TS. Heat it in a water bath. The characteristic odor disappears, and an odor of 1-butanol is evolved. Cool, and add 10 ml of water and 0.5 ml of diluted hydrochloric acid (1 in 4). The solution responds to test (3) for Acetate in the Qualitative Tests.

Purity

(1) Refractive index n_D^{20}: 1.392–1.395.

(2) Specific gravity 0.880–0.884.

(3) Clarity of solution Clear (2.0 ml, 70% (vol) ethanol 3.0 ml).

(4) Acid Value Not more than 1.0 (Flavoring Substances Tests).

Assay Weigh accurately about 0.5 g of Butyl Acetate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 58.08 mg of $C_6H_{12}O_2$

Butyl Butyrate

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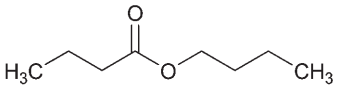


$C_8H_{16}O_2$ Mol. Wt. 144.21

Butyl butanoate [109-21-7]

Content Butyl Butyrate contains not less than 98.0% of butyl butyrate ($C_8H_{16}O_2$).

Description Butyl Butyrate is a colorless to light yellow, transparent liquid having a fruity odor.

Identification To 1 ml of Butyl Butyrate, add 5 ml of ethanolic 10% potassium hydroxide TS. Heat it in a water bath while shaking. The fruity odor disappears, and an odor of 1-butanol develops. Cool, and acidify with diluted sulfuric acid (1 in 20). An odor of butyric acid is evolved.

Purity

(1) Refractive index n_D^{20}: 1.405–1.407

(2) Specific gravity 0.867–0.872.

(3) Clarity of solution Clear (1.0 ml, 70% (vol) ethanol 4.0 ml).

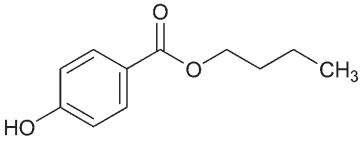
(4) Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Weigh accurately about 0.7 g of Butyl Butyrate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 72.11 mg of $C_8H_{16}O_2$

Butyl *p*-Hydroxybenzoate

パラオキシ安息香酸ブチル



$C_{11}H_{14}O_3$ Mol. Wt. 194.23

Butyl 4-hydroxybenzoate [94-26-8]

Content Butyl *p*-Hydroxybenzoate, when dried, contains not less than 99.0% of butyl *p*-hydroxybenzoate ($C_{11}H_{14}O_3$).

Description Butyl *p*-Hydroxybenzoate occurs as colorless crystals or a white crystalline powder. It is odorless.

Identification

(1) To 0.5 g of Butyl *p*-Hydroxybenzoate, add 10 ml of sodium hydroxide solution (1 in 25), boil for 30 minutes, evaporate to about 5 ml. Cool, acidify with diluted sulfuric acid (1 in 20), and collect the precipitate formed by filtration. Wash it thoroughly with water, and dry at 105°C for 1 hour. The melting point is 213–217°C.

(2) To 0.05 g of Butyl *p*-Hydroxybenzoate, add 2 drops of acetic acid and 5 drops of sulfuric acid, and warm for 5 minutes. An odor of butyl acetate is evolved.

Purity

(1) Melting point 69–72°C.

(2) Free acid Not more than 0.55% as *p*-hydroxybenzoic acid.

Weigh 0.75 g of Butyl *p*-Hydroxybenzoate, add 15 ml of water, heat in a boiling water bath for 1 minute, cool, and filter. The filtrate is acidic or neutral. Measure 10 ml of the filtrate, and add 0.20 ml of 0.1 mol/L sodium hydroxide and 2 drops of methyl red TS. A yellow color develops.

(3) Sulfate Not more than 0.024% as SO_4.

Sample Solution Weigh 1.0 g of Butyl *p*-Hydroxybenzoate, add 100 ml of boiling water, and heat for 5 minutes while shaking well. After cooling, add water to make 100 ml, and filter. Use 40 ml of the filtrate as the sample solution.

Control Solution Use 0.20 ml of 0.005 mol/L sulfuric acid.

(4) Heavy metals Not more than 10 µg/g as Pb.

Test Solution Weigh 2.0 g of Butyl *p*-Hydroxybenzoate, dissolve it in 25 ml of acetone, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution To 2.0 ml of Lead Standard Solution, add 25 ml of acetone, 2 ml of diluted acetic acid (1 in 20), and water to make 50 ml.

(5) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g,

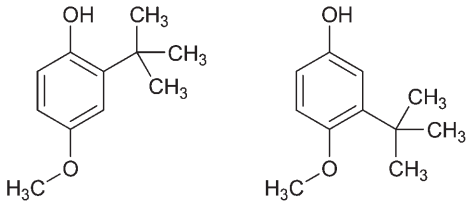
Method 3, Apparatus B).
Loss on Drying Not more than 0.50% (5 hours).
Residue on Ignition Not more than 0.10% .
Assay Weigh accurately about 2 g of Butyl *p*-Hydroxybenzoate, previously dried, add exactly 40 ml of 1 mol/L sodium hydroxide, and boil for 30 minutes. After cooling, titrate the excess alkali with 0.5 mol/L sulfuric acid (indicator: 5 drops of bromothymol blue TS). The color at the endpoint is that produced when the same indicator is added to phosphate buffer (pH 6.5). Perform a blank test in the same manner.

Each ml of 1 mol/L sodium hydroxide = 194.2 mg of $C_{11}H_{14}O_3$

Butylated Hydroxyanisole

BHA

ブチルヒドロキシアニソール



$C_{11}H_{16}O_2$ Mol. Wt. 180.24
Mixture of 2-(1,1-dimethylethyl)-4-methoxyphenol and 3-(1,1-dimethylethyl)-4-methoxyphenol [25013-16-5]
Description Butylated Hydroxyanisole occurs as colorless or slightly yellow-brown crystals or lumps or as a white crystalline powder having a slight characteristic odor.
Identification

(1) To 2–3 ml of a solution of Butylated Hydroxyanisole in ethanol (1 in 100), add 2–3 drops of sodium borate solution (1 in 50) and crystals of 2,6-dichloroquinonechloroimide, and shake. A purple-blue color develops.

(2) Proceed as directed in Identification (2) for Butylated Hydroxytoluene.

Purity

(1) Melting point 57–65°C.

(2) Clarity and color of solution Colorless and clear (0.50 g, ethanol 10 ml).

(3) Sulfate Not more than 0.019% as SO_4.

Test Solution Weigh 0.50 g of Butylated Hydroxyanisole, dissolve it in 35 ml of acetone, and add 1 ml of diluted hydrochloric acid (1 in 4) and water to make 50 ml.

Control Solution To 0.20 ml of 0.005 mo/l sulfuric acid, add 35 ml of acetone, 1 ml of diluted hydrochloric acid (1 in 4), and water to make 50 ml.

(4) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

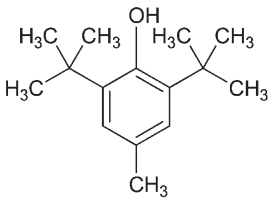
(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(6) *p*-Hydroxyanisole Weigh 1.0 g of Butylated Hydroxyanisole, dissolve it in 20 ml of a 1:1 mixture of diethyl ether/petroleum benzine, add 10 ml of water and 1 ml of sodium hydroxide solution (1 in 25), shake well, allow to stand, and collect the lower layer. To this solution, add 20 ml of a 1:1 mixture of diethyl ether/petroleum benzine, shake well, allow to stand, collect the lower layer, and add water to make 500 ml. Transfer 1.0 ml of this solution into a Nessler tube, and add 2 ml of sodium hydroxide solution (1 in 25), 5 ml of boric acid solution (3 in 100), and water to make 30 ml. Add 5 ml of 4-amino-antipyrine solution (1 in 1,000), shake, add 1 ml of potassium ferricyanide solution (1 in 100), shake again, add water to make 50 ml, and allow to stand for 15 minutes. The color of the solution is not darker than that of a solution prepared by diluting 0.6 ml of Cobaltous Chloride Colorimetric Standard Stock Solution to 50 ml with water.
Residue on Ignition Not more than 0.050%.

Butylated Hydroxytoluene

BHT

ジブチルヒドロキシトルエン



$C_{15}H_{24}O$ Mol. Wt. 220.35
2,6-Bis(1,1-dimethylethyl)-4-methylphenol [128-37-0]
Description Butylated Hydroxytoluene occurs as colorless crystals or as a white crystalline powder or lumps. It is odorless or has a slight characteristic odor.
Identification

(1) To 5 mg of Butylated Hydroxytoluene, add 1–2 drops of a solution of 5-nitroso-8-hydroxyquinoline in sulfuric acid (1 in 100). It dissolves, producing a yellow color, which changes to red-brown.

(2) To 1 ml of a solution of Butylated Hydroxytoluene in ethanol (1 in 30), add 3–4 drops of iron(III) chloride solution (1 in 500). No color develops. To this solution, add α,α′-dipyridyl crystals. A red color develops. Before the test, perform a blank test for iron(III) chloride solution to confirm that no color develops.

Purity

(1) Melting point 69–72°C.

(2) Clarity and color of solution Colorless and clear (1.0 g, ethanol 10 ml).

(3) Sulfate Not more than 0.019% as SO_4.

Test Solution Weigh 0.50 g of Butylated Hydroxytoluene, add 30 ml of water, heat in a water bath for 5 minutes with occasional shaking, cool, and filter.

Control Solution Use 0.20 ml of 0.005 mol/L sulfuric acid.

(4) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(6) *p*-Cresol Not more than 0.10% as *p*-cresol.

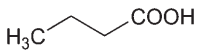
Sample Solution Weigh 1.0 g of Butylated Hydroxytoluene, add 10 ml of water and 1 ml of ammonia solution, heat in a water bath for 3 minutes with occasional shaking. Cool, and filter. Wash the residue on the filter paper with a small amount of water, combine the washings and the filtrate, and add water to make 100 ml.

Procedure Measure 3.0 ml of the test solution, transfer into a Nessler tube, add 1 ml of a solution of phosphomolybdic acid in ethanol (1 in 20) and 0.2 ml of ammonia TS, and shake. Add water to make 50 ml, and allow to stand for 10 minutes. The color of the solution is not darker than that of the solution prepared in the same manner as the test solution, using 3.0 ml of *p*-cresol solution (1 in 100,000).

Residue on Ignition Not more than 0.05%.

Butyric Acid

酪酸



$C_4H_8O_2$ Mol. Wt. 88.11

Butanoic acid [107-92-6]

Content Butyric Acid contains not less than 98.0% of butyric acid ($C_4H_8O_2$).

Description Butyric Acid is a colorless, transparent liquid having a characteristic odor.

Identification

(1) To 1 ml of Butyric Acid, add 2 ml of water. It dissolves, and the resulting solution is strongly acidic.

(2) To 1 ml of Butyric Acid, add 1 ml of ethanol and 3 drops of sulfuric acid, and warm in warm water. An odor of ethyl butyrate is evolved.

Purity

(1) Refractive index n_D^{20}: 1.398–1.401.

(2) Specific gravity 0.958–0.961.

(3) Sulfate Not more than 0.002% as SO_4 (10 g, Control solution 0.005 mol/L sulfuric acid 0.40 ml).

Assay Weigh accurately about 1 g of Butyric Acid, add 40 ml of water, and titrate with 1 mol/L sodium hydroxide (indicator: 2 drops of phenolphthalein TS).

Each ml of 1 mol/L sodium hydroxide = 88.11 mg of $C_4H_8O_2$

Calcinated Eggshell Calcium

卵殻焼成カルシウム

Definition Calcinated Eggshell Calcium* is obtained by calcinating eggshells. It consists mainly of calcium oxide.

Contain Calcinated Eggshell Calcium, when ignited, contains the equivalent of not less than 95.0% of calcium oxide (CaO = 56.08).

Description Calcinated Eggshell Calcium occurs as a white to grayish white powder.

Identification

(1) Moisten about 1 g of Calcinated Eggshell Calcium with water. It generates heat. Then add about 5 ml of water. The resulting suspension is alkali.

(2) To 1 g of Calcinated Eggshell Calcium, add 20 ml of water and 10 ml of acetic acid (1 in 3) to dissolve, and neutralize with ammonia TS. The resulting solution responds to the tests for Calcium Salt as described in the Quantitative Tests.

Purity

(1) Hydrochloric acid-insoluble substance Not more than 0.50%.

To 5.0 g of Calcinated Eggshell Calcium, add 100 ml of water, and then add hydrochloric acid dropwise while shaking until the sample no longer dissolves. Boil for 5 minutes, cool, and filter through a filter paper (No. 5C). Wash the residue well on the filter with water until the washings are free from chloride, ignite the residue with the filter paper in a crucible, and weigh the residue.

(2) Carbonate To 2.0 g of Calcinated Eggshell Calcium, add 50 ml of water, shake well, and add 25 ml of hydrochloric acid (1 in 4). It does not bubble vigorously.

(3) Heavy metals Not more than 10 µg/g as Pb.

Test Solution Dissolve 2.0 g of Calcinated Eggshell Calcium in 20 ml of diluted hydrochloric acid (1 in 4) to dissolve, and evaporate to dryness on a water bath. To the residue, add about 40 ml of water to dissolve, filter if necessary, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution To 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(4) Arsenic Not more than 4.0 µg/g as As_2O_3.

Test Solution Dissolve 0.50 g of Calcinated Eggshell Calcium in 5 ml of diluted hydrochloric acid (1 in 4).

Apparatus Use Apparatus B.

Residue on Ignition Not less than 10.0% (900°C, 30 minutes).

Assay Weigh accurately about 1.5 g of Calcinated Eggshell Calcium, previously ignited, dissolve it in 30 ml of diluted hydrochloric acid (1 in 4), and add water to make exactly 250 ml. Using this solution as the test solution, proceed as directed in Method 1 for Calcium Salt Determination.

Each ml of 0.05 mol/L EDTA = 2.804 mg of CaO

Calcinated Shell Calcium

貝殻焼成カルシウム

Definition Calcinated Shell Calcium* is obtained by calcinating shells. It consists mainly of calcium oxide.

Contain Calcinated Shell Calcium, when ignited, contains the equivalent of not less than 91.0% of calcium oxide (CaO

* Calcinated Eggshell Calcium is one of the substances belonging to the "Calcinated Calcium" category. "Calcinated Calcium" is defined in the List of Existing Food Additives as a substance that is obtained by calcinating sea urchin shells, shells, reef corals, whey, bones, or eggshells and that consists mainly of calcium.

* Calcinated Shall Calcium is one of the substances belonging to the "Calcinated Calcium" category. For the definition of Calcinated Calcium, see the footnote for the Calcinated Eggshell Calcium.

= 56.08).

Description Calcinated Shell Calcium occurs as white to grayish white lumps, granules, or powder.

Identification

(1) Suspend 1 g of Calcinated Shell Calcium in 5 ml of water. The resulting liquid is alkali.

(2) To 1 g of Calcinated Shell Calcium, add 20 ml of water and 10 ml of acetic acid (1 in 3) to dissolve, and neutralize with ammonia TS. The resulting solution responds to the tests for Calcium Salt as described in the Quantitative Tests.

Purity

(1) Hydrochloric acid-insoluble substance Not more than 0.50%.

Weigh 5.0 g of Calcinated Shell Calcium, add 100 ml of water, and add hydrochloric acid dropwise while shaking until the sample no longer dissolves. Boil for 5 minutes, cool, and filter through a filter paper (No. 5C). Wash the residue well on the filter with hot water until the washings are free of chloride, ignite the residue along with the filter paper, and weigh the residue.

(2) Carbonate To 1.0 g of Calcinated Shell Calcium, add a little volume of water, crush it, mix well with 50 ml of water, and allow to stand. Decant off the supernatant milky liquid, and add an excess amount of diluted hydrochloric acid (1 in 4) to the residue. It does not bubble vigorously.

(3) Heavy metals Not more than 10 μg/g as Pb.

Test Solution Weigh 2.0 g of Calcinated Shell Calcium, add 20 ml of diluted hydrochloric acid (1 in 4) to dissolve, and evaporate on a water bath to dryness. To the residue, add about 40 ml of water to dissolve, filter if necessary, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Measure exactly 2 ml of Lead Standard Solution, and add 2 ml of acetic acid and water to make 50 ml.

(4) Arsenic Not more than 4.0 μg/g as As_2O_3.

Standard Solution Weigh 0.50 g of Calcinated Shell Calcium, and add 5 ml of diluted hydrochloric acid (1 in 4) to dissolve.

Apparatus Use Apparatus B.

Residue on Ignition Not less than 10.0% (900ºC, 30 minutes).

Assay Weigh accurately about 1.5 g Calcinated Shell Calcium, previously ignited, add 30 ml of diluted of hydrochloric acid (1 in 4), and dissolve while heating. After cooling, add water to exactly 250 ml. Using this solution as the test solution, proceed as directed in Method 1 for Calcium Salt Determination.

Each ml of 0.05 mol/L EDTA = 2.804 mg of CaO

Calcium Alginate

アルギン酸カルシウム

Calcium Alginate [9005-35-0]

Content Calcium Alginate, when dried, contains 89.6–104.5% of calcium alginate.

Description Calcium Alginate occurs in white to yellowish white filamentous, granular, or powdered form.

Identification

(1) To 0.25 g of Calcium Alginate, add 50 ml of sodium carbonate solution (1 in 400) while stirring. Warm the mixture at 60–70°C for 20 minutes with occasional shaking to make it homogeneous, and cool. Use this solution as the test solution. Proceed as directed in Identification (1) for Ammonium Alginate.

(2) Ignite 1 g of Calcium Alginate at 550–600°C for 3 hours. To the residue, add 10 ml of water and 5 ml of acetic acid (1 in 3) to dissolve, and filter if necessary. Boil it, cool, and neutralize with ammonia TS. The solution obtained responds to all the tests for Calcium Salt described in the Qualitative Tests.

Purity

(1) Lead Not more than 5.0 μg/g as Pb (2.0 g, Method 1).

(2) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Loss on Drying Not more than 15.0% (105°C for 4 hour).

Microbial Limit Proceed as directed in the Microbial Limit Test for Ammonium Alginate.

Assay Proceed as directed in the Assay for Alginic Acid.

Each ml of 0.25 mol/L sodium hydroxide = 27.38 mg of calcium alginate

Calcium Carbonate

炭酸カルシウム

$CaCO_3$ Mol. Wt. 100.09

Calcium carbonate [471-34-1]

Content Calcium Carbonate, when dried, contains 98.0–102.0% of calcium carbonate ($CaCO_3$).

Description Calcium Carbonate occurs as a fine white powder. It is odorless.

Identification To 1 g of Calcium Carbonate, add 10 ml of water and 7 ml of diluted acetic acid (1 in 4). It effervesces and dissolves. When boiled and neutralized with ammonia TS, this solution responds to all tests for Calcium Salt in the Qualitative Tests.

Purity

(1) Hydrochloric acid-insoluble substances Not more than 0.20%.

Weigh 5.0 g of Calcium Carbonate, add 10 ml of water, then gradually add 12 ml of hydrochloric acid dropwise while stirring, and add water to make 200 ml. Filter through a filter paper for quantitative analysis (5C), wash thoroughly the residue on the filter paper with boiling water until the washings are free of chloride, incinerate together with the filter paper, and weigh the residue.

(2) Free alkali Weigh 3.0 g of Calcium Carbonate, add 30 ml of freshly boiled and cooled water, shake for 3 minutes, and filter the solution. To 20 ml of the filtrate, add 2 drops of phenolphthalein TS. A pink color develops, and it disappears when 0.20 ml of 0.1 mol/L hydrochloric acid is added.

(3) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of Calcium Carbonate, dissolve it in 8 ml of diluted hydrochloric acid (1 in 4), and add water to make about 20 ml. Add, dropwise, ammonia TS while shaking until the solution is slightly turbid appears. Add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution To exactly 2 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to

make 50 ml.

(4) Alkali metals and magnesium Not more than 1.0%. Weigh 1.0 g of Calcium Carbonate, dissolve by gradually adding 30 ml of diluted hydrochloric acid (1 in 10), and let the carbon dioxide out by boiling. Cool, neutralize with ammonia TS, add 60 ml of an ammonium oxalate solution (1 in 25), and heat on a water bath for 1 hour. After cooling, add water to make 100 ml, stir thoroughly, and filter. Measure 50 ml of the filtrate, add 0.5 ml of sulfuric acid, evaporate to dryness, and ignite to constant weight, and weigh the residue.

(5) Barium Not more than 0.030% as Ba.

Test Solution Weigh 1.0 g of Calcium Carbonate, dissolve it in 8 ml of diluted hydrochloric acid (1 in 4), add water to make 20 ml.

Procedure Add 2 g of sodium acetate, 1 ml of diluted acetic acid (1 in 20), and 0.5 ml of potassium chromate solution (1 in 20) to the test solution, and allow to stand for 15 minutes. The solution is not more turbid than a control solution prepared as follows: To 0.30 ml of Barium Standard Solution, add water to make 20 ml, and then treat in the same manner as the test solution.

(6) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.50 g of Calcium Carbonate, moisten with 1 ml of water, and dissolve it in 4 ml of hydrochloric acid (1 in 4).

Apparatus Use Apparatus B.

Loss on Drying Not more than 2.0% (200°C, 4 hours).

Assay Weigh accurately about 1 g of Calcium Carbonate, previously dried. Add it gradually to 10 ml of diluted hydrochloric acid (1 in 4) to dissolve, and add water to make exactly 100 ml. Use this solution as the test solution. Proceed as directed in Method 1 under Calcium Salt Determination.

Each ml of 0.05 mol/L EDTA = 5.004 mg of $CaCO_3$

Calcium Carboxymethylcellulose

Calcium Cellulose Glycolate

カルボキシメチルセルロースカルシウム

[9050-04-8]

Description Calcium Carboxymethylcellulose occurs as a white to light yellow powder or fibrous substance. It is odorless.

Identification

(1) Determine the absorption spectrum of Calcium Carboxymethylcellulose, previously dried as directed in the Potassium Bromide Disk Method in Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) Ignite 1 g of Calcium Carboxymethylcellulose at 550–600ºC for 3 hours. To the residue obtained, add 10 ml of water and 5 ml of diluted acetic acid (1 in 3), and filter if necessary. Boil the solution, cool, and neutralize with ammonia TS. The solution responds to all tests for Calcium Salt in the Qualitative Tests.

Purity

(1) Free alkali Weigh 1.0 g of Calcium Carboxymethylcellulose, add 50 ml of freshly boiled and cooled water, shake well, and then add 2 drops of phenolphthalein TS. No pink color develops.

(2) Chloride Not more than 0.35% as Cl.

Sample Solution Weigh 0.10 g of Calcium Carboxymethylcellulose, add 10 ml of water, stir thoroughly, and add 2 ml of a sodium hydroxide solution (1 in 25). Shake, allow to stand for 10 minutes, and make the solution slightly acidic with diluted nitric acid (1 in 10). Add 0.5 ml of hydrogen peroxide, and heat in a water bath for 30 minutes. After cooling, add water to make 100 ml, and filter through a dry filter paper. Use 20 ml of the filtrate as the sample solution.

Control Solution Prepare the control solution with 0.20 ml of 0.01 mol/L hydrochloric acid.

(3) Sulfate Not more than 0.96% as SO_4.

Sample Solution Weigh 0.10 g of Calcium Carboxymethylcellulose, add 10 ml of water, stir thoroughly, and add 2 ml of sodium hydroxide solution (1 in 25). Shake, allow to stand for 10 minutes, and make slightly acidic with diluted hydrochloric acid (1 in 4). Add 0.5 ml of hydrogen peroxide, and heat in a water bath for 30 minutes. Use 20 ml of the filtrate as the sample solution.

Control Solution Prepare the control solution with 0.40 ml of 0.005 mol/L sulfuric acid.

(4) Lead Not more than 2.0 μg/g (5.0 g, Method 1).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Loss on Drying Not more than 10.0% (105°, 3 hours).

Residue on Ignition 10.0–20.0% (dried sample, 1 g).

Calcium Chloride

塩化カルシウム

$CaCl_2 \cdot nH_2O$ (n = 2, 1, 1/2, 1/3, or 0)

Mol. Wt. dihydrate 147.01
anhydrous 110.98

Calcium chloride dihydrate [10035-04-8]
Calcium chloride monohydrate
Calcium chloride hemihydrate
Calcium chloride 1/3 hydrate
Calcium chloride [10043-52-4]

Content Calcium Chloride contains not less than 70.0% of calcium chloride ($CaCl_2$).

Description Calcium Chloride occurs as white crystals, powder, flakes, granules, or lumps. It is odorless.

Identification Calcium Chloride responds to all tests for Calcium Salt and for Chloride in the Qualitative Tests.

Purity

(1) Clarity of solution Slightly turbid (1.0 g, water 20 ml).

(2) Free acid and free alkali Weigh 1.0 g of Calcium Chloride, dissolve it in 20 ml of freshly boiled and cooled water, add 2 drops of phenolphthalein TS, and perform the following test with this solution:

(i) If the solution is colorless, add 2.0 ml of 0.02 mol/L sodium hydroxide. A pink color develops.

(ii) If the solution is pink, add 2.0 ml of 0.02 mol/L hydro-

chloric acid. The color disappears.

(3) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(4) Alkali metals and magnesium Not more than 5.0%.

Weigh 1.0 g of Calcium Chloride, dissolve it in 50 ml of water, mix with 0.50 g of ammonium chloride, and boil for 1 minute. Quickly add 40 ml of an oxalic acid solution (3 in 50), stir vigorously to form a precipitate, immediately add 2 drops of methyl red TS, and then add ammonia TS dropwise to make slightly alkaline, and cool. Transfer the solution into a 100-ml measuring cylinder, add water to make 100 ml, allow to stand for 4 hours to overnight, and filter the supernatant through a dried filter paper. Measure 50 ml of the filtrate, add 0.5 ml of sulfuric acid, evaporate to dryness, ignite to constant weight, and weigh the residue.

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

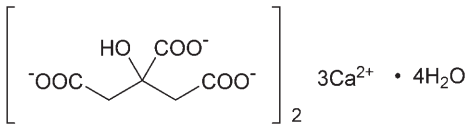
Assay Weigh accurately about 1.5 g of Calcium Chloride, dissolve it in 50 ml of water, and add water to make exactly 100 ml. Perform the test, using this solution as the test solution. Proceed as directed in Method 1 under Calcium Salt Determination.

Each ml of 0.05 mol/L EDTA = 5.549 mg of $CaCl_2$

Calcium Citrate

Tricalcium Citrate

クエン酸カルシウム



$C_{12}H_{10}Ca_3O_{14}·4H_2O$ Mol. Wt. 570.49

Tricalcium bis(2-hydroxypropane-1,2,3-tricarboxylate) tetrahydrate [anhydrous 813-94-5]

Content Calcium Citrate, when dried, contains not less than 97.0% of calcium citrate ($C_{12}H_{10}Ca_3O_{14}$ = 498.43).

Description Calcium Citrate occurs as a white powder. It is odorless.

Identification

(1) Ignite Calcium Citrate at 300–400°C for 1 hour. The residue obtained responds to all tests for Calcium Salt in the Qualitative Tests.

(2) To 0.5 g of Calcium Citrate, add 10 ml of water and 2.5 ml of diluted nitric acid (1 in 10) to dissolve. The solution responds to test (2) for Citrate in the Qualitative Tests.

Purity

(1) Hydrochloric acid-insoluble substances Not more than 0.060%.

Weigh 5.0 g of Calcium Citrate, add 10 ml of hydrochloric acid and 50 ml of water, and heat on a water bath for 30 minutes. Add water to make 200 ml, and filter through a filter paper for quantitative analysis (5C). Wash the residue on the filter paper thoroughly with boiling water, ignite at 300–400°C for 1 hour together with the filter paper, and weight the residue.

(2) pH 5.5–8.0 (5% suspension).

(3) Chloride Not more than 0.007% as Cl.

Test Solution Weigh 1.0 g of Calcium Citrate, add 10 ml of diluted nitric acid (1 in 10), dissolve while heating, cool, and add water to make 50 ml.

Control Solution To 0.20 ml of 0.01 mol/L hydrochloric acid, add 6 ml of diluted nitric acid (1 in 10) and water to make 50 ml.

(4) Sulfate Not more than 0.024% as SO_4.

Test Solution Weigh 1.0 g of Calcium Citrate, add 10 ml of diluted hydrochloric acid (1 in 4), and dissolve while heating. Cool, and add water to make 50 ml.

Control Solution To 0.5 ml of 0.005 mol/L sulfuric acid, add 1 ml of diluted hydrochloric acid (1 in 4) and water to make 50 ml.

(5) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(6) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.50 g of Calcium Citrate, add 5 ml of diluted hydrochloric acid (1 in 4), and dissolve while heating.

Apparatus Use Apparatus B.

Loss on Drying 10.0–14.0% (150°C, 4 hours).

Assay Weigh accurately about 1 g of Calcium Citrate, previously dried, dissolve it in 10 ml of diluted hydrochloric acid (1 in 4), and add water to make exactly 50 ml. Proceed as directed in Method 1 under Calcium Salt Determination, using this solution as the test solution.

Each ml of 0.05 mol/L EDTA = 8.307 mg of $C_{12}H_{10}Ca_3O_{14}$

Calcium Dihydrogen Phosphate

Calcium Phosphate, Monobasic
Monocalcium Phosphate
Primary Calcium Phosphate

リン酸二水素カルシウム

$Ca(H_2PO_4)_2·nH_2O$ (n = 1 or 0) Mol. Wt. monohydrate 252.07
anhydrous 234.05

Calcium bis(dihydrogenphosphate) monohydrate [7758-23-8]

Calcium bis(dihydrogenphosphate)

Content Calcium Dihydrogen Phosphate, when dried, contains 95.0–105.0% of calcium dihydrogen phosphate ($Ca(H_2PO_4)_2$).

Description Calcium Dihydrogen Phosphate occurs as colorless to white crystals or as a white powder.

Identification

(1) Moisten Calcium Dihydrogen Phosphate with silver nitrate solution (1 in 50). A yellow color develops.

(2) To 0.1 g of Calcium Dihydrogen Phosphate, add 20 ml of water, and shake. Filter, and add 5 ml of ammonium oxalate solution (1 in 30). A white precipitate is formed.

Purity

(1) Clarity of solution Very slightly turbid.

Test Solution Weigh 2.0 g of Calcium Dihydrogen Phosphate, add 18 ml of water and 2.0 ml of hydrochloric acid, and dissolve by heating in a water bath for 5 minutes.

(2) Free acid and secondary salt Weigh 1.0 g of Calcium

Dihydrogen Phosphate, add 3 ml of water, and mix well. Add 100 ml of water, shake, and add 1 drop of methyl orange TS. A red color develops. Add 1.0 ml of 1 mol/L sodium hydroxide. The solution turns yellow.

(3) Carbonate Weigh 2.0 g of Calcium Dihydrogen Phosphate, add 5 ml of water, and boil. Cool, and add 2 ml of hydrochloric acid. No effervescence occurs.

(4) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of Calcium Dihydrogen Phosphate, add 5 ml of water and 5 ml of diluted hydrochloric acid (1 in 4), dissolve by heating, and cool. Add ammonia TS until a slight precipitate is formed, dissolve the precipitate by adding a small amount of diluted hydrochloric acid (1 in 4) dropwise, and filter if necessary through a filter paper for quantitative analysis (5C). Add 10 ml of hydrochloric acid–ammonium acetate buffer (pH 3.5) and water to make 50 ml.

Control Solution To 2.0 ml of Lead Standard Solution, add 10 ml of hydrochloric acid-ammonium acetate buffer (pH 3.5) and water to make 50 ml.

(5) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.50 g of Calcium Dihydrogen Phosphate, and dissolve it in 5 ml of diluted hydrochloric acid (1 in 4).

Apparatus Use Apparatus B.

Loss on Drying Not more than 17.0% (180°C, 3 hours).

Assay Weigh accurately about 0.8 g of Calcium Dihydrogen Phosphate, previously dried, dissolve it in 6 ml of diluted hydrochloric acid (1 in 4), and add water to make exactly 200 ml. Proceed as directed in Method 2 under Calcium Salt Determination, using this solution as the test solution.

Each ml of 0.02 mol/L EDTA = 4.681 mg of $Ca(H_2PO_4)_2$

Calcium Dihydrogen Pyrophosphate

Acid Calcium Pyrophosphate
Calcium Acid Pyrophosphate
Calcium Dihyrogen Diphosphate

ピロリン酸二水素カルシウム

$CaH_2P_2O_7$ Mol. Wt. 216.04

Calcium dihydrogendiphosphate [14866-19-4]

Content Calcium Dihydrogen Pyrophosphate, when dried, contains not less than 90.0% of calcium dihydrogen pyrophosphate ($CaH_2P_2O_7$).

Description Calcium Dihydrogen Pyrophosphate occurs as white crystals or powder.

Identification

(1) To 0.5 g of Calcium Dihydrogen Pyrophosphate, add 10 ml of water, and shake. The resulting solution is acidic.

(2) To 0.2 g of Calcium Dihydrogen Pyrophosphate, add 5 ml of diluted nitric acid (1 in 10), and dissolve it by warming. Then add 2 ml of ammonium molybdate TS, and warm. A yellow precipitate is formed.

(3) To 0.3 g of Calcium Dihydrogen Pyrophosphate, add 9 ml of water and 1 ml of diluted hydrochloric acid (1 in 4), dissolve it by warming, cool, and filter. To the filtrate, add 3 ml of ammonium oxalate solution (1 in 30). A white precipitate is formed. The precipitate dissolves on the addition of 5 ml of diluted hydrochloric acid (1 in 30).

Purity

(1) Hydrochloric acid-insoluble substances Not more than 0.40%.

Weigh accurately a glass filter (1G4), previously dried at 110°C for 30 minutes and allowed to cool in a desiccator. Weigh 5.0 g of Calcium Dihydrogen Pyrophosphate, add 100 ml of diluted hydrochloric acid (1 in 4), and allow to stand for 1 hour with occasional shaking. Collect the insoluble substances by filtration with the glass filter, wash with 30 ml of water, and dry at 110°C for 2 hours together with the glass filter. Allow to cool in a desiccator, and weigh accurately the glass filter containing the residue.

(2) Orthophosphate Weigh 1.0 g of Calcium Dihydrogen Pyrophosphate, and add 2–3 drops of silver nitrate solution (1 in 50). No brilliant yellow color develops.

(3) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of Calcium Dihydrogen Pyrophosphate, add 3.5 ml of diluted hydrochloric acid (1 in 4) and 30 ml of water, dissolve by boiling, cool, and filter. To the filtrate, add ammonia TS dropwise while shaking until a slight precipitate is formed, dissolve the precipitate by adding a small amount of diluted hydrochloric acid (1 in 4) dropwise, and filter if necessary through a filter paper for quantitative analysis (5C). Add 10 ml of hydrochloric acid-ammonium acetate buffer (pH 3.5), then add water to make 50 ml.

Control Solution To 2.0 ml of Lead Standard Solution, add 10 ml of hydrochloric acid-ammonium acetate buffer (pH 3.5) and water to make 50 ml.

(4) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.50 g of Calcium Dihydrogen Pyrophosphate, and dissolve it in 5 ml of diluted hydrochloric acid (1 in 4).

Apparatus Use Apparatus B.

Loss on Drying Not more than 5.0% (150°C, 4 hours).

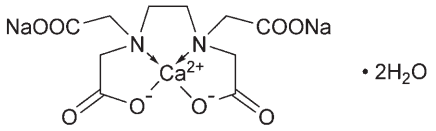
Assay Weigh accurately about 0.7 g of Calcium Dihydrogen Pyrophosphate, previously dried, add 20 ml of diluted hydrochloric acid (1 in 4), and boil. After cooling, add water to make exactly 200 ml, and proceed with the resulting solution, as directed in Method 2 under Calcium Salt Determination.

Each ml of 0.02 mol/L EDTA = 4.321 mg of $CaH_2P_2O_7$

Calcium Disodium Ethylenediaminetetraacetate

Calcium Disodium EDTA

エチレンジアミン四酢酸カルシウム二ナトリウム



$C_{10}H_{12}CaN_2Na_2O_8 \cdot 2H_2O$ Mol. Wt. 410.30
Disodium (ethylenediaminetetraacetato)calciate(2-) dihydrate [anhydrous 62-33-9]

Content Calcium Disodium Ethylenediaminetetraacetate, when calculated on the anhydrous basis, contains 97.0–102.0% of calcium disodium ethylenediaminetetraacetate ($C_{10}H_{12}CaN_2Na_2O_8$ = 374.27).

Description Calcium Disodium Ethylenediaminetetraacetate occurs as a white to whitish crystalline powder or granules. It is odorless and has a slightly salty taste.

Identification

(1) A solution of Calcium Disodium Ethylenediaminetetraacetate (1 in 20) responds to test (2) for Calcium Salt and to all tests for Sodium Salt in the Qualitative Tests.

(2) Add 0.05 g of Calcium Disodium Ethylenediaminetetraacetate to 5 ml of water, previously mixed with 2 drops of ammonium thiocyanate solution (2 in 25) and 2 drops of iron(III) chloride solution (1 in 10). The red color of the solution disappears.

Purity

(1) pH 6.5–8.0.

Test Solution Weigh 1.0 g of Calcium Disodium Ethylenediaminetetraacetate, and dissolve it in water to make 15 ml.

(2) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

(4) Magnesium-chelating substance Weigh 1.0 g of Calcium Disodium Ethylenediaminetetraacetate, dissolve it in 5 ml of water, add 5 ml of ammonia–ammonium chloride buffer (pH 10.7), and titrate with 0.1 mol/L magnesium acetate (indicator: 5 drops of eriochrome black T TS). The volume consumed is not more than 2.0 ml.

Water Content Not more than 13.0% (0.3 g, Direct Titration).

Assay Weigh accurately about 1 g of Calcium Disodium Ethylenediaminetetraacetate into a 250-ml volumetric flask, and dissolve it in water to make exactly 250 ml. Measure exactly 25 ml of this solution, adjust the pH to about 2 with diluted nitric acid (1 in 10), and titrate with 0.01 mol/L bismuth nitrate (indicator: 3 drops of xylenol orange TS) until a red color develops. Calculate on the anhydrous basis.

Each ml of 0.01 mol/L bismuth nitrate = 3.743 mg of $C_{10}H_{12}CaN_2Na_2O_8$

Calcium Ferrocyanide

Calcium Hexacyanoferrate(II)

フェロシアン化カルシウム

$Ca_2[Fe(CN)_6] \cdot 12H_2O$ Mol. Wt. 508.29
Calcium hexacyanoferrate(II) dodecahydrate [anhydrous 13821-08-4]

Content Calcium Ferrocyanide contains not less than 99.0% of calcium ferrocyanide ($Ca_2[Fe(CN)_6] \cdot 12H_2O$).

Description Calcium Ferrocyanide occurs as yellow crystals or crystalline powder.

Identification

(1) Proceed as directed in Identification (1) for Potassium Ferrocyanide.

(2) Calcium Ferrocyanide responds to all tests for Calcium Salt in the Qualitative Tests.

Purity

(1) Cyanide Proceed as directed in Purity (1) for Potassium Ferrocyanide.

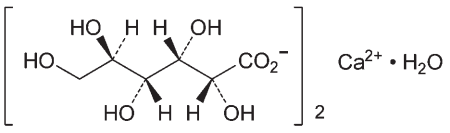
(2) Ferricyanide Proceed as directed in Purity (2) for Potassium Ferrocyanide.

Assay Weigh accurately about 1 g of Calcium Ferrocyanide, and dissolve it in 200 ml of water. To this solution, add 10 ml of sulfuric acid, and titrate with 0.02 mol/L potassium permanganate. The endpoint is when the red color of the solution persists for 30 seconds.

Each ml of 0.02 mol/L potassium permanganate = 50.83 mg of $Ca_2[Fe(CN)_6] \cdot 12H_2O$

Calcium Gluconate

グルコン酸カルシウム



$C_{12}H_{22}CaO_{14} \cdot H_2O$ Mol. Wt. 448.39
Monocalcium bis(D-gluconate) monohydrate [anhydrous 99-28-5]

Content Calcium Gluconate, when dried, contains 98.0–104.0% of calcium gluconate ($C_{12}H_{22}CaO_{14} \cdot H_2O$).

Description Calcium Gluconate occurs as a white crystalline powder or granular powder. It is odorless and tasteless.

Identification

(1) To 1 ml of a solution of Calcium Gluconate (1 in 40), add 1 drop of iron(III) chloride solution (1 in 10). A dark yellow color develops.

(2) Measure 5 ml of a solution of Calcium Gluconate in warm water (1 in 10), and proceed as directed in Identification (2) for Glucono-δ-Lactone.

(3) A solution of Calcium Gluconate (1 in 40) responds to all tests for Calcium Salt in the Qualitative Tests.

Purity

(1) Clarity of solution Almost clear.

Test Solution Weigh 1.0 g of Calcium Gluconate, add 20 ml of water, and dissolve by warming to 60°C.

(2) pH 6.0–8.0. Measure the pH of the following solution: Add 20 ml of water to 1.0 g Calcium Glunonate, dissolve by heating at 60°C, and cool.

(3) Chloride Not more than 0.071% (0.30 g, Control solution 0.01 mol/L hydrochloric acid 0.60 ml).

(4) Sulfate Not more than 0.048% (0.50 g, Control solution 0.005 mol/L sulfuric acid 0.50 ml).

(5) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(6) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.50 g of Calcium Gluconate, add 5 ml of water, and dissolve while warming. Add 5 ml of sulfuric acid (3 in 50) and 1 ml of Bromine TS, and concentrate to 5 ml by heating on a water bath.

Apparatus Use Apparatus B.

(7) Sucrose or reducing sugars Proceed as directed in Purity (6) for Glucono-δ-Lactone.

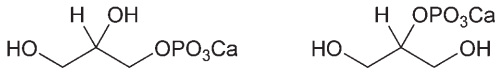
Loss on Drying Not more than 0.50% (80°C, 2 hours).

Assay Weigh accurately about 2.5 g of Calcium Gluconate, previously dried, dissolve it in 25 ml of diluted hydrochloric acid (1 in 4), and add water to make exactly 50 ml. Using this solution, proceed as directed in Method 1 under Calcium Salt Determination.

Each ml of 0.05 mol/L EDTA = 22.42 mg of $C_{12}H_{22}CaO_{14}{\cdot}H_2O$

Calcium Glycerophosphate

グリセロリン酸カルシウム



$C_3H_7CaO_6P$ Mol. Wt. 210.14

Mixture of monocalcium 2,3-dihydroxylpropanyl phosphate and monocalcium 1,3-dihydroxypropan-2-yl phosphate [27214-00-2]

Content Calcium Glycerophosphate, when calculated on the dried basis, contains not less than 98.0% of calcium glycerophosphate ($C_3H_7CaO_6P$).

Description Calcium Glycerophosphate occurs as a white powder. It is odorless and has a slightly bitter taste.

Identification To 1 g of Calcium Glycerophosphate, add 10 ml of water of 5°C or below, and shake well. Use this solution as the test solution.

(1) Boil the test solution. White crystals are deposited.

(2) To 3 ml of the test solution, add 2–3 drops of lead acetate TS. A white, curd-like precipitate is formed. The precipitate dissolves on the addition of 3 ml of nitric acid.

(3) The test solution responds to all tests for Calcium Salt and for Glycerophosphate in the Qualitative Tests.

Purity

(1) Clarity of solution Very slightly turbid (1.0 g, water 50 ml).

(2) Ethanol-soluble substance Not more than 1.0%.

Weigh 1.0 g of Calcium Glycerophosphate, add 25 ml of absolute ethanol, shake, and filter. Evaporate the filtrate on a water bath, dry the residue at 60°C for 1 hour, and weight the mass.

(3) Free alkali Weigh 1.0 g of Calcium Glycerophosphate, dissolve it in 60 ml of water, add 5 drops of phenolphthalein TS, and titrate with 0.05 mol/L sulfuric acid. The consumed volume is not more than 1.5 ml.

(4) Chloride Not more than 0.071% as Cl (0.25 g, Control solution 0.01 mol/L hydrochloric acid 0.50 ml).

(5) Sulfate Not more than 0.048% as SO_4 (0.50 g, Control solution 0.005 mol/L sulfuric acid 0.50 ml).

(6) Phosphate Not more than 0.040% as PO_4.

Weigh 1.0 g of Calcium Glycerophosphate, dissolve it in 10 ml of diluted nitric acid (1 in 10), add 10 ml of cold ammonium molybdate TS, and allow to stand for 10 minutes. The solution is not more turbid than a control solution prepared as directed below .

Control Solution Weigh 0.192 g of monopotassium phosphate, dissolve it in 100 ml of water, measure 3.0 ml of this solution, add diluted nitric acid (1 in 10) to make 100 ml. Measure 10 ml of this solution, add 10 ml of cold ammonium molybdate TS, and allow to stand for 10 minutes.

(7) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 0.50 g of Calcium Glycerophosphate, dissolve it in 3 ml of diluted acetic acid (1 in 20), and add water to make 50 ml.

Control Solution To 1.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(8) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 1.0 g of Calcium Glycerophosphate, dissolve it in 25 ml of water, add 1 ml of sulfuric acid and 10 ml of sulfurous acid, evaporate to about 2 ml, and add water to make 10 ml. Use 5 ml of this solution as the test solution.

Apparatus Use Apparatus B.

Loss on Drying Not more than 13.0% (0.5g, 150°C, 4 hours).

Assay Weigh accurately about 1 g of Calcium Glycerophosphate, previously dried, dissolve it in 10 ml of diluted hydrochloric acid (1 in 4), and add water to make exactly 50 ml. Using this solution as the test solution, proceed as directed in Method 1 under Calcium Salt Determination.

Each ml of 0.05 mol/L EDTA = 10.51 mg of $C_3H_7CaO_6P$

Calcium Hydroxide

Slaked Lime

水酸化カルシウム

$Ca(OH)_2$ Mol. Wt. 74.09

Calcium hydroxide [1305-62-0]

Content Calcium Hydroxide contains not less than 95.0% of calcium hydroxide ($Ca(OH)_2$).

Description Calcium Hydroxide occurs as a white powder.

Identification

(1) To Calcium Hydroxide, add 3–4 times as much water as the sample amount. It becomes muddy and alkaline.

(2) To 1 g of Calcium Hydroxide, add 20 ml of water and

6 ml of diluted acetic acid (1 in 3) to dissolve. The solution responds to all tests for Calcium Salt in the Qualitative Tests.

Purity

(1) Hydrochloric acid-insoluble substances Not more than 0.50%.

Weigh 2.0 g of Calcium Hydroxide, add 10 ml of hydrochloric acid and 20 ml of water to dissolve, and boil. After cooling, add water to make 200 ml, filter through a filter paper for quantitative analysis (5C), wash the residue on the filter paper with boiling water until the washings are free from chloride. Incinerate the residue with the filter paper, and weigh the residue.

(2) Carbonate Weigh 2.0 g of Calcium Hydroxide, add 50 ml of water, shake well, and add 25 ml of diluted hydrochloric acid (1 in 4). No remarkable effervescence occurs.

(3) Heavy metals Not more than 40 μg/g as Pb.

Test Solution Weigh 0.50 g of Calcium Hydroxide, dissolve it in 10 ml of diluted hydrochloric acid (1 in 4), and evaporate to dryness on a water bath. To the residue, add 2 ml of diluted acetic acid (1 in 20) and 20 ml of water, filter if necessary, and add water to make 50 ml.

Control Solution To 2.0 ml of Lead Standard Solution, measured exactly, add 2.0 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(4) Alkali metals and magnesium Not more than 6.0%.

Weigh 0.50 g of Calcium Hydroxide, dissolve it in 30 ml of diluted hydrochloric acid (1 in 10), and boil for 1 minute. Quickly add 40 ml of oxalic acid solution (3 in 50), and proceed as directed in Purity (4) for Calcium Chloride.

(5) Barium Not more than 0.030% as Ba.

Test Solution Weigh 1.50 g of Calcium Hydroxide, dissolve it in 15 ml of diluted hydrochloric acid (1 in 4), add water to make 30 ml, and filter. Use 20 ml of the filtrate as the test solution.

Procedure To the test solution, add 2 g of sodium acetate, 1 ml of diluted acetic acid (1 in 20), and 0.5 ml of potassium chromate solution (1 in 20), and allow to stand for 15 minutes. The solution is not more turbid than a control solution prepared as follows: To 0.30 ml of Barium Standard Solution, add water to make 20 ml, and then treat in the same manner as the test solution.

(6) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.50 g of Calcium Hydroxide, dissolve it in 5 ml of diluted hydrochloric acid (1 in 4).

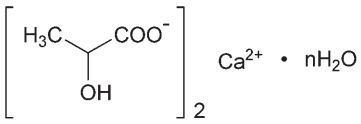
Apparatus Use Apparatus B.

Assay Weigh accurately about 2 g of Calcium Hydroxide, dissolve it in 30 ml of diluted hydrochloric acid (1 in 4), and add water to make exactly 250 ml. Proceed as directed in Method 1 under Calcium Salt Determination, using this solution as the test solution.

Each ml of 0.05 mol/L EDTA = 3.705 mg of $Ca(OH)_2$

Calcium Lactate

乳酸カルシウム



(n=5, 3, 1, or 0)

$C_6H_{10}CaO_6 \cdot nH_2O$ (n=5, 3, 1, or 0)

Mol. Wt. pentahydrate 308.29
anhydrous 218.22

Monocalcium bis(2-hydroxypropanoate) pentahydrate [5743-47-5]

Monocalcium bis(2-hydroxypropanoate) trihydrate [139061-06-6]

Monocalcium bis(2-hydroxypropanoate) monohydrate

Monocalcium bis(2-hydroxypropanoate) [814-80-2]

Content Calcium Lactate, when calculated on the dried basis, contains 97.0–101.0% of calcium lactate ($C_6H_{10}CaO_6$).

Description Calcium Lactate occurs as a white powder or granules. It is odorless or has a slight, characteristic odor.

Identification A solution of Calcium Lactate (1 in 20) responds to all tests for Calcium Salt and for Lactate in the Qualitative Tests.

Purity

(1) Clarity and color of solution Colorless and clear.

Test Solution Weigh 1.0 g of Calcium Lactate, add 20 ml of water, and dissolve by heating in a water bath.

(2) pH 6.0–8.0.

Test Solution Weigh 1.0 g of Calcium Lactate, add 20 ml of water, dissolve by heating in a water bath, and cool.

(3) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of Calcium Lactate, add 2 ml of diluted acetic acid (1 in 20) and about 35 ml of water, dissolve by heating on a water bath, and add water to make 50 ml.

Control Solution To 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(4) Alkali metals and magnesium Not more than 1.0%.

Weigh 1.0 g of Calcium Lactate, dissolve it in about 40 ml of water, add 0.5 g of ammonium chloride, and boil. Add about 20 ml of ammonium oxalate solution (1 in 25), heat on a water bath for 1 hour, cool, add water to make 100 ml, and filter. Measure 50 ml of the filtrate, add 0.5 ml of sulfuric acid, evaporate to dryness, ignite at 450–550°C to constant weigh, and weigh the residue.

(5) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.50 g of Calcium Lactate, and add 2 ml of water and 3 ml of hydrochloric acid to dissolve.

Apparatus Use Apparatus B.

(6) Volatile fatty acid Weigh 0.5 g of Calcium Lactate, add 1 ml of sulfuric acid, and heat in a water bath. No butyric acid-like odor is evolved.

Loss on Drying Not more than 30.0% (120°C, 4 hours).

Assay Weigh accurately about 2 g of Calcium Lactate, dissolve it in 20 ml of diluted hydrochloric acid (1 in 4), and add water to make exactly 100 ml. Using this solution as the

test solution, proceed as directed in Method 1 under Calcium Salt Determination, and calculate on the dried basis.

Each ml of 0.05 mol/L EDTA = 10.91 mg of $C_6H_{10}CaO_6$

Calcium Monohydrogen Phosphate

Calcium Phosphate, Dibasic
Dicalcium Phosphate
Secondary Calcium Phosphate

リン酸一水素カルシウム

$CaHPO_4 \cdot nH_2O$ (n=2, 1½, 1, ½, or 0)

Mol. Wt. dihydrate 172.09
anhydrous 136.06

Calcium hydrogenphosphate dihydrate [7789-77-7]
Calcium hydrogenphosphate sesquihydrate
Calcium hydrogenphosphate monohydrate
Calcium hydrogenphosphate hemihydrate
Calcium hydrogenphosphate [7757-93-9]

Content Calcium Monohydrogen Phosphate, when dried, contains 98.0–103.0% of calcium monohydrogen phosphate ($CaHPO_4$).

Description Calcium Monohydrogen Phosphate occurs as white crystals or powder.

Identification

(1) Moisten Calcium Monohydrogen Phosphate with silver nitrate solution (1 in 50). A yellow color develops.

(2) To 0.1 g of Calcium Monohydrogen Phosphate, add 5 ml of diluted acetic acid (1 in 4), boil, cool, and filter. To the filtrate, add 5 ml of ammonium oxalate solution (1 in 30). A white precipitate is formed.

Purity

(1) Clarity of solution Very slightly turbid.

Test Solution Weigh 2.0 g of Calcium Monohydrogen Phosphate, add 16 ml of water and 4.0 ml of hydrochloric acid, and dissolve by heating in a water bath for 5 minutes.

(2) Carbonate Weigh 2.0 g of Calcium Monohydrogen Phosphate, add 5 ml of water, and boil. Cool, and add 2 ml of hydrochloric acid. No effervescence occurs.

(3) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of Calcium Monohydrogen Phosphate, add 5 ml of water and 5 ml of diluted hydrochloric acid (1 in 4), and dissolve by heating. After cooling, add ammonia TS until a slight precipitate is formed, dissolve the precipitate by adding a small amount of diluted hydrochloric acid (1 in 4) dropwise, and filter if necessary through a filter paper for quantitative analysis (5C). Add 10 ml of hydrochloric acid–ammonium acetate buffer (pH 3.5) and water to make 50 ml.

Control Solution To 2.0 ml of Lead Standard Solution, add 10 ml of hydrochloric acid–ammonium acetate buffer (pH 3.5) and water to make 50 ml.

(4) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.50 g of Calcium Monohydrogen Phosphate, and dissolve it in 5 ml of diluted hydrochloric acid (1 in 4).

Apparatus Use Apparatus B.

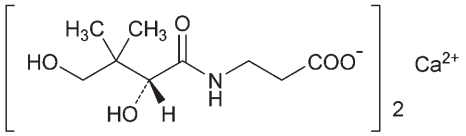
Loss on Drying Not more than 22.0% (200°C, 3 hours).

Assay Weigh accurately about 0.4 g of Calcium Monohydrogen Phosphate, previously dried, dissolve it in 12 ml of diluted hydrochloric acid (1 in 4), and add water to make exactly 200 ml. Proceed as directed in Method 2 under Calcium Salt Determination, using this solution as the test solution.

Each ml of 0.02 mol/L EDTA = 2.721 mg of $CaHPO_4$

Calcium Pantothenate

パントテン酸カルシウム



$C_{18}H_{32}CaN_2O_{10}$ Mol. Wt. 476.53

Monocalcium bis{3-[(2*R*)-2,4-dihydroxy-3,3-dimethylbutanoylamino]propanoate} [137-08-6]

Content Calcium Pantothenate, when calculated on the dried basis, contains 5.7–6.0% of nitrogen (N = 14.01) and 8.2–8.6% of calcium (Ca = 40.08).

Description Calcium Pantothenate occurs as a white powder. It is odorless and has a slightly bitter taste.

Identification

(1) Dissolve 0.05 g of Calcium Pantothenate in 5 ml of sodium hydroxide solution (1 in 25), and add 1 drop of cupric sulfate solution (1 in 10). A blue-purple color develops.

(2) To 0.05 g of Calcium Pantothenate, add 5 ml of sodium hydroxide solution (1 in 25), and boil for 1 minute. Cool, and add 2 ml of diluted hydrochloric acid (1 in 4) and 2 drops of iron(III) chloride solution (1 in 10). A dark yellow color develops.

(3) Calcium Pantothenate solution (1 in 20) responds to all tests for Calcium Salt in the Qualitative Tests.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +25.0 to +28.5° (after dried, 1.25 g, water, 25 ml).

(2) pH 7.0–9.0.

Test Solution Weigh 2.0 g of Calcium Pantothenate, and add water to make 10 ml.

(3) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

(5) Alkaloid Weigh 0.050 g of Calcium Pantothenate, dissolve it in 5 ml of water, and add 0.5 ml of ammonium molybdate TS and 0.5 ml of phosphoric acid (1 in 10). No white turbidity appears.

Loss on Drying Not more than 5.0% (105°C, 3 hours).

Assay

(1) Nitrogen Weigh accurately about 0.05 g of Calcium Pantothenate, proceed as directed in the Semi-micro Kjeldahl Method under Nitrogen Determination, and calculate on the dried basis.

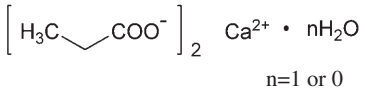
(2) Calcium Weigh accurately about 2.5 g of Calcium Pantothenate, add 5 ml of diluted hydrochloric acid (1 in 4)

and 20 ml of water to dissolve, and then add water to make exactly 50 ml. Proceed as directed in Method 1 under Calcium Salt Determination, using this solution as the test solution, and calculate on the dried basis.

Each ml of 0.05 mol/L EDTA = 2.004 mg of Ca

Calcium Propionate

プロピオン酸カルシウム



n=1 or 0

$C_6H_{10}CaO_4 \cdot nH_2O$ (n=1 or 0) Mol. Wt. monohydrate 204.23
anhydrous 186.22

Monocalcium dipropanoate monohydrate
Monocalcium dipropanoate [4075-81-4]

Content Calcium Propionate, when dried, contains not less than 98.0% of calcium propionate ($C_6H_{10}CaO_4$).

Description Calcium Propionate occurs as white crystals, powder or granules. It is odorless or has a slight, characteristic odor.

Identification

(1) To 5 ml of a solution of Calcium Propionate (1 in 10), add 5 ml of diluted sulfuric acid (1 in 10), and heat. A characteristic odor is evolved.

(2) Calcium Propionate responds to all tests for Calcium Salt in the Qualitative Tests.

Purity

(1) Water-insoluble substances Not more than 0.30%.

Weigh 10.0 g of Calcium Propionate, add 100 ml of water, and allow to stand for 1 hour with occasional shaking. Filter the insoluble substances through a glass filter (1G4), wash with 30 ml of water, and dry at 180°C for 4 hours, and weigh the residue.

(2) Free acid and free alkali Weigh 2.0 g of Calcium Propionate, dissolve it in 20 ml of freshly boiled and cooled water, and add 2 drops of phenolphthalein TS and 0.30 ml of 0.1 mol/L hydrochloric acid. The solution is colorless. On the addition of 0.6 ml of 0.1 mol/L sodium hydroxide, the color of the solution changes to red.

(3) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Drying Not more than 9.5% (120°C, 2 hours).

Assay Weigh accurately about 1 g of Calcium Propionate, previously dried, and dissolve it in water to make exactly 100 ml. Measure exactly 25 ml of this solution, add 75 ml of water and 15 ml of sodium hydroxide solution (1 in 10), and allow to stand for about 1 minute. Add 0.1 g of NN indicator, and immediately titrate with 0.05 mol/L EDTA until the red color completely disappears and the solution turns blue.

Each ml of 0.05 mol/L EDTA = 9.311 mg of $C_6H_{10}CaO_4$

Calcium 5′-Ribonucleotide

5′-リボヌクレオチドカルシウム

Definition Calcium 5′-Ribonucleotide is a mixture of calcium 5′-inosinate, calcium 5′-guanylate, calcium 5′-cytidylate, and calcium 5′-uridylate, or a mixture of calcium 5′-inosinate and calcium 5′-guanylate.

Content Calcium 5′-Ribonucleotide, when calculated on the anhydrous basis, contains 97.0–102.0% of calcium 5′-ribonucleotide, of which not less than 95.0% consists of calcium 5′-inosinate and calcium 5′-guanylate.

Description Calcium 5′-Ribonucleotide occurs as white to whitish crystals or powder. It is odorless and has a slight, characteristic taste.

Identification

(1) To 0.1 g of Calcium 5′-Ribonucleotide, add 200 ml of water, and dissolve by heating in a water bath, and cool. To 1 ml of resulting solution, add 0.2 ml of a solution of orcinol in ethanol (1 in 10), then add 3 ml of a solution of ferric ammonium sulfate in hydrochloric acid (1 in 1,000), and heat in a water bath for 10 minutes. A green color develops.

(2) Dissolve 0.1 g of Calcium 5′-Ribonucleotide in 200 ml of diluted hydrochloric acid (1 in 4). To 2 ml of the resulting solution, add 0.1 g of zinc dust, and proceed as directed in Identification (2) for Disodium 5′-Ribonucleotide.

(3) To 0.1 g of Calcium 5′-Ribonucleotide, add 500 ml of water, dissolve by heating in a water bath, and cool. To 1 ml of the resulting solution, add 1 ml of diluted hydrochloric acid (1 in 4), and heat in a water bath for 10 minutes. Cool, and add 0.5 ml of Folin's TS and 2 ml of sodium carbonate saturated solution. A blue color develops.

(4) To 0.1 g of Calcium 5′-Ribonucleotide, add 5 ml of water and 5 ml of nitric acid, boil gently for 10 minutes. Cool, and neutralize with ammonia solution or ammonia TS. The solution responds to test (2) for Phosphate in the Qualitative Tests.

(5) To 0.1 g of Calcium 5′-Ribonucleotide, add 200 ml of water, dissolve by heating in a water bath, and cool. The solution responds to all tests for Calcium Salt in the Qualitative Tests.

Purity

(1) pH 7.0–8.0.

Test Solution Weigh 0.10 g of Calcium 5′-Ribonucleotide, add 200 ml of water, dissolve while heating in a water bath, and cool.

(2) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of Calcium 5′-Ribonucleotide, transfer into a crucible, cover the sample with 1 g of ammonium sulfate, and add 0.5 ml of water. Carbonize by heating gently, add 3 drops of sulfuric acid and 3 drops of nitric acid when white fumes are no longer evolved, and incinerate by heating gradually. After cooling, add 1 ml of hydrochloric acid and 0.2 ml of nitric acid, and evaporate to dryness on a water bath. Repeat this procedure three times. To the residue, add 1 ml of diluted hydrochloric acid (1 in 4) and 15 ml of water, and heat on a water bath for 10 minutes. After cooling, add 1 drop of phenolphthalein TS, and add ammonia TS dropwise until a slightly pink color develops. Add 2 ml of diluted acetic acid (1 in 20), and filter. Wash the residue on the filter paper with a small amount of water, combine the

filtrate and the washings, and add water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, transfer into a crucible, and then proceed as directed for the test solution.

(3) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.50 g of Calcium 5′-Ribonucleotide, and dissolve it in 5 ml of diluted hydrochloric acid (1 in 4).

Apparatus Use Apparatus B.

(4) Water-soluble substances Not more than 16%.

Weigh 1.0 g of Calcium 5′-Ribonucleotide, add 50 ml of water, allow to stand for 10 minutes with occasional shaking, and filter through a dry filter paper for quantitative analysis (5C). Measure 25 ml of the filtrate, and evaporate to dryness. Dry the residue at 105°C for 1 hour, and weigh.

Water Content Not more than 23.0% (0.15 g, Back Titration).

Before titrating, add water determination TS in excess, and stir for 20 minutes.

Assay Calculate the content of calcium 5′-ribonucleotide and the total content of calcium 5′-inosinate ($C_{10}H_{11}CaN_4O_8P$) and calcium 5′-guanylate ($C_{10}H_{12}CaN_5O_8P$) by the formulae from the values of I_{Ca}, G_{Ca}, and P_{Ca} obtained in (1), (2), and (3) below.

$$\text{Content (\%) of calcium 5′-ribonucleotide} = \frac{I_{Ca} + G_{Ca} + P_{Ca}}{100 - \text{Water content(\%)}} \times 100$$

$$\text{Content (\%) of calcium 5′-inosinate } (C_{10}H_{11}CaN_4O_8P) \text{ and calcium 5′-guanylate } (C_{10}H_{12}CaN_5O_8P) = \frac{I_{Ca} + G_{Ca}}{100 - \text{Water content(\%)}} \times 100$$

(1) Calcium 5′-inosinate Weigh accurately about 0.65 g of Calcium 5′-Ribonucleotide, dissolve it in diluted hydrochloric acid (1 in 100) to make exactly 500 ml, and use this solution as the sample solution. Proceed as directed in Assay (1) for Disodium 5′-Ribonucleotide to determine the content of disodium 5′-inosinate ($C_{10}H_{11}N_4Na_2O_8P$). Multiply the content (%) by 0.985 to determine the content I_{Ca} (%) of calcium 5′-inosinate ($C_{10}H_{11}CaN_4O_8P$).

(2) Calcium 5′-guanylate Measure accurately 1 ml of the sample solution obtained in (1) above, and proceed as directed in Assay (2) for Disodium 5′-Ribonucleotide to determine the content of disodium 5′-guanylate ($C_{10}H_{12}N_5Na_2O_8P$). Multiply the content (%) by 0.986 to determine the content G_{Ca} (%) of calcium 5′-guanylate ($C_{10}H_{12}CaN_5O_8P$).

(3) Calcium 5′-cytidylate and calcium 5′-uridylate Weigh accurately about 1.5 g of Calcium 5′-Ribonucleotide, dissolve it in 10 ml of diluted hydrochloric acid (1 in 10), add 1 ml of monosodium phosphate solution (3 in 5), adjust the pH to 7.0 with sodium hydroxide solution (1 in 25), and filter. Wash the residue on the filter paper with 10 ml of water, combine the filtrate and the washings, and add water to make exactly 50 ml. Use this solution as the sample solution. Proceed as directed in Assay (3) for Disodium 5′-Ribonucleotide to determine the content of disodium 5′-cytidylate ($C_9H_{12}N_3Na_2O_8P$) and disodium 5′-uridylate ($C_9H_{11}N_2Na_2O_9P$). Multiply the content (%) by 0.984 to obtain the total content P_{Ca} (%) of calcium 5′-cytidylate ($C_9H_{12}CaN_3O_8P$) and calcium 5′-uridylate ($C_9H_{11}CaN_2O_9P$).

Calcium Stearate

ステアリン酸カルシウム

Definition Calcium Stearate is a mixture of calcium salts consisting principally of stearic acid and palmitic acid.

Content Calcium Stearate, when calculated on the dried basis, contains 6.4–7.1% of calcium (Ca = 40.08).

Description Calcium Stearate occurs as a white, light, bulky powder. It is odorless or has a faint, characteristic odor.

Identification

(1) To 3.0 g of Calcium Stearate, add 20 ml of diluted hydrochloric acid (1 in 2) and 30 ml of diethyl ether, shake vigorously for 3 minutes, and allow to stand. The separated water layer responds to test (1) for calcium in the Qualitative Tests.

(2) Collect the diethyl ether layer obtained in Identification (1), and wash sequentially with a 20-ml potion of dilute hydrochloric acid, a 10 ml-portion of dilute hydrochloric acid, and a 20-ml potion of water. Evaporate the diethyl ether on a water bath. The melting point of the residue is not lower than 54°C.

Purity

(1) Heavy metals Not more than 10 μg/g as Pb.

Test Solution Take 1.0 g of Calcium Stearate, carefully heat gently at first, and continue to heat by gradually rising the temperature to incineration. After cooling, add 2 ml of hydrochloric acid, and evaporate on a water bath to dryness. To the residue, add 20 ml of water and 2 ml of dilute acetic acid, warm for 2 minutes, cool, and filter. Wash the residue with 15 ml of water, combine the filtrate and washings, and add water to make 50 ml.

Control Solution Evaporate 2 ml of hydrochloric acid on a water bath to dryness, and add 2 ml of dilute acetic acid, 1.0 ml of Standard Lead Solution, and water to make 50 ml.

(2) Arsenic Not more than 4.0 μg/g.

Test Solution To 0.50 g of Calcium Stearate, add 5 ml of hydrochloric acid (1 in 2) and 20 ml of chloroform, shake vigorously for 3 minutes, allow to stand, and collect the water layer.

Apparatus Use Apparatus B.

Procedure Perform the test as directed in the Arsenic Limit Test.

(3) Free fatty acids Not more than 3.0% as stearic acid.

Weigh accurately about 2 g of Calcium Stearate into a 100-ml Erlenmeyer flask, and add 50 ml of acetone. Heat under a condenser for 10 minutes in a water bath, and cool. Filter it through two-ply paper filter for quantitative analysis (5C), wash the inside of the flask, the residue, and the filter paper with 50 ml of acetone, and combine the washings with the filtrate. Add 2–3 drops of phenolphthalein TS and 5 ml of water, and titrate with 0.1 mol/L sodium hydroxide. Perform a blank test using a mixture of 100 ml of acetone and 5 ml of water.

Each ml of 0.1 mol/L sodium hydroxide = 28.45 mg $C_{18}H_{36}O_2$

Loss on Drying Not more than 4.0% (105°C, 3 hours).

Assay Weigh accurately about 0.5 g of Calcium Stearate in a crucible. Carefully heat gently at first, and then ignite in an electric furnace at 700°C for 3 hours to incineration. After cooling, add 10 ml of hydrochloric acid to the residue, and

warm on a water bath for 10 minutes. Transfer the contents of the crucible into a flask with the aid of two 10-ml potions of warm water and one 5-ml potion of warm water. Add sodium hydroxide TS until the solution is slightly turbid. Next, add 25 ml of 0.05 mol/L EDTA, 10 ml of ammonia–ammonium chloride buffer solution (pH 10.7), 4 drops of eriochrome black T TS, and 5 drops of methyl yellow TS. Immediately titrate the excess EDTA with 0.05 mol/L magnesium chloride. The endpoint is when the green color of the solution disappears and a red color is produced. Separately, perform a blank test.

Each ml of 0.05 mol/L EDTA = 2.004 mg of Ca

Calcium Stearoyl Lactylate

Calcium Stearoyl-2-lactylate

ステアロイル乳酸カルシウム

[5793-94-2]

Definition Calcium Stearoyl Lactylate is a mixture of calcium salts of stearoyl lactylic acids and minor proportions of related acids and calcium salts of these related acids. Its principal component is calcium salts of stearoyl lactylic acids.

Description Calcium Stearoyl Lactylate occurs as a white to yellowish powder or solid. It is odorless or has a characteristic odor.

Identification

(1) Ignite 1 g of Calcium Stearoyl Lactylate at 500°C for 1 hour, and dissolve the residue obtained in 5 ml of diluted hydrochloric acid (1 in 4). The solution responds to all tests for Calcium Salt in the Qualitative Tests.

(2) To 2 g of Calcium Stearoyl Lactylate, add 10 ml of diluted hydrochloric acid (1 in 4), stir thoroughly, heat in a water bath, and filter while hot. Collect the residue on the filter paper, add 30 ml of sodium hydroxide solution (1 in 25), and heat in a water bath at a temperature of not less than 95°C for 30 minutes while stirring. After cooling, add 20 ml of diluted hydrochloric acid (1 in 4), and extract twice with 30 ml of diethyl ether each time. Combine the diethyl ether extracts, wash with 20 ml of water, then dehydrate with anhydrous sodium sulfate, and filter. Heat the filtrate on a water bath, remove the diethyl ether by evaporation, and measure the melting point of the residue. The melting point is 54–69°C.

(3) Calcium Stearoyl Lactylate responds to the test for Lactate in the Qualitative Tests.

Purity

(1) Acid value 50–86.

Test Solution Weigh about 0.5 g of powdered Calcium Stearoyl Lactylate, and dissolve it in 20 ml of a 1:1 mixture of ethanol/diethyl ether mixture.

Procedure Proceed as directed in the Acid Value Test in the Fats and Related Substances Tests. The endpoint is when a pink color persists for 20 seconds.

(2) Ester value 125–164 (Fats and Related Substances Tests).

For the acid value, use the value measured in Purity (1). For the saponification value, weigh accurately about 1 g of Calcium Stearoyl Lactylate, and proceed as directed in the Saponification Value Test in the Fats and Related Substances Tests. In the saponification value test, take care to avoid the adhesion of the precipitate to the wall of the flask when ethanolic potassium hydroxide TS is added, and perform the titration while the solution is hot.

(3) Total lactic acid 32–38% as lactic acid ($C_3H_6O_3$).

Test Solution Transfer 0.2 g of Calcium Stearoyl Lactylate, accurately weighed, into a 100-ml flask, add 10 ml of ethanolic potassium hydroxide TS and 10 ml of water, and heat under a reflux condenser in a water bath for 45 minutes. Rinse the flask and condenser with 40 ml of water, add the rinses to the flask, and heat until the amount of the solution is not more than one-third of the original volume. Mix with 6 ml of diluted sulfuric acid (1 in 2), add 25 ml of petroleum ether, shake well, transfer the entire amount to a separating funnel, and allow to stand to let it separate into two layers. Transfer the water layer to a 100-ml volumetric flask, wash the petroleum ether layer twice with 20 ml of water each time, adding the washings to the volumetric flask, and then add water to make exactly 100 ml. Measure accurately 1 ml of this solution, add water to make exactly 100 ml.

Calibration Curve Measure exactly 5 ml, 7 ml, and 10 ml of Lithium Lactate Standard Solution, and add water to each to make exactly 100 ml. Transfer exactly 1 ml of each solution into separate test tubes with a stopper, measure the absorbances of them in the same manner as for the test solution, and prepare a calibration curve.

Procedure Measure accurately 1 ml of the test solution, place into a test tube with a stopper, add 1 drop of cupric sulfate solution (1 in 8), and mix. Rapidly add 9 ml of sulfuric acid, place the stopper loosely, heat for exactly 5 minutes in a water bath at 90°C, and immediately cool to 20°C in ice water. Add 0.2 ml of *p*-phenylphenol TS, shake well, and keep in a water bath at 30°C for 30 minutes. During that time, shake the contents two or three times. Heat in a water bath at 90ºC for exactly 90 seconds, immediately cool to room temperature in ice water, allow to stand for 30 minutes, and measure the absorbance at a wavelength of 570 nm. Use a reference solution prepared in the same manner as for the test solution, using 1.0 ml of water instead of the test solution.

Determine the amount (mg) of lactic acid in the test solution from the calibration curve and the absorbance of the test solution, and calculate the content of total lactic acid ($C_3H_6O_3$) by the formula:

$$\text{Amount (\%) of total lactic acid}(C_3H_6O_3) = \frac{\text{Weigth (mg) of lactic acid in the test solution}}{\text{Weigth (g) of the sample} \times 10} \times 100$$

(4) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Residue on Ignition 14.3–17.7% (800ºC).

Calcium Sulfate

硫酸カルシウム

$CaSO_4 \cdot 2H_2O$ Mol. Wt. 172.17
Calcium sulfate dihydrate [7778-18-9]
Content Calcium Sulfate contains 98.0–105.0% of calcium sulfate ($CaSO_4 \cdot 2H_2O$).
Description Calcium Sulfate occurs as a white crystalline powder.
Identification To 1 g of Calcium Sulfate, add 100 ml of water, shake well, and filter. The filtrate responds to all tests for Calcium Salt and for Sulfate in the Qualitative Tests.
Purity
(1) Clarity of solution Almost clear.
Test Solution Weigh 0.20 g of Calcium Sulfate, add 10 ml of diluted hydrochloric acid (1 in 4), and dissolve by heating.
(2) Free alkali Weigh 0.5 g of Calcium Sulfate, add 100 ml of water, shake, and filter. Measure 10 ml of the filtrate, and add 1 drop of phenolphthalein TS. No pink color develops.
(3) Chloride Not more than 0.21% as Cl.
Sample Solution Weigh 0.20 g of Calcium Sulfate, add 20 ml of water, shake well, and filter. Use 5 ml of the filtrate as the sample solution.
Control Solution Use 0.30 ml of 0.01 mol/L hydrochloric acid.
(4) Carbonate Weigh 0.5 g of Calcium Sulfate, and add 5 ml of diluted hydrochloric acid (1 in 4). No effervescence occurs.
(5) Heavy metals Not more than 20 μg/g as Pb.
Test Solution Weigh 1.0 g of Calcium Sulfate, add 10 ml of water and 2 ml of hydrochloric acid, dissolve by boiling, cool, and filter. Neutralize the filtrate with ammonia TS, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml, and filter if necessary.
Control Solution To 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.
(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 2, Apparatus B).
Loss on Ignition 18.0–24.0%.
Assay Weigh accurately about 1 g of Calcium Sulfate, add 40 ml of diluted hydrochloric acid (1 in 4), dissolve by heating on a water bath, and cool. Add water to make exactly 100 ml. Proceed as directed in Method 1 under Calcium Salt Determination, using this solution as the test solution.
Each ml of 0.05 mol/L EDTA = 8.609 mg of $CaSO_4 \cdot 2H_2O$

Candelilla Wax

カンデリラロウ

Definition Candelilla Wax is obtained from the stems of the candelilla plant *Euphorbia antisyphilitica* Zuccarini or *Euphorbia cerifera* Alcocer and consists mainly of hentriacontane.
Description Candelilla Wax occurs as a light yellow to brown solids having a luster. When heat it, an aroma is evolved.
Identification Determine the absorption spectrum of Candelilla Wax as directed in the Potassium Bromide Disk Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.
Purity
(1) Melting point 68–73ºC.
(2) Acid value 12–22.
Weigh accurately about 3 g of Candelilla Wax, dissolve it in 80 ml of a 5:3 mixture of ethanol/xylene, and use the solution obtained as the test solution. Proceed as directed in the Acid Value Test in the Fats and Related Substances Tests. When turbidity is produced while cool, titrate while warming.
(3) Saponification value 43–65.
Proceed as directed in Purity test (3) for Carnauba Wax.
(4) Ester value 31–43 (Fats and Related Substances Tests).
(5) Heavy metals Not more than 40 μg/g as Pb (0.50 g, Method 2, Control solution Lead Standard Solution 2.0 ml).
(6) Lead Not more than 10 μg/g as Pb (1.0 g, Method 1).
(7) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).
Residue on Ignition Not more than 0.30% .

Caramel I (plain)

カラメル I

Definition Caramel I (plain) is produced by heating edible carbohydrates, including starch hydrolysates, molasses, and sugars, with or without acids or alkalis. No ammonium or sulfite compounds are used.
Description Caramel I (plain) occurs as a dark brown to black powder, lumps, paste, or liquid. It is odorless or has a slight characteristic odor. It is tasteless or has a slight characteristic taste.
Identification
(1) A solution of Caramel I (plain) (1 in 100) is light brown to black-brown.
(2) Prepare a solution of Caramel I (plain) with an approximate absorbance of 0.5 at a wavelength of 560 nm by transferring an appropriate amount of the sample into a 100-ml volumetric flask. Add 0.025 mol/L hydrochloric acid to make exactly 100 ml. Centrifuge if necessary, and refer to the supernatant as solution A. To 20 ml of solution A, add 0.20 g (0.7 meq/g exchange-capacity; the amount of use should be adjusted in proportion to the cellulose-exchange capacity) of weakly basic diethylaminoethyl cellulose anion exchanger, shake well, and centrifuge. Collect the supernatant, and refer to as solution B. Determine the absorbances (A_A and A_B) of solution A and solution B in a 1-cm cell at a wavelength of 560 nm, using 0.025 mol/L hydrochloric acid as the reference. $(A_A–A_B)/A_A$ is not more than 0.75.
(3) Weigh 0.20–0.30 g of Caramel I (plain), and add 0.025 mol/L hydrochloric acid to make exactly 100 ml. Centrifuge

if necessary, and refer to the supernatant as solution C. To 40 ml of solution C, add 2.0 g (0.85 meq/g exchange-capacity, amount of use should be adjusted in proportion to the cellulose-exchange capacity) of strongly acidic phosphorylated cellulose cation exchanger, shake well, and centrifuge. Collect the supernatant, and refer to it as solution D. Determine the absorbances (A_C and A_D) of solution C and solution D in a 1-cm cell at a wavelength of 560 nm, using 0.025 mol/L hydrochloric acid as the reference. $(A_C - A_D)/A_C$ is not more than 0.50.

Purity

(1) Heavy metals Not more than 25 µg/g as Pb (2.0g, Method 2, Control solution Lead standard Solution 5.0ml).

(2) Lead Not more than 2.0 µg/g as Pb (5.0g, Method 1).

(3) Arsenic Not more than 1.0 µg /g as As_2O_3 (2.0g, Method 3, Apparatus B).

(4) Solid content Not less than 55%.

Weigh exactly 30.0 g of sea sand, transfer to a weighing dish, and weigh accurately the total weight (W_S). Weigh accurately 1.5 to 2.0 g (W_C) of Caramel I (plain), transfer to the weighing dish, mix thoroughly with small amount of water, and evaporate to dryness on a water bath. Dry under reduced pressure at 60ºC for 5 hours until a constant weight is attained, weigh accurately the weight (W_f), and calculate the solid content by the formula:

$$\text{Solid content (\%)} = \frac{W_f - W_S}{W_c} \times 100$$

(5) Total sulfur Not more than 0.3% (on a solid basis).

Place 1 to 3 g of magnesium oxide or 6.4–19.2 g of magnesium nitrate, 1 g of white soft sugar, and 50 ml of nitric acid in an evaporating dish. Add 5–10 g of Caramel I (plain), and evaporate on a water bath to paste. Place the evaporating dish in an unheated electric muffle (ordinary temperature), and gradually heat (not higher than 525ºC) until all nitrogen dioxide fumes are driven off. Cool the evaporating dish, add hydrochloric acid (1 in 2.5) to dissolve, and neutralize the residue, then add an excess of 5ml of hydrochloric acid (1 in 2.5). Filter, heat to boiling, and add 5 ml of 10% barium chloride solution dropwise. Evaporate to 100 ml, allow to stand overnight, filter using filer paper for quantitative analysis (5C), and wash with warm water. Transfer the filter paper and the residues in a previously weighed crucible, ignite to constant weight, and weigh accurately the barium sulfate. Calculate the total sulfur by the formula below, and calculate on the solid basis. Perform a blank test in the same manner.

$$\text{Total sulfur (\%)} = \frac{\text{Barium sulfate (g)} \times 0.1374}{\text{Amount of sample (g)}} \times 100$$

(6) Total nitrogen Not more than 4.0% (on a solid basis).

Weigh accurately about 1 g of Caramel I (plain), and proceed as directed in the Kjeldahl Method under Nitrogen Determination.

(7) 4-Methylimidazole Not to be detected.

Test Solution Weigh accurately an amount of Caramel I (plain) equivalent to about 10 g of solids into a 150-ml polypropylene beaker, add 5 ml of 3.0 mol/L sodium hydroxide, mix, and adjust the pH to 12 or more. To the beaker, add 20 g of diatomaceous earth for chromatography, and mix the contents until a semidry mixture is obtained. Fill the mixture into a glass tube for chromatography (with a teflon stopcock, about 2 cm in internal diameter) whose bottom is filled with the glass wool so that the mixture is about 25 cm high. Rinse the polypropylene beaker with ethyl acetate, and pour the contents into the glass tube. When the ethyl acetate reaches the bottom of the glass tube, close the stopcock, and allow to stand for 5 minutes. Open the stopcock, and pour ethyl acetate into the glass tube until the total volume of effluent is about 200 ml. To the effluent collected, add exactly 1 ml of the internal standard solution. Transfer the contents to an eggplant-shape flask, and evaporate the ethyl acetate at below 35ºC. Dissolve the residue by adding acetone, and make exactly 5 ml.

Standard Solution Weigh accurately about 0.02 g of 4-methylimidazole, add exactly 20 ml of the internal standard, and add acetone to dissolve it and make exactly 100 ml.

Internal Standard To 0.050 g of 2-methylimidazole, add ethyl acetate to dissolve, and make exactly 50 ml.

Procedure Analyze 5 µl portions of the test solution and the standard solution by gas chromatography using the operating conditions given below. No peak of 4-methylimidazole is observed for the test solution.

Operating Conditions

Detector: Flam-ionization detector.

Column: A glass tube of 4 mm internal diameter and 1 m length.

Column packing material

Liquid phase: A mixture of 7.5% polyethylene glycol 20M/2% potassium hydroxide of the amount of the solid support

Solid support: 150- to 160-µm diatomaceous earth for gas chromatography.

Column temperature: 180ºC.

Injection port temperature: 200ºC.

Carrier gas: Nitrogen.

Flow rate: 50 ml/minute.

Caramel II (caustic sulfite process)

カラメル II

Definition Caramel II (caustic sulfite process) is produced by heating edible carbohydrates, including starch hydrolysates, molasses, and sugars, in the presence of sulfite compounds, with or without acids or alkalis. No ammonium compounds are used.

Description Caramel II (caustic sulfite process) occurs as a dark brown to black powder, lumps, paste, or liquid. It is odorless or has a slight characteristic odor. It is tasteless or has a slight characteristic taste.

Identification

(1) A solution of Caramel II (caustic sulfite process) (1 in 100) is light brown to black-brown.

(2) Proceed as directed in Identification (2) for Caramel I (plain). The value is not less than 0.50.

(3) Weigh 0.10 g of Caramel II (caustic sulfite process), add water to make exactly 100 ml, and centrifuge if necessary. Refer to the supernatant as solution A. Measure 5 ml of solution A, add water to make exactly 100 ml, and refer to as

solution B. Determine the absorbance (A_A) of solution A in a 1-cm cell at a wavelength of 560 nm against water and the absorbance (A_B) of solution B in a 1-cm cell at a wavelength of 280 nm against water. $A_A \times 20 / A_A$ is not less than 50.

Purity

(1) Heavy metals Not more than 25 μg/g as Pb (2.0g, Method 2, Control solution Lead Standard Solution 5.0ml).

(2) Lead Not more than 2.0 μg/g as Pb (5.0g, Method 1).

(3) Arsenic Not more than 1.0 μg/g as As_2O_3 (2.0g, Method 3, Apparatus B).

(4) Solid content Not less than 65%.

Proceed as directed in Purity (4) for Caramel I (plain).

(5) Total sulfur Not more than 2.5% (on a solid basis).

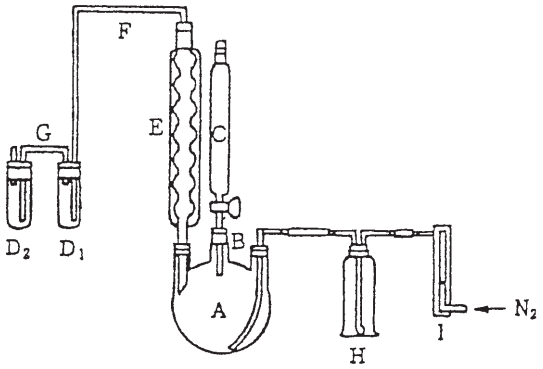
Proceed as directed in Purity (5) for Caramel I (plain).

(6) Total Nitrogen Not more than 0.2% (on a solid basis).

Proceed as directed in Purity (6) for Caramel I (plain).

(7) Sulfur dioxide Not more than 0.2% (on a solid basis).

(i) Apparatus An outline is shown in the figure below.



A: Three-neck flask (1L)
B: Stopper (silicon)
C: Separator (100-ml capacity cylindrical separator)
D_1, D_2: Receivers (50-ml capacity centrifuge tubes)
E: Allihn condensor (300-mm diameter)
F, G: Joint tube
H: Glass scrubber (250-ml capacity)
I: Flow meter

(ii) Method Place 180 ml of water and 25 ml of phosphoric acid (1 in 4) into three-neck flask A. Place 20 ml of the hydrogen peroxide solution TS in each of receivers D_1 and D_2. Heat three-neck flask A while passing through nitrogen gas (from which oxygen has been removed by alkaline pyrogallol solution) at a rate of 200±10 ml/minute and controlling the temperature of the mantle heater to make the water droplet from condenser E at 80 to 90 drops/minute Boil for about three minutes and cool. Weigh accurately about 10 g of Caramel II (caustic sulfite process), and transfer immediately to three neck flask A. While flowing the nitrogen gas as described above at a rate of 200±10 ml/minute, heat to boil gently three neck flask A, and continue to heat for 60 minutes. Then stop supplying water to condenser E, and continue to heat for a while until water droplet appears on the inside of connecting tube F near condenser E and the temperature of the upper part of condenser E reaches 60 to 70ºC. Remove receivers D_1 and D_2, rinse connecting tubes G and F with small amount of water, and transfer the condensed fluid in the receivers to a beaker. Add two drops of methyl red TS, then add 1 mol/L sodium hydroxide solution until the solution changes to yellow. To this solution add 4 drops of 1 mol/L hydrochloric acid, boil, and add gradually 2 ml of barium chloride solution (1 in 6). Heat the solution on the water bath for one hour, cool, and allow to stand overnight. Filter the solution using a filter paper for quantitative analysis (5C), wash the residues on the filter paper with warm water until the washings do not respond to the test of chloride. Dry the residues as well as the filter paper, ignite to constant weight, weigh accurately as the barium sulfate, and calculate according to the formula below. Calculate further on the basis of solid content.

$$\text{Content (\%) of sulfer dioxide } (SO_2) = \frac{\text{Weight (g) of barium sulfate} \times 0.2745}{\text{Weight (g) of the sample}} \times 100$$

Caramel III (ammonia process)

カラメル III

Definition Caramel III (ammonia process) is produced by heating edible carbohydrates, including starch hydrolysates, molasses, and sugars, in the presence of ammonium compounds, with or without acids or alkalis. No sulfite compounds are used.

Description Caramel III (ammonia process) occurs as a dark brown to black powder, lumps, paste, or liquid. It is odorless or has a slight characteristic odor. It is tasteless or has a slight characteristic taste.

Identification

(1) A solution of Caramel III (ammonia process) (1 in 100) is light brown to black-brown.

(2) Proceed as directed in Identification (2) for Caramel I (plain). The value is not more than 0.50.

(3) Proceed as directed in Identification (3) for Caramel I (plain). The value is not less than 0.50.

Purity

(1) Heavy metals Not more than 25 μg/g as Pb (2.0g, Method 2, Control solution Lead Standard Solution 5.0 ml).

(2) Lead Not more than 2.0 μg/g as Pb (5.0g, Method 1).

(3) Arsenic Not more than 1.0 μg/g as As_2O_3 (2.0g, Method 3, Apparatus B).

(4) Solid content Not less than 53%.

Proceed as directed in Purity (4) for Caramel I (plain).

(5) Ammoniacal nitrogen Not more than 0.4% (on a solid basis).

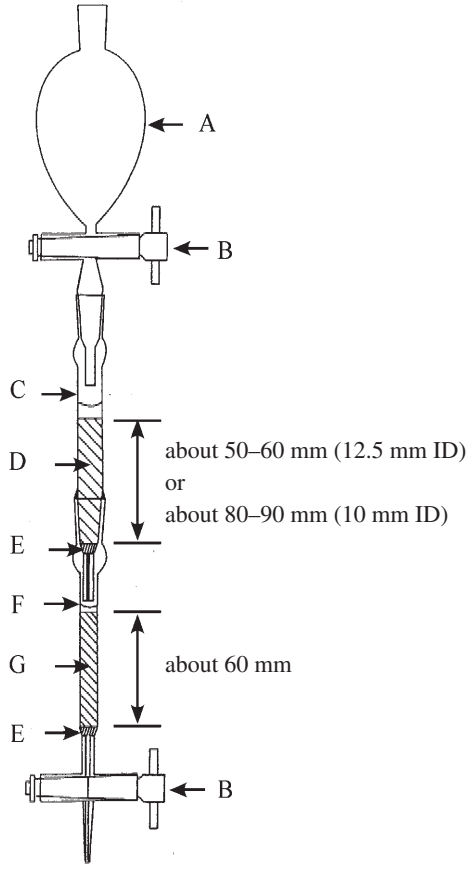
Add 25 ml of 0.05 mol/L sulfuric acid to a 500-ml receiving flask, and connect it to a distillation apparatus consisting of a Kjeldahl connecting bulb and a condenser such that the condenser delivery tube is immersed beneath the surface of the acid solution in the receiving flask. Weigh accurately about 2 g of Caramel III (ammonia process), transfer into an 800-ml Kjeldahl digestion flask, and to the flask add 2 g of magnesium oxide, 200 ml of water, and several boiling chips. Shake the digestion flask well to mix the contents, and then quickly connect it to the distillation apparatus. Heat the digestion flask to boiling, and collect about 100 ml of distillate into the receiving flask. Rinse the tip of the delivery tube with 2–3 ml of water, collecting the rinses into the receiving flask, then add 4 or 5 drops of methyl red TS,

and titrate with 0.1 mol/L sodium hydroxide. Express to the volume (ml) of 0.1 mol/L sodium hydroxide consumed as S. Conduct a blank test in the same manner, and express to the volume (ml) consumed as B. Calculate the content of ammoniacal nitrogen in the sample using the following formula. Determine the content on the basis of solid content.

$$\text{Content (\%) of ammoniacal nitrogen} = \frac{(B - S) \times 0.0014}{\text{Weight (g) of the sample}} \times 100$$

(6) Total sulfur Not more than 0.3% (on a solid basis).
Proceed as directed in Purity (5) for Caramel I (plain).

(7) Total nitrogen Not more than 6.8% (on a solid basis).
Weigh accurately about 0.5 g of Caramel III (ammonia process), and proceed as directed in the Kjeldahl Method under Nitrogen Determination.



A: Dropping funnel (100 ml capacity)
B: Teflon stopcock
C: Glass column (12.5 mm internal diameter, 150 mm length including the joint parts; or 10 mm internal diameter, 200 mm length including the joint parts)
D: Weakly acidic cation-exchange resin (fine particle)
E: Cotton stopper
F: Glass column (10 mm internal diameter, 175 mm length including the joint parts)
G: Strongly acidic cation-exchange resin (fine particle)

(8) 4-Methylimidazole Not more than 0.30 mg/g (on a solid basis).

Prepare a test solution as directed in Purity (7) for Caramel I (plain).

Standard Solutions Weigh accurately about 0.02 g, 0.06 g, and 0.1 g of 4-methylimidazole, and add exactly 20 ml of the internal standard to each. Dissolve separately by adding acetone, and make exactly 100 ml of each.

Internal Standard To 0.050 g of 2-methylimidazole, add ethyl acetate to dissolve, and make exactly 50 ml.

Procedure Analyze 5 µl portions of the test solution and the standard solutions by gas chromatography using operating conditions specified in Purity (7) for Caramel I (plain). Measure the peak area ratios of 4-methylimidazole to 2-methylimidazole for the standard solutions and the concentrations of 4-methylimidazole in the standard solutions, and prepare a calibration curve. Calculate the 4-methylimidazole content from the peak area ratio of 4-methylimidazole to 2-methylimidazole for the test solution and the calibration curve.

(9) 2-Acetyl-4-tetrahydroxybutylimidazole Not more than 40 µg/g (on a solid basis).

(i) Apparatus Use a combination column as illustrated in the left column. All joint parts should be made from ground glass.

(ii) Method

Test solution Weigh accurately 0.20–0.25 g of Caramel III (ammonia process), and dissolve it in 3 ml of water. Use this as the sample solution. Quantitatively transfer the sample solution directly to glass column C of the combination column. Wash the column with about 100 ml of water. Disconnect the column, and connect dropping funnel A to glass column F. Next, run 0.5 mol/L hydrochloric acid through column F. Discard the first 10 ml of eluate, and collect the following 35 ml of eluate. Concentrate the solution to dryness at 40ºC, 2.0 kPa. Dissolve the syrup residue in 250 µl of carbonyl-free methanol and add 250 µl of 2,4-dinitrophenylhydrazine hydrochloride TS. Transfer the reaction mixture to a septum-capped vial, and allow to stand for 5 hours at room temperature.

Standard Solutions To 1 ml of hydrochloric acid, add 0.50 g of 2,4-dinitrophenylhydrazine, and stir. Add 10 ml of ethanol, and heat on a water bath until the 2,4-dinitrophenylhydrazine dissolves completely. To the hot solution, add 0.1 g of 2-acetyl-4-tetrahydroxybutylimidazole. 2-Acetyl-4-tetrahydroxybutylimidazole-2,4-dinitrophenylhydrazone starts crystallizing in a few minutes. Cool to room temperature, and separate the crystals obtained by filtration when the crystallization is complete. Purify the 2-acetyl-4-tetrahydroxybutylimidazole-2,4-dinitrophenylhydrazone by recrystallization from an ethanol solution containing hydrochloric acid at a ratio of one drop per 5 ml of ethanol. Separate the purified crystals through filtration, and dry in a desiccator. Weigh accurately about 0.01 g of the dried crystals, and dissolve them in carbonyl-free methanol to make exactly 100 ml. Dilute appropriate portions of this solution with carbonyl-free methanol, and prepare standard solutions containing 2-acetyl-4-tetrahydroxybutylimidazole-2,4-dinitrophenylhydrazone at 0 µg/ml, 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, and 100 µg/ml.

Procedure Analyze 5 µl portions of the test solution and the standard solutions by liquid chromatography using the conditions given below. Measure the peak areas of the standard solutions, and prepare a calibration curve. Measure the

peak area of the test solution, and then determine the amount of 2-acetyl-4-tetrahydroxybutylimidazole. One hundred μg/ml of 2-acetyl-4-tetrahydroxybutylimidazole-2,4-dinitrophenylhydrazone is equivalent to 47.58 μg/ml of 2-acetyl-4-tetrahydroxybutylimidazole.

Operating Conditions

Detector: Ultraviolet spectrophotometer (wavelength 385 nm).

Column: A stainless steel tube of 4.6 mm internal diameter and 25 cm length.

Column packing material: 10-μm octylsilanized silica gel for liquid chromatography.

Column temperature: Room temperature.

Mobile phase: A 1:1 mixture of 0.1 mol/L phosphoric acid/methanol.

Flow rate: Adjust so that the retention time of 2-acetyl-4-tetrahydroxybutylimidazole-2,4-dinitrophenylhydrazone is 6.3±0.1 minutes.

Caramel IV (sulfite ammonia process)

カラメル IV

Definition Caramel IV (sulfite ammonia process) is produced by heating edible carbohydrates, including starch hydrolysates, molasses, and sugars, in the presence of both sulfite and ammonia compounds, with or without acids or alkalis.

Description Caramel IV (sulfite ammonia process) occurs as a dark brown to black powder, lumps, paste, or liquid. It is odorless or has a slight characteristic odor. It is tasteless or has a slight characteristic taste.

Identification

(1) A solution of Caramel IV (sulfite ammonia process) (1 in 100) is light brown to black-brown.

(2) Proceed as directed in Identification (2) for Caramel I (plain). The value is not less than 0.50.

(3) Proceed as directed in Identification (3) for Caramel II (sulfite process). The value is not more than 50.

Purity

(1) Heavy metals Not more than 25 μg/g as Pb (2.0g, Method 2, Control solution Lead standard solution 5.0 ml).

(2) Lead Not more than 2.0 μg/g (5.0g, Method 1).

(3) Arsenic Not more than 1.0 μg/g as As_2O_3 (2.0g, Method 3, Apparatus B).

(4) Solid content Not less than 40%.

Proceed as directed in Purity (4) for Caramel I (plain).

(5) Ammoniacal nitrogen Not more than 2.8% (on a solid basis).

Proceed as directed in Purity (5) for Caramel III (ammonia process).

(6) Total sulfur Not more than 10.0% (on a solid basis).

Proceed as directed in Purity (5) for Caramel I (plain).

(7) Total Nitrogen Not more than 7.5% (on a solid basis).

Proceed as directed in Purity (7) for Caramel III (ammonia process)

(8) Sulfur dioxide Not more than 0.5% (on a solid basis).

Proceed as directed in Purity (7) for Caramel II (caustic sulfite process).

(9) 4-Methylimidazole Not more than 1.0mg/g (on a solid basis).

Proceed as directed in Purity (8) for Caramel III (ammonia process). For the standard solutions, prepare according to the following method: Weigh accurately about 0.02 g, 0.06 g, 0.1 g, and 0.2 g of 4-methylimidazole, add exactly 20 ml of the internal standard to each, and separately dissolve by adding acetone to make exactly 100 ml of each.

Carbon Dioxide

Carbonic Acid Gas

二酸化炭素

CO_2 Mol. Wt. 44.01

Carbon dioxide [124-38-9]

Content Carbon Dioxide contains not less than 99.5% (vol) of carbon dioxide (CO_2).

Description Carbon Dioxide is a colorless gas. It is odorless.

Identification Pass Carbon Dioxide through calcium hydroxide TS. A white precipitate is formed. Collect the precipitate, and add diluted acetic acid (1 in 4). It dissolves with effervescence.

Purity The amounts of Carbon Dioxide to be weighed in the purity tests are expressed as numbers of milliliters at a temperature of 20ºC and an atmospheric pressure of 101.3 kPa.

(1) Free acid Transfer 50 ml of freshly boiled and cooled water into a Nessler tube. Insert a gas induction tube (with the internal diameter of about 1 mm) into the Nessler tube, keep the end of the gas induction tube at a position within 2 mm above the bottom of the Nessler tube, pass 1,000 ml of Carbon Dioxide through in 15 minutes, and add 0.1 ml of methyl orange TS. The color of solution is not darker than that of a control solution prepared by adding 0.1 ml of methyl orange TS and 50 ml of freshly boiled and cooled water to 1.0 ml of 0.01 mol/L hydrochloric acid.

(2) Hydrogen phosphide, hydrogen sulfide, and reducing organic substances

Transfer 25 ml of silver nitrate–ammonia TS and 3 ml of ammonia TS into a Nessler tube, and pass 1,000 ml of Carbon Dioxide through in the same manner as (1) above, protecting from light. No brown color develops.

(3) Carbon monoxide Analyze 5 ml portions of Carbon Dioxide, collected using a gas measuring tube or injection syringe for gas chromatography, by gas chromatography under the conditions given below. The peak is not observed at the peak position corresponding to carbon monoxide.

Operating Conditions

Detector: Thermal conductivity detector. The height of the peak on the recording paper is not less than 50% of the full scale when 4 ml of hydrogen or helium containing 0.02% (vol) of nitrogen is introduced.

Column: A glass or stainless steel tube of 3–4 mm internal diameter and 1–3 m length.

Column packing material: 297- to 500-μm zeolite for gas chromatography.

Column temperature: A constant temperature around 40°C.

Carrier gas: Hydrogen or helium.

Flow rate: A constant flow rate of 30–80 ml/minute.

Assay For sampling, follow the requirements directed under Purity. Transfer potassium hydroxide solution (1 in 3) into a gas pipet with a suitable capacity. Exactly take an amount not less than 100 ml of Carbon Dioxide into a gas burette with a capacity of not less than 100 ml, previously filled with sodium chloride solution (3 in 10). Transfer it into the gas pipet, and shake well. When the volume of the gas not absorbed is constant, measure the volume and express as V (ml), and calculate the content by the formula:

$$\text{Content (\%vol) of carbon dioxide } (CO_2) = \frac{\text{Volume (ml) of the sample} - \text{V (ml)}}{\text{Volume (ml) of the sample}} \times 100$$

Carnauba Wax

Brazil Wax

カルナウバロウ

[8015-86-9]

Definition Carnauba Wax is obtained from the leaves of the Brazilian wax palm *Copernicia prunifera* H.E.Moore (*Copernicia cerifera* Martius) and consists mainly of ceryl hydroxycerotate.

Description Carnauba Wax occurs as a light yellow to light brown, hard, brittle solid having clear fracture faces, and has an aroma.

Identification Determine the absorption spectrum of Carnauba Wax as directed in the Potassium Bromide Disk Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Melting point 80–86°C.

(2) Acid value Not more than 10.

Weigh accurately about 1 g of Carnauba Wax, dissolve with 80 ml of a 5:3 mixture of ethanol/xylene, and use as the test solution. Proceed as directed in the Acid Value Test in the Fats and Related Substances Tests. When turbidity is produced while the solution is cool, titrate while warming.

(3) Saponification value 78–95.

Weigh accurately about 1 g of Carnauba Wax, add exactly 50 ml of a 5:3 mixture of ethanol/xylene and exactly 25 ml of 0.5 mol/L ethanolic potassium hydroxide solution. Heat under a reflux condenser for 1 hour with occasional shaking.

Proceed as directed in the Saponification Value Test in the Fats and Related Substances Tests.

(4) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(5) Lead Not more than 10 μg/g as Pb (1.0 g, Method 1).

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Residue on Ignition Not more than 0.25%.

Carob Bean Gum

Locust Bean Gum

カロブビーンガム

Definition Carob Bean Gum is obtained by grinding or dissolving, and precipitating the seed endosperm of the locust tree *Ceratonia siliqua* Linné. It may contain sucrose, glucose, lactose, dextrin, or maltose.

Description Carob Bean Gum occurs as a white to light yellow-brown powder or granules. It is odorless or has slightly odor.

Identification

(1) To 2 g of Carob Bean Gum, add 4 ml of 2-propanol, and stir vigorously. Add 200 ml of water with vigorous stirring, and continue the stirring until the gum is completely dispersed. A slightly viscous solution is formed. Heat 100 ml of the solution on the water bath for about 10 minutes, and cool to room temperature. The solution is more viscous than before heating.

(2) To 10 ml of the viscous solution obtained finally in Identification (1), add 2 ml of sodium borate solution (1 in 20), mix, and allow to stand. A gel is formed.

Purity

(1) Protein Not more than 7.0%.

Weigh accurately about 0.2 g of Carob Bean Gum, and proceed as directed in the semi-micro Kjeldahl Method under Nitrogen Determination.

Each ml of 0.005 mol/L sulfuric acid = 0.8754 mg of protein

(2) Acid-insoluble substances Not more than 4.0%.

Proceed as directed in Purity (5) for Processed Eucheuma Algae.

(3) Lead Not more than 2.0 μg/g as Pb (5.0 g, Method 1).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(5) Starch Weigh 0.10 g of Carob Bean Gum, add 10 ml of water, and heat. After cooling, add 2 drops of Iodine TS. No blue color develops.

(6) 2-Propanol Not more than 1.0%.

(i) Apparatus Use the apparatus specified in Purity (9) for Processed Eucheuma Algae.

(ii) Method

Test Solution Weigh accurately about 2 g of Carob Bean Gum in eggplant-shaped flask A, add 200 ml of water, a few boiling chips, and 1 ml of silicon resin, and stir well. Place exactly 4 ml of the internal standard solution in volumetric flack E, and assemble the apparatus. Moisten the joint parts with water. Distill at a rate of 2 to 3 ml/minute, taking care not to allow bubbles to get in delivery tube C, and collect about 90 ml of distillate. To the distillate, add water to make exactly 100 ml. Use *tert*-butanol solution (1 in 1,000) as the internal standard solution.

Standard Solution Weigh accurately about 0.5 g of 2-propanol, and add water to make exactly 50 ml. Measure exactly 5 ml of this solution, and add water to make exactly 50 ml. Next, measure exactly 20 ml of the second solution and 4 ml of the internal standard solution in a 100-ml volumetric flask, and add water to volume.

Procedure Analyze 2.0 μl portions of the test solution and

the standard solution by gas chromatography using the operating conditions below. Determine the peak area ratios (Q_T and Q_S) of 2-propanol to *tert*-butanol for the test solution and the standard solution, respectively. Obtain the content of 2-propanol by the formula:

$$\text{Content (\%) of 2-propanol} = \frac{\text{Weight (g) of 2-propanol}}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 4$$

Operating Conditions

Detector: Flame-ionization detector.

Column: A glass tube of 3 mm internal diameter and 2 m length.

Column packing material: 180- to 250-μm styrene-divinylbenzene porous polymer for gas chromatography.

Column temperature: A constant temperature at about 120°C.

Injection port: A constant temperature at about 200°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust so that the retention time of 2-propanol is about 10 minutes.

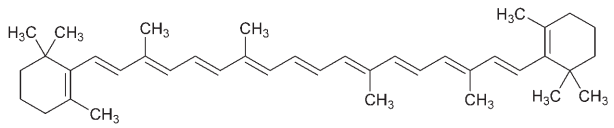
Loss on Drying Not more than 14.0% (105ºC, 5 hours).

Ash Not more than 1.2% (800ºC, 3–4 hours).

Microbial limit Proceed as directed in the Microbial Limit Tests. The total bacterial count is not more than 10,000/g, and *Escerichia coli* is negative.

β-Carotene

β-カロテン



$C_{40}H_{56}$ Mol. Wt. 536.87

(1*E*,3*E*,5*E*,7*E*,9*E*,11*E*,13*E*,15*E*,17*E*)-3,7,12,16-Tetramethyl-1,18-bis(2,6,6-trimethylcyclohex-1-en-1-yl) octadeca-1,3,5,7,9,11,13,15,17-nonaene [7235-40-7]

Content β-Carotene, when dried, contains not less than 96.0% of β-carotene ($C_{40}H_{56}$).

Description β-Carotene occurs as red-purple to dark red crystals or crystalline powder having a slight, characteristic odor and taste.

Identification

(1) A 1 in 1,000 solution of β-Carotene in a 1:1 mixture of acetone/cyclohexane is orange. Dilute this solution with acetone to make a 1 in 25 solution. To 5 ml of the solution obtained, add 1 ml of 5% sodium nitrite solution and 1 ml of 0.5 mol/L sulfuric acid. The color of the solution immediately disappears.

(2) To 0.5 ml of a 1 in 250 solution of β-Carotene in a 1:1 mixture of acetone/cyclohexane, add 1,000 ml of cyclohexane. The solution exhibits absorption maxima at wavelengths of 454–456 nm and 482–484 nm.

Purity

(1) Melting point 176–183ºC (in a sealed tube under reduced pressure, decomposition).

(2) Clarity of solution Clear (0.10 g, a 1:1 mixture of acetone/cyclohexane 10 ml).

(3) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 μg/g as AsO_3 (0.50 g, Method 3, Apparatus B).

(5) Absorbance ratio Weigh accurately about 0.04 g of β-Carotene, previously dried, dissolve it in 10 ml of a 1:1 mixture of acetone/cyclohexane, and add cyclohexane to make exactly 100 ml. Measure exactly 5 ml of this solution, add cyclohexane to make exactly 100 ml, and use the solution obtained as the test solution. Measure exactly 10 ml of the test solution, add cyclohexane to make exactly 100 ml, and use this solution as the diluted test solution. Measure the absorbances (A_1 and A_2) of the test solution at wavelengths of 340 nm and 362 nm, respectively, and the absorbances (A_3, A_4, and A_5) of the diluted test solution at wavelengths of 434 nm, 455 nm, and 483 nm, respectively. A_2/A_1 is not less than 1.00, $(A_4 \times 10)/A_1$ is not less than 15.0, A_4/A_3 is 1.30–1.60, and A_4/A_5 is 1.05–1.25.

Loss on Drying Not more than 1.0% (reduced pressure, 4 hours).

Residue on Ignition Not more than 0.10%.

Assay Measure the absorbance (A) of the diluted test solution used in Purity (5) at the absorption maximum at a wavelength of 454–456 nm, and calculate the content by the formula:

$$\text{Content (\%) of β-carotene } (C_{40}H_{56}) = \frac{200}{\text{Weight (g) of the sample}} \times \frac{A}{2{,}500} \times 100$$

Storage Standards Store in a hermetic, light-resistant container under inert gas.

Carrot Carotene

ニンジンカロテン

Definition Carrot Carotene is obtained from the roots of the carrot plant *Daucus carota* Linné and consists mainly of carotene. It may contain edible fats or oils.

Content (Color Value) Carrot Carotene contains the equivalent of not less than 0.80% of β-carotene ($C_{40}H_{56}$ = 536.87) and the equivalent of 95–115% of the labeled content; or its Color Value ($E^{10\%}_{1cm}$) is not less than 200 and in the range of 95–115% of the labeled value.

Description Carrot Carotene is a red-brown to brown turbid oily substance having a slightly characteristic odor.

Identification

(1) Weigh the equivalent of 1 g of Carrot Carotene with a Color Value 200, and dissolve it in 10 ml of a 1:1 mixture of acetone/cyclohexane. An orange color develops.

(2) Dilute the solution of Carrot Carotene prepared in Identification (1) with acetone to make a 1 in 25 solution. To 5 ml the solution obtained, add 1 ml of 5% sodium nitrate

solution and 1 ml of 0.5 mol/L sulfuric acid. The solution is discolored immediately.

(3) A solution of Carrot Carotene in cyclohexane exhibits an absorption maximum at a wavelength of 445–460 nm or 465–485 nm, or absorption maxima at both of 445–460nm and 465–485nm.

Purity

(1) Heavy metals Not more than 20 μg/g as Pb (1.0g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 10 μg/g as Pb (1.0g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50g, Method 3, Apparatus B).

Assay (Color Value Test) Proceed as directed in the Color Value Test, using the conditions given blow. Determine the color value or calculate the content of β-carotene by dividing the color value by 250.

Operating Conditions

Solvent: Cyclohexane.

Wavelength: Maximum absorption wavelength of 445–460 nm.

Carthamus Red

ベニバナ赤色素

Definition Carthamus Red is obtained from the flowers of the safflower plant *Carthamus tinctorius* Linné and consists mainly of carthamin. It may contain dextrin or lactose.

Color Value The Color Value ($E^{10\%}_{1cm}$) of Carthamus Red is not less than 500 and is in the range of 90–110% of the labeled value.

Description Carthamus Red occurs as a dark red to dark purple powder, lumps, or paste having a slightly characteristic odor.

Identification

(1) Weigh the equivalent of 0.1 g of Carthamus Red with a Color Value 500, and dissolve it in 200 ml of dimethylformamide. The solution is red and exhibits an absorption maximum at a wavelength of 525–535nm.

(2) Weigh the equivalent of 0.01 g of Carthamus Red with a Color Value 500, and dissolve it in 50 ml of water. The solution is red. This solution turns dark yellow when made alkaline with sodium hydroxide solution (1 in 25), and it turns red when made acidic with dilute hydrochloric acid.

(3) Weigh the equivalent of 1 g of Carthamus Red with a Color Value 500, dissolve it in 10 ml of dimethylformamide solution, and use the resulting solution as the test solution. Analyze a 2-μl portion of the test solution by thin-layer chromatography, using a 4:2:1 mixture of 1-butanol/water/acetic acid as the developing solvent. No control solution is used. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110ºC for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the original line, and air-dry the plate. An orange-red spot is observed at an R_f value of about 0.4. The spot emits a red-purple fluorescence when irradiated with ultraviolet light (around 255 nm).

Purity

(1) Heavy metals Not more than 40 μg/g as Pb (0.50 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 10 μg/g as Pb (1.0g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Color Value Test Proceed as directed in the Color Value Test, using the following conditions.

Operating Conditions

Solvent: Dimethylformamide.

Wavelength: Maximum absorption wavelength of 525–535 nm.

Carthamus Yellow

ベニバナ黄色素

Definition Carthamus Yellow is obtained from the flowers of the safflower plant *Carthamus tinctorius* Linné and consists mainly of safflower yellows. It may contain dextrin or lactose.

Color Value The Color Value ($E^{10\%}_{1cm}$) of Carthamus Yellow is not less than 100 and is in the range of 90–110% of the labeled value.

Description Carthamus Yellow occurs as a yellow to dark brown powder, lumps, paste, or liquid having a slightly characteristic odor.

Identification (1) Weigh the equivalent of 0.1 g of Carthamus Yellow with a Color Value 100, and dissolve it in 100 ml of citrate buffer (pH 5.0). The solution is yellow. It exhibits an absorption maximum at a wavelength of 400–408 nm.

(2) To the solution prepared in Identification (1), add sodium hydroxide solution (1 in 25) to make it alkaline. The color becomes orangish.

(3) Weigh the equivalent of 1 g of Carthamus Yellow with a Color Value of 100, dissolve it in 1 ml of water, and add 10 ml of methanol. After mixing, centrifuge at 3,000 rpm for 10 minutes, and use the supernatant as the test solution. Analyze a 2 μl portion of the test solution by thin-layer chromatography, using a 4:2:1 mixture of 1-butanol/water/acetic acid as the developing solvent. No control solution is used. Use a thin-layer plate coated with microcrystalline cellulose for thin-layer chromatography as the solid support and then dried at 60–80ºC for 20 minutes. Stop the development when the solvent front has ascended to a point about 10 cm above the original line, and air-dry the plate. Two or more yellow spots are observed at R_f values of about 0.20–0.50.

Purity

(1) Heavy metals Not more than 40 μg/g as Pb (0.50 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 10 μg/g as Pb (1.0g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Color Value Test Proceed as directed in the Color Value Test, using the following conditions:

Operating Conditions

Solvent: Citrate buffer (pH 5.0).

Wavelength: Maximum absorption wavelength of 400–

408 nm.

Casein

カゼイン

Content Casein, when dried, contains 13.8–16.0% of nitrogen (N = 14.01).
Description Casein occurs as a white to light yellow powder, granules or flakes. It is odorless and tasteless, or has a slight, characteristic odor and taste.
Identification

(1) Dissolve 0.1 g of Casein in 10 ml of sodium hydroxide solution (1 in 10), and add 8 ml of diluted acetic acid (1 in 2). A white, cotton-like precipitate is formed.

(2) Dissolve 0.1 g of Casein in 10 ml of sodium hydroxide solution (1 in 10), add 1 drop of cupric sulfate solution (1 in 8), and shake. A blue precipitate is formed, and the solution is purple.

(3) Ignite 0.1 g of Casein at 450–550ºC. Fumes are produced, and a characteristic odor develops. When the fumes are no longer evolved, stop heating, and cool. To the black residue, add 5 ml of diluted nitric acid (1 in 10), dissolve while warming, and filter. To the filtrate, add 1 ml of ammonium molybdate TS, and warm. A yellow precipitate is formed.

Purity

(1) Color and clarity of solution Colorless and slightly turbid.

Test Solution Dry Casein in a vacuum desiccator for 4 hours, and make into a fine powder. Weigh 0.1 g of the powder, add 30 ml of water, shake, and allow to stand for about 10 minutes. Add 2 ml of sodium hydroxide solution (1 in 250), dissolve while warming at 60ºC for 1 hour and shaking occasionally, cool, and add water to make 100 ml.

(2) pH 3.7–6.5.

Test Solution Weigh 1.0 g of Casein, add 50 ml of water, shake for 10 minutes, and filter.

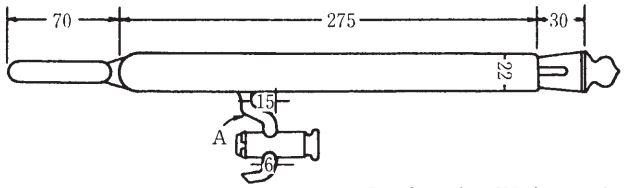
(3) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Water-soluble substances Not more than 1.0%.

Weigh 1.5 g of Casein, add 30 ml of water, shake for 10 minutes, and filter. Measure 20 ml of the filtrate, evaporate to dryness on a water bath, dry at 100ºC to constant weight, and weigh.

(5) Fat Not more than 1.5%.

Dry a flask at 100ºC for 30 minutes, allow to cool in a desiccator, and weigh accurately. Weigh accurately about 2.5 g of Casein into another flask, add 15 ml of diluted hydrochloric acid (2 in 3), dissolve while gently heating directly, and heat in a water bath for 20 minutes. After cooling, add 10 ml of ethanol, transfer the contents into a Rörig tube, add 25 ml of diethyl ether, and shake vigorously for 1 minute. Add 25 ml of petroleum ether, shake vigorously for 30 seconds, and allow to stand. Filter the upper-layer solution, taken from side branch tube A, through a filter paper into the flask previously prepared. Repeat the extraction two times, using 15 ml of diethyl ether and 15 ml of petroleum ether each time, add the upper-layer solution to the flask, and evaporate the diethyl ether and petroleum ether on a water bath. Dry the residue at 98–100ºC for 4 hours, allow to cool in a desiccator, and weigh the flask with the content accurately.



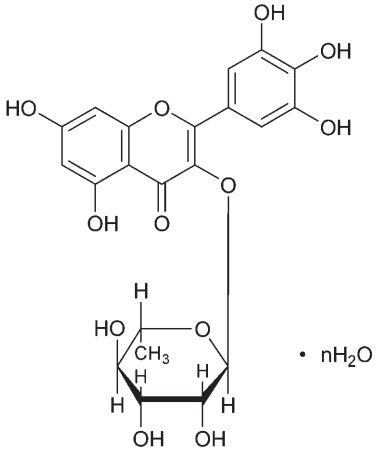
Rörig tube (Unit: mm)

Loss on Drying Not more than 12.0% (100ºC, 3 hours).
Residue on Ignition Not more than 2.5% (dried sample).
Assay Weigh accurately about 0.15 g of Casein, previously dried, and proceed as directed in the Kjeldahl Method under Nitrogen Determination.

Each ml of 0.1 mol/L sulfuric acid = 1.401 mg of N

Chinese Bayberry Extract

ヤマモモ抽出物



$C_{21}H_{20}O_{12}\cdot nH_2O$
5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl)-4-oxo-4*H*-chromen-7-yl α-L-rhamnopyranoside hydrate
[myricitrin, anhydrous 17912-87-7]
Definition Chinese Bayberry Extract is obtained from the fruits, bark, or leaves of Chinese bayberry, *Myrica rubra* Siebold et Zuccarini, by extraction. It consists mainly of myricitrin.
Content Chinese Bayberry Extract, when calculated on the anhydrous basis, contains 95.0–105.0% of myricitrin ($C_{21}H_{20}O_{12}$ = 464.38).
Description Chinese Bayberry Extract occurs as a pale yellow powder or lumps having a slight characteristic odor.
Identification

(1) Dissolve 5 mg of Chinese Bayberry Extract in 10 ml of ethanol. A light yellow to brown color develops. On the addition of 1–2 drops of iron(III) chloride–hydrochloric acid TS, the color changes to greenish black.

(2) Dissolve 5 mg of Chinese Bayberry Extract in 5 ml of

ethanol. A light yellow to brown color develops. On the addition of 2 ml of hydrochloric acid and 0.05 g of magnesium dust, the color gradually changes to red.

(3) A solution of 0.01 g of Chinese Bayberry Extract in 1,000 ml of methanol exhibits absorption maxima at wavelengths of about 257 nm and 354 nm.

Purity

(1) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 5.0 μg/g as Pb (2.0 g, Method 1).

(3) Arsenic Not more than 2.0 μg/g as As_2O_3 (1.0 g, Method 3, Apparatus B).

(4) Methanol Not more than 50 μg/g.

(i) Apparatus Use the apparatus specified in Purify (4) for Enju Extract.

(ii) Method

Test Solution Weigh accurately about 5 g of Chinese Bayberry Extract into eggplant-shaped flask A. Add 100 ml of boric acid-sodium hydroxide buffer, mix well, and add a few boiling chips. Put exactly 2 ml of the internal standard in volumetric flask E, and set up the apparatus. Moisten the joint parts with water, distill the mixture at a rate of 2 to 3 ml/minute, and collect about 45 ml of distillate. To the distillate, add water to make exactly 50 ml. Use *tert*-butanol solution (1 in 1,000) as the internal standard.

Standard Solution Weigh accurately about 0.5 g of methanol, and add water to make exactly 100 ml. Measure exactly 5 ml of this solution, and add water to make exactly 100 ml. Then place exactly 2 ml of the resulting solution and 4 ml of the internal standard solution in a volumetric flask, and add water to make exactly 100 ml.

Procedure Analyze 2.0 μl portions of the test solution and the standard solution by gas chromatography using the operating conditions given below. Determine the peak area ratios (Q_T and Q_S) of methanol to *tert*-butanol for the test solution and the standard solution, and calculate the methanol content by the formula:

$$\text{Content (μg/g) of methanol} = \frac{\text{Weight (g) of methanol}}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 500$$

Operating Conditions

Detector: Flame-ionization detector.

Column: A glass tube of 3 mm internal diameter and 2 m length.

Column packing material: 180- to 250-μm styrene-divinylbenzene porous polymer for gas chromatography.

Column temperature: A constant temperature at about 120°C.

Injection port: A constant temperature at about 200°C.

Injection: Total loop fill.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust so that the retention time of methanol is about 2 minutes.

Water Content Not more than 8.0% (0.2 g, Direct Titration).

Assay

Test Solution and Standard Solution Weigh accurately about 0.05 g each of Chinese Bayberry Extract and myricitrin for assay, and separately dissolve them in methanol to make 2 solutions of exactly 100 ml each. To exactly 5 ml of each solution, add an 800:200:1 mixture of water/acetonitrile/phosphoric acid to make exactly 50 ml. Use these solutions as the test solution and the standard solution, respectively.

Procedure Analyze 20 μl portions of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Measure the peak areas (A_T and A_S) of myricitrin for the test solution and the standard solution, and calculate the myricitrin content using the following formula. Separately, determine the water content of myricitrin for assay by Direct Titration.

$$\text{Content (\%) of myricitrin } (C_{21}H_{20}O_{12}) = \frac{\text{Anhydrous basis weight (g) of myricitrin for assay}}{\text{Anhydrous basis weight (g) of the sample}} \times \frac{A_T}{A_S} \times 100$$

Operating Conditions

Detector: ultraviolet spectrophotometer (determination wavelength: 254 nm).

Column: A stainless steel tube of 3–6 mm internal diameter and 15–25 cm length.

Column packing material: 5- to 10-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 40°C.

Mobile phase: An 800:200:1 mixture of water/acetonitrile/phosphoric acid.

Flow rate: Adjust so that the retention time of myricitrin is about 8–12 minutes.

Chlorophyll

クロロフィル

Definition Chlorophyll is obtained from green plants and consists mainly of chlorophylls. It may contain edible fats or oils.

Content (Color Value) The Color Value ($E^{10\%}_{1cm}$) of Chlorophyll is not less than 600 and is in the range of 90–110% of the labeled value.

Description Chlorophyll occurs as a green to dark green powder, lumps, paste, or liquid having a characteristic odor.

Identification

(1) Weigh the equivalent of 1 g of Chlorophyll with a Color Value 600, and dissolve it in 100 ml of hexane. A green color develops. Add 0.5 ml of hydrochloric acid, and mix thoroughly. The color of the solution changes to greenish yellow.

(2) Weigh the equivalent of 1 g of Chlorophyll with a Color Value 600, and dissolve it in 100 ml of ethyl acetate. A red fluorescence is emitted.

(3) A solution of Chlorophyll in hexane exhibits absorption maxima at wavelengths of both 410–430nm and 660–670nm.

(4) Weigh the equivalent of 1 g of Chlorophyll with a Color Value 600, dissolve it in 30 ml of hexane, and use this solution as the test solution. Analyze a 2-μl portion of the test solution by thin-layer chromatography, using a 10:1:1 mixture of hexane/acetone/*tert*-butanol as the developing solvent. No control solution is used. Use a thin-layer plate

coated with silica gel for thin-layer chromatography as the solid support and then dried at 110ºC for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the original line, and air-dry the plate. Three spots are observed: a yellow-green spot (chlorophyll b) at an R_f value of about 0.3, a green spot (chlorophyll a) at an R_f value of about 0.4, and a gray spot (pheophytin) at an R_f value of about 0.65. These spots emit red fluorescence when irradiated with ultraviolet light (around 366 nm) in a dark place. Also, two additional spots are observed: a yellow (xanthophyll) spot at an R_f of approximate 0.25 and a yellow-orange (β-carotene) spot at an R_f of approximate 0.95. These spots do not emit fluorescence when irradiated with ultraviolet light (around 366 nm) in a dark place.

Purity

(1) Heavy metals Not more than 40 μg/g as Pb (0.50 g, Method 2, Control solution Lead standard solution 2.0 ml).

(2) Lead Not more than 10 μg/g as Pb (1.0g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50g, Method 3, Apparatus B).

Color Value Test Proceed as directed in the Color Value Test, using the conditions below.

Operating Conditions

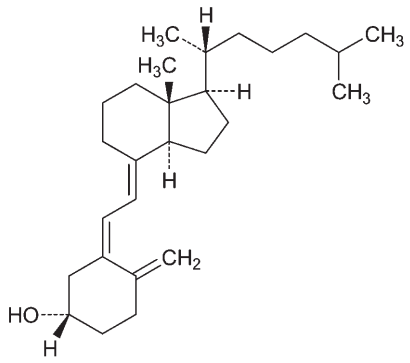
Solvent: Hexane.

Wavelength: Maximum absorption wavelength of 660–670 nm.

Cholecalciferol

Vitamin D_3

コレカルシフェロール



$C_{27}H_{44}O$ Mol. Wt. 384.64

(3*S*,5*Z*,7*E*)-9,10-Secocholesta-5,7,10(19)-trien-3-ol [67-97-0]

Description Cholecalciferol occurs as white crystals. It is odorless.

Identification

(1) Proceed as directed in Identification (1) for Ergocalciferol.

(2) Proceed as directed in Identification (2) for Ergocalciferol. The melting point is 133–135ºC.

Purity

(1) Specific absorbance $E^{1\%}_{1cm}$ (265 nm): 450–490.

Test Solution Weigh about accurately about 0.1 g of Cholecalciferol, dissolve it in ethanol to make exactly 200 ml, measure exactly 2 ml of the solution, and add ethanol to make exactly 100 ml.

(2) Specific rotation $[\alpha]_D^{20}$: +103.0 to +112.0º (0.1 g, ethanol, 20 ml).

(3) Melting point 84–88ºC.

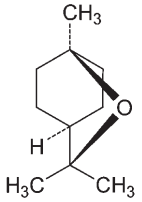
(4) 7-Dehydrocholesterol Weigh 0.010 g of Cholecalciferol, dissolve it in 2 ml of 90% (vol) ethanol, add a solution prepared by weighing 0.020 g of digitonin and dissolving in 2 ml of 90% (vol) ethanol, and allow to stand for 18 hours. No precipitate is formed.

Storage Standards Store in a cold place in a hermetic, light-resistant container under inert gas.

1,8-Cineole

Eucalyptol

1,8-シネオール



$C_{10}H_{18}O$ Mol. Wt. 154.25

1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octane [470-82-6]

Content 1,8-Cineole contains not less than 85.0% of 1,8-cineole ($C_{10}H_{18}O$).

Description 1,8-Cineole is a colorless or light yellow transparent liquid having a eucalyptus leaf-like odor.

Identification Determine the absorption spectrum of 1,8-Cineole as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Refractive index n_D^{20}: 1.454–1.462.

(2) Angular rotation α_D^{20}: – 3.0 to +10.0°.

(3) Specific gravity 0.915–0.929.

(4) Clarity of solution Clear (2.0 ml, 70% (vol) ethanol 6.0 ml).

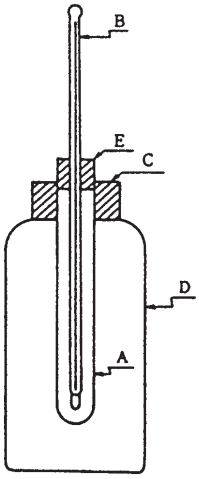
(5) Phellandrene Measure 2.5 ml of 1,8-Cineole, dissolve it in 5 ml of petroleum benzine, add 10 ml of sodium nitrite solution (1 in 20), and gradually add 5 ml of acetic acid. No crystals are deposited within 10 minutes.

(6) Resorcinol To 1.0 ml of 1,8-Cineole, add 5 ml of water, 4 ml of sodium borate (1 in 500), and a small-sized crystal of 2,6-dichloroquinonechloroimide, and shake the mixture. No blue or blue-purple is produced.

Assay Use the apparatus, outlined on the next page, consisting of a test tube (A) (about 15-mm diameter, about 8- to 16-cm length), a thermometer (B), cork stoppers (C and E), and a wide-mouth glass bottle (D).

Percentage of 1,8-cineole

Temperature	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
47	80.0	80.2	80.4	80.6	80.8	81.1	81.3	81.5	81.7	81.9
48	82.1	82.3	82.5	82.7	82.9	83.2	83.4	83.6	83.8	84.0
49	84.2	84.4	84.6	84.8	85.0	85.3	85.5	85.7	85.9	86.0
50	86.3	86.6	86.8	87.1	87.3	87.6	87.8	88.1	88.3	88.6
51	88.8	89.1	89.3	89.6	89.8	90.1	90.3	90.6	90.8	91.1
52	91.3	91.6	91.8	92.1	92.3	92.6	92.8	93.1	93.3	93.6
53	93.8	94.1	94.3	94.6	94.8	95.1	95.3	95.6	95.8	96.1
54	96.3	96.6	96.9	97.2	97.5	97.8	98.1	98.4	98.7	99.0
55	99.3	99.7	100.0							

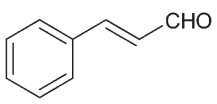


Weigh exactly 3.0 g of 1,8-Cineole into the test tube, add 2.1 g of previously melted *o*-cresol while heating, and fix the thermometer with cork stopper E so that the mercury bulb is slightly below the center of the solution. Gently stir the solution with the thermometer, and read the temperature when crystals start being deposited. Heat the test tube to fuse the crystals completely, and insert the test tube into the wide-mouth bottle through cork stopper C. Lower the temperature gradually. When the crystals start being deposited again or when the temperature reaches the initially recorded value, vigorously rub the tube wall by moving the thermometer vertically. The temperature slightly rises, and then shows a constant value for a while. Record the reading of the thermometer at that time. Repeat the above operation several times, and determine the 1,8-cineole content from the maximum temperature obtained using the given table.

Cinnamaldehyde

Cinnamic Aldehyde

シンナムアルデヒド



C_9H_8O Mol. Wt. 132.16

(2*E*)-3-Phenylprop-2-enal [14371-10-9]

Content Cinnamaldehyde contains not less than 98.0% of cinnamaldehyde (C_9H_8O).

Description Cinnamaldehyde is a colorless to light yellow, transparent liquid having a cinnamon-like odor.

Identification Determine the absorption spectrum of Cinnamaldehyde as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum of Cinnamaldehyde. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Refractive index n_D^{20}: 1.619–1.625.

(2) Specific gravity 1.051–1.056.

(3) Clarity of solution Clear (1.0 ml, 60% (vol) ethanol 7.0 ml).

(4) Acid value Not more than 5.0 (Flavoring Substances Tests).

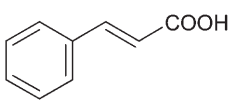
(5) Halogenated compounds Proceed as directed for Halogenated Compounds in the Flavoring Substances Tests.

Assay Weigh accurately about 1 g of Cinnamaldehyde, and proceed as directed in Method 1 in the Aldehyde and Ketone Content Test under the Flavoring Substances Tests. In the test, allow the mixture to stand for 15 minutes before titrating.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 66.08 mg of C_9H_8O

Cinnamic Acid

ケイ皮酸



$C_9H_8O_2$ Mol. Wt. 148.16

(2*E*)-3-Phenylprop-2-enoic acid [140-10-3]

Content Cinnamic Acid, when dried, contains not less than 99.0% of cinnamic acid ($C_9H_8O_2$).

Description Cinnamic Acid occurs as a white crystalline powder having a characteristic odor.

Identification

(1) To 0.5 g of Cinnamic Acid, add 1 ml of sulfuric acid,

and dissolve while heating in a water bath. The resulting solution is yellow-green. When heated further, it turns dark red.

(2) Dissolve 0.1 g of Cinnamic Acid in 2 ml of potassium hydroxide solution (1 in 15), add 5 ml of potassium permanganate solution (1 in 300), and warm in water bath. An odor of benzaldehyde is evolved.

Purity

(1) Melting point 132–135ºC.

(2) Clarity of solution

Clear (1.0 g, ethanol 7.0 ml).

Clear (0.20 g, anhydrous sodium carbonate solution (1 in 8) 2.0 ml and water 8.0 ml).

(3) Heavy metals Not more than 10 μg/g as Pb.

Test Solution Weigh 2.0 g of Cinnamic Acid, add 2 ml of diluted acetic acid (1 in 20) and ethanol to make 50 ml.

Control Solution To 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and ethanol to make 50 ml.

(4) Arsenic Not more than 4 μg/g as As_2O_3 (0.50 g, Method 4, Apparatus B).

(5) Halogenated compounds Proceed as directed in the Flavoring Substances Tests.

Loss on Drying Not more than 1.0% (4 hours).

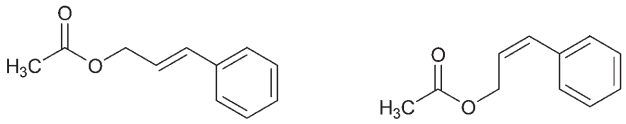
Residue on Ignition Not more than 0.05%.

Assay Weigh accurately about 0.2 g of Cinnamic Acid, previously dried, dissolve it in 10 ml of neutralized ethanol and 10 ml of water, and titrate with 0.1 mol/L sodium hydroxide (indicator: 3 drops of phenolphthalein TS).

Each ml of 0.1 mol/L sodium hydroxide = 14.82 mg of $C_9H_8O_2$

Cinnamyl Acetate

酢酸シンナミル



$C_{11}H_{12}O_2$ Mol. Wt. 176.21

3-Phenylprop-2-en-1-yl acetate [103-54-8]

Content Cinnamyl Acetate contains not less than 98.0% of cinnamyl acetate ($C_{11}H_{12}O_2$).

Description Cinnamyl Acetate is a colorless or slightly yellowish, transparent liquid having a characteristic odor.

Identification To 1 ml of Cinnamyl Acetate, add 5 ml of ethanolic 10% potassium hydroxide TS. Heat under a reflux condenser in a water bath for 30 minutes. The characteristic odor disappears. Cool, and add 5 ml of water and 1.2 ml of diluted hydrochloric acid (1 in 4). The solution responds to test (3) for Acetate in the Qualitative Tests.

Purity

(1) Refractive index n_D^{20}: 1.539–1.543.

(2) Specific gravity 1.053–1.057.

(3) Clarity of solution Clear (1.0 ml, 70% (vol) ethanol 6.0 ml).

(4) Acid value Not more than 1.0 (Flavoring Substances Tests).

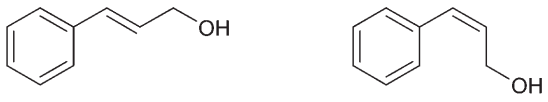
Assay Weigh accurately about 1 g of Cinnamyl Acetate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 88.11 mg of $C_{11}H_{12}O_2$

Cinnamyl Alcohol

Cinnamic Alcohol

シンナミルアルコール



$C_9H_{10}O$ Mol. Wt. 134.18

3-Phenylprop-2-en-1-ol [104-54-1]

Content Cinnamyl Alcohol contains not less than 98.0% of cinnamyl alcohol ($C_9H_{10}O$).

Description Cinnamyl Alcohol occurs as a colorless to light yellow liquid or as white to light yellow crystalline lumps having a characteristic odor.

Identification To 0.2 g of Cinnamyl Alcohol, add 5 ml of potassium permanganate solution (1 in 20) and 1 ml of diluted sulfuric acid (1 in 25). An odor of cinnamaldehyde is evolved.

Purity

(1) Congealing point Not less than 31ºC.

(2) Clarity of solution Clear.

Test Solution Weigh 1.0 g of Cinnamyl Alcohol, add 3.0 ml of 50% (vol) ethanol, and dissolve by warming to 35ºC.

(3) Acid value Not more than 1.0 (Flavoring Substances Tests).

(4) Cinnamaldehyde Not more than 1.5% as cinnamaldehyde (C_9H_8O = 132.16).

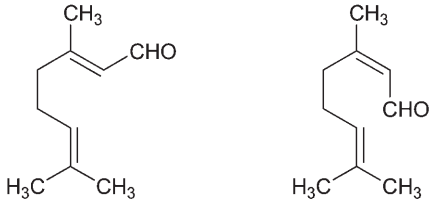
Weigh accurately about 5 g of Cinnamyl Alcohol, and proceed as directed in Method 1 in the Aldehyde and Ketone Content Test in the Flavoring Substances Tests. In the test, allow the mixture to stand for 15 minutes before titrating.

Assay Proceed as directed in Method 2 under the Alcohol Content Test in the Flavoring Substances Tests, using 0.5 g of the sample.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 67.09 mg of $C_9H_{10}O$

Citral

シトラール



$C_{10}H_{16}O$ Mol. Wt. 152.23

Mixture of (2*E*)-3,7-dimethylocta-2,6-dienal (*trans*-isomer) and (2*Z*)-3,7-dimethylocta-2,6-dienal (*cis*-isomer)

[5392-40-5]

Content Citral contains not less than 96.0% of citral ($C_{10}H_{16}O$).

Description Citral is a colorless to light yellow liquid having a lemon-like odor.

Identification Determine the absorption spectrum of Citral as directed in the Liquid Film Method in Infrared Spectrophotometry, and compare with the Reference Spectrum of Citral. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Refractive index n_D^{20}: 1.486–1.490.

(2) Specific gravity 0.880–0.894.

(3) Clarity of solution Clear (1.0 ml, 60% (vol) ethanol 10 ml).

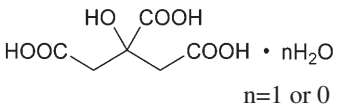
(4) Acid Value Not more than 5.0 (Flavoring Substances Tests).

Assay Weigh accurately about 1 g of Citral, and proceed as directed in Method 2 in the Aldehyde and Ketone Content Test in the Flavoring Substances Tests. In the test, allow the mixture to stand for 15 minutes before titrating.

Each ml of 0.5 mol/L hydrochloric acid = 76.12 mg of $C_{10}H_{16}O$

Citric Acid

クエン酸



n=1 or 0

$C_6H_8O_7 \cdot nH_2O$ (n=1 or 0) Mol. Wt. monohydrate 210.14

anhydrous 192.12

2-Hydroxypropane-1,2,3-tricarboxylic acid monohydrate [5949-29-1]

2-Hydroxypropane-1,2,3-tricarboxylic acid [77-92-9]

Definition Citric Acid occurs in two forms: the crystalline form (monohydrate) called Citric Acid (crystal), and the anhydrous form called Citric Acid (anhydrous).

Content Citric Acid, when calculated on the anhydrous basis, contains not less than 99.5% of citric acid ($C_6H_8O_7$).

Description Citric Acid occurs as colorless, transparent crystals, granules, or lumps or as a white powder. It is odorless and has a strongly acid taste.

Identification

(1) A solution of Citric Acid (1 in 10) is acidic.

(2) Citric Acid responds to all tests for Citrate in the Qualitative Tests.

Purity

(1) Sulfate Not more than 0.048% as SO_4 (0.50 g, Control solution 0.005 mol/L sulfuric acid 0.50 ml).

(2) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(3) Calcium Weigh 1.0 g of Citric Acid, dissolve it in 10 ml of water, neutralize with ammonia TS, and add 1 ml of ammonium oxalate solution (1 in 30). No turbidity appears.

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

(5) Oxalate Weigh 1.0 g of Citric Acid, dissolve it in 10 ml of water, and add 2 ml of calcium chloride solution (2 in 25). No turbidity appears.

(6) Isocitric acid

Test Solution Weigh 0.5 g of Citric Acid, heat at 105ºC for 3 hours, cool, and dissolve it in 10 ml of acetone.

Analyze 5 μl of the test solution by paper chromatography without using any control solution. For the filter paper, use a No. 2 filter paper for chromatography. Stop the development when the developing solvent ascends to a point about 25 cm above the base line. Then air-dry the filter paper, and spray it with bromophenol blue TS for citric acid. No more than one spot is observed. As the developing solvent, use the upper part of an 8:3:2 mixture of 1-butanol/formic acid/water that has been allowed to stand over night.

(7) Polycyclic aromatic hydrocarbon

Test Solution Weigh 25 g of Citric Acid, add 30 ml of water, and dissolve while warming to about 50ºC. Cool, perform extraction three times with three 20-ml portions of hexane for ultraviolet absorption spectrum measurement, and centrifuge at about 2,500–3,000 rpm for about 10 minutes each time. Combine all of the hexane layers, and evaporate the hexane to 1–2 ml. After cooling, add hexane for ultraviolet absorption spectrum measurement to make 10 ml.

Procedure Measure the absorbance of the test solution in the wavelength range of 260–350 nm. It is not more than 0.05. In the measurement, use a reference solution prepared as directed for the test solution without using the sample.

(8) Readily carbonizable substances To 0.5 g of Citric Acid, add 5 ml of sulfuric acid, and dissolve while heating at about 90±1ºC for 1 hour. The color of the solution is not darker than that of Matching Fluid K.

Residue on Ignition Not more than 0.10%.

Water Content

Crystal: Not more than 8.8% (0.2 g, Direct Titration).

Anhydrous: Not more than 0.5% (2 g, Direct Titration).

Assay Weigh accurately about 1.5 g of Citric Acid, and dissolve it in water to make exactly 250 ml. Measure exactly 25 ml of this solution, and titrate with 0.1 mol/L sodium hydroxide (indicator: 2–3 drops of phenolphthalein TS). Calculate on the anhydrous basis.

Each ml of 0.1 mol/L sodium hydroxide = 6.404 mg of $C_6H_8O_7$

Citronellal

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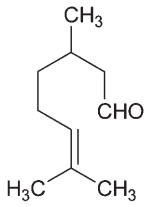
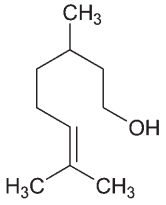


$C_{10}H_{18}O$ Mol. Wt. 154.25
3,7-Dimethyloct-6-enal [106-23-0]
Content Citronellal contains not less than 85.0% of citronellal ($C_{10}H_{18}O$).
Description Citronellal is a colorless, transparent liquid having a characteristic odor.
Identification To 1 ml of Citronellal, add 2 ml of sodium hydrogen sulfite TS and 2 drops of anhydrous sodium carbonate solution (1 in 8), and shake. The mixture forms white crystalline lumps, generating heat. Add 10 ml of sodium hydrogen sulfite TS, and heat in a water bath while shaking. The crystalline lumps dissolve.
Purity
(1) Refractive index n_D^{20}: 1.446–1.452.
(2) Specific gravity 0.852–0.859.
(3) Clarity of solution Clear (1.0 ml, 70% (vol) ethanol 5.0 ml).
(4) Acid value Not more than 3.0 (Flavoring Substances Tests).
Assay Weigh accurately about 1 g of Citronellal, and proceed as directed in Method 2 in the Aldehyde and Ketone Content Test under the Flavoring Substances Tests. In the procedure allow the mixture to stand for 15 minutes before titrating.
Each ml of 0.5 mol/L hydrochloric acid = 77.12 mg of $C_{10}H_{18}O$

Citronellol

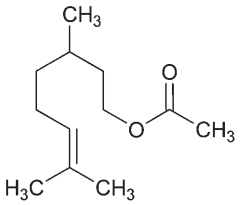
シトロネロール



$C_{10}H_{20}O$ Mol. Wt. 156.27
3,7-Dimethyloct-6-en-1-ol [106-22-9]
Content Citronellol contains not less than 94.0% of citronellol ($C_{10}H_{20}O$).
Description Citronellol is a colorless, transparent liquid having a characteristic odor.
Identification To 1 ml of Citronellol, add 1 ml of acetic anhydride and 1 drop of phosphoric acid, and keep the solution at a lukewarm temperature for 10 minutes. Add 1 ml of water, shake in warm water for 5 minutes, and cool. Add anhydrous sodium carbonate solution (1 in 8) to make it slightly alkaline. An odor of citronellyl acetate is evolved.
Purity
(1) Refractive index n_D^{20}: 1.453–1.462.
(2) Specific gravity 0.853–0.863.
(3) Clarity of solution Clear (2.0 ml, 70% (vol) ethanol 4.0 ml).
(4) Acid value Not more than 1.0 (Flavoring Substances Tests).
(5) Ester value Not more than 4.0 (5 g, Flavoring Substances Tests).
(6) Aldehyde Weigh exactly 5 g of Citronellol, and proceed as directed in Method 2 in the Aldehyde and Ketone Content Test in Flavoring Substances Tests. The volume of consumed 0.5 mol/L hydrochloric acid is not more than 0.7 ml.
Assay Proceed as directed in Method 1 for Alcohol Content in the Flavoring Substances Tests, using about 1 g of acetylated oil.

Citronellyl Acetate

酢酸シトロネリル



$C_{12}H_{22}O_2$ Mol. Wt. 198.30
3,7-Dimethyloct-6-en-1-yl acetate [150-84-5]
Content Citronellyl Acetate contains not less than 95.0% of citronellyl acetate ($C_{12}H_{22}O_2$).
Description Citronellyl Acetate is a colorless, transparent liquid having a characteristic odor.
Identification To 1 ml of Citronellyl Acetate, add 5 ml of ethanolic 10% potassium hydroxide hydroxide TS, and heat in a water bath for 10 minutes. The characteristic odor disappears, and an odor of citronellol is evolved. Cool, and add 2 ml of water and 2 ml of diluted hydrochloric acid (1 in 4). The solution responds to test (3) for Acetate in the Qualitative Tests.
Purity
(1) Refractive index n_D^{20}: 1.443–1.451.
(2) Specific gravity 0.888–0.894.
(3) Clarity of solution Clear (1.0 ml, 70% (vol) ethanol 7.0 ml).
(4) Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Weigh accurately about 1.5 g of Citronellyl Acetate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 99.15 mg of $C_{12}H_{22}O_2$

Citronellyl Formate

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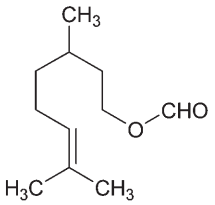


$C_{11}H_{20}O_2$ Mol. Wt. 184.28

3,7-Dimethyloct-6-en-1-yl formate [105-85-1]

Content Citronellyl Formate contains not less than 86.0% of citronellyl formate ($C_{11}H_{20}O_2$).

Description Citronellyl Formate is a colorless, transparent liquid having a characteristic odor.

Identification

(1) To 1 ml of Citronellyl Formate, add 10 ml of ethanolic 10% potassium hydroxide TS, and heat in a water bath for 5 minutes while shaking. The characteristic odor disappears, and an odor of citronellol is evolved.

(2) Proceed as directed in Identification (2) for Geranyl Formate.

Purity

(1) Refractive index n_D^{20}: 1.444–1.450.

(2) Specific gravity 0.891–0.900.

(3) Clarity of solution Clear (1.0 ml, 80% (vol) ethanol 3.0 ml).

(4) Acid value Not more than 1.0 (Flavoring Substances Tests).

Titrate while cooling in ice water until a light pink color persists for 10 seconds.

Assay Weigh accurately about 1 g of Citronellyl Formate, and perform the tests as directed in the Saponification Value Test and the Acid Value Test, respectively, in the Flavoring Substances Tests. Calculate the content by the formula:

$$\text{Content (\%) of citronellyl formate } (C_{11}H_{20}O_2) = \frac{\text{Saponification value} - \text{Acid value}}{561.1} \times 184.3$$

Cochineal Extract

Carminic Acid

コチニール色素

Definition Cochineal Extract is obtained from the cochineal insect *Dactylopius coccus* Costa (*Coccus cacti* Linnaeus) and consists mainly of carminic acid.

Color Value The Color Value ($E_{1cm}^{10\%}$) of Cochineal Extract is not less than 80 and is in the range of 95–115% of the labeled value.

Description Cochineal Extract occurs as a red to dark red powder, lumps, liquid, or paste having a slightly characteristic odor.

Identification

(1) Weigh the equivalent of 0.5 g of Cochineal Extract with a Color Value 80, dissolve it in 1000 ml of 0.1 mol/L hydrochloric acid, and centrifuge. The supernatant is orange and exhibits an absorption maximum at a wavelength of 490–497 nm.

(2) Weigh the equivalent of 1 g of Cochineal Extract with a Color Value 80, and mix with 100 ml of water. A red to dark red-brown color develops. When made alkaline with sodium hydroxide solution (1 in 25), the solution turns purple to purple-red.

Purity

(1) Heavy metals Not more than 40 μg/g as Pb (0.50 g, Method 2, Control solution Lead standard solution 2.0 ml).

(2) Lead Not more than 10 μg/g as Pb (1.0g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(4) Protein Not more than 2.2%.

Weigh accurately about 1 g of Cochineal Extract and proceed as directed in the Semi-micro Kjeldahl Method under Nitrogen Determination.

Each ml of 0.005 mol/L sulfuric acid = 0.8754 mg of protein

Color Value Test Proceed as directed in the Color Value Test using the conditions below.

Operating Conditions

Solvent: 0.1 mol/L hydrochloric acid.

Wavelength: Maximum absorption wavelength of 490–497 nm.

Copper Chlorophyll

Copper Complexes of Chlorophylls

銅クロロフィル

Description Copper Chlorophyll occurs as a blue-black to green-black powder, flakes, lumps, or viscous substances having a characteristic odor.

Identification

(1) Proceed as directed in Identification (1)(ii) for Sodium Copper Chlorophyllin.

(2) Dissolve 0.010 g of Copper Chlorophyll in 50 ml of diethyl ether, add 2 ml of a solution of sodium hydroxide in methanol (1 in 100), and shake. Heat under a reflux condenser on a water bath for 30 minutes. Cool, perform extraction 3 to 5 times with 10 ml of water each time, combine the extracts, add phosphate buffer (pH 7.5) to make 200 ml, and measure the absorbance of this solution. The solution exhibits absorption maxima at wavelengths of 403–407 nm and 630–640 nm. When the absorbances at the absorption maxima are expressed as A_1 and A_2, respectively, A_1/A_2 is not more than 4.0.

Purity

(1) Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength near 405 nm): Not less than 62.0 (on the dried basis).

This test should be protected from direct light, and the apparatus used in the test should be light-resistant.

Weigh accurately about 0.1 g of Copper Chlorophyll, dissolve it in 50 ml of diethyl ether, add 10 ml of a solution of sodium hydroxide in methanol (2 in 100), and shake. Heat under a reflux condenser on a water bath for 30 minutes. Cool, perform extraction four times with 20 ml of water each time, combine the extracts, and add water to make exactly 100 ml. Filter this solution, measure exactly 5.0 ml of the filtrate, add phosphate buffer (pH 7.5) to make exactly 100 ml, and quickly measure absorbance.

(2) Inorganic copper salt Not more than 0.03% as Cu.

Test Solution Weigh 1.0 g of Copper Chlorophyll, and dissolve it in 60 ml of acetone.

Procedure Proceed as directed in Purity (3) for Sodium Copper Chlorophyllin.

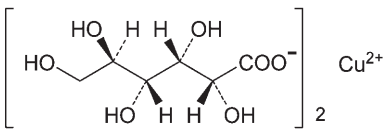
(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(4) Chlorophyllin salt Weigh 1.0 g of Copper Chlorophyll, dissolve it in 30 ml of diethyl ether, add 20 ml of water, and shake. Allow to stand, filter the aqueous layer through a filter paper moistened with water. The filtrate is colorless.

Loss on Drying Not more than 3.0% (105℃, 2 hours).

Copper Gluconate

グルコン酸銅



$C_{12}H_{22}CuO_{14}$ Mol. Wt. 453.84

Monocopper(II) bis(D-gluconate)

Content Copper Gluconate contains 98.0–102.0% of copper gluconate ($C_{12}H_{22}CuO_{14}$).

Description Copper Gluconate occurs as a light blue powder.

Identification

(1) Copper Gluconate responds to tests (1) and (3) for Cupric Salt in the Qualitative Tests.

(2) Measure 5 ml of a solution of Copper Gluconate in warm water (1 in 10), and proceed as directed in Identification (2) for Glucono-δ-Lactone.

Purity

(1) Clarity of solution Almost Clear (1.0 g, water 10 ml).

(2) Lead Not more than 10 μg/g as Pb.

Test Solution Weigh 1.0 g of Copper Gluconate, and add water to make 20 ml.

Control Solution To 1.0 ml of Lead Standard Solution, add water to make 20 ml.

Procedure Proceed as directed in Method 1 in the Lead Limit Test.

(3) Arsenic Not more than 4.0 μg/g (0.50 g, Method 1, Apparatus B).

(4) Reducing sugars Not more than 1.0% as D-glucose.

Weigh 1.0 g of Copper Gluconate, transfer it into a 250-ml Erlenmeyer flask, dissolve it in 10 ml of water, and add 25 ml of alkaline cupric citrate TS. Cover with a small beaker, boil gently for exactly 5 minutes, and cool quickly to room temperature. To this solution, add 25 ml of diluted acetic acid (1 in 10) and exactly 10 ml of 0.05 mol/L iodine, then add 10 ml of diluted hydrochloric acid (1 in 4) and 3 ml of starch TS, and titrate the excess iodine with 0.1 mol/L sodium thiosulfate. The volume of the sodium thiosulfate solution consumed is not less than 6.3 ml.

Assay Weigh accurately about 1.5 g of Copper Gluconate, transfer it into a flask with a ground-glass stopper, dissolve it in about 100 ml of water, add 2 ml of acetic acid and 5 g of potassium iodide, immediately stopper tightly, and allow to stand in a dark place for 5 minutes. Titrate this solution with 0.1 mol/L sodium thiosulfate until the color of the solution changes to a light yellow color, dissolve 2 g of ammonium thiocyanate, add 3 ml of starch TS, and titrate again with 0.1 mol/L sodium thiosulfate until the color of the solution changes to an opaque color. Perform a blank test in the same manner, and make any necessary correction.

Each ml of 0.1 mol/L sodium thiosulfate = 45.38 mg of $C_{12}H_{22}CuO_{14}$

Crude Magnesium Chloride (Sea Water)*

粗製海水塩化マグネシウム

Definition Crude Magnesium Chloride is obtained by precipitating and separating both potassium chloride and sodium chloride from sea water. It consists mainly of magnesium chloride.

Content Crude Magnesium Chloride contains the equivalent of 12.0–30.0% of magnesium chloride ($MgCl_2$ = 95.21).

Description Crude Magnesium Chloride is a colorless to light yellow liquid having a bitter taste.

Identification

(1) To Crude Magnesium Chloride, add sodium hydroxide TS. A white gelatinous precipitate is produced. On the addition of iodine TS, the precipitate is stained dark brown and

*Note: The standards for Crude Magnesium Chloride (Sea Water) shown in this monograph were newly established and published in Official Gazzette in March 2007. These standards were to take effect on April 1, 2008. However, the enforcement of them has been postponed until the date the Minister of Health, Labour and Welfare decides.

does not dissolve in an excess amount of sodium hydroxide.

(2) Crude Magnesium Chloride responds to Test (1) for Chloride as described in the Quantitative Tests.

Purity

(1) Sulfate Not more than 4.8% as SO_4.

Sample Solution Weigh 0.25 g of Crude Magnesium Chloride, and dissolve it in water to make 100 ml. Use 2.0 ml of this solution for the test.

Control Solution Use 0.50 ml of 0.005 mol/L sulfuric acid.

(2) Bromide Not more than 2.5% as Br.

Test Solution Weigh 1.0 g of Crude Magnesium Chloride, and dissolve it in water to make 500 ml. Measure 10 ml of this solution, and add water to make 100 ml. Next, measure 2 ml of the second solution, add 3 ml of water, 2 ml of dilute phenol red TS, and 1 ml of chloramine T TS (1 in 10,000), immediately mix, and allow to stand for 2 minutes. Add 0.15 ml of 0.1 mol/L sodium thiosulfate, mix, and add water to make 10 ml.

Control Solution Weigh exactly 2.979 g of potassium bromide, previously dried at 110°C for 4 hours, and dissolve it in water to make exactly 1,000 ml. Measure exactly 1 ml of this solution, and add water to make exactly 1,000 ml. Next, measure 5 ml of the second solution, add 2 ml of dilute phenol red TS and 1 ml of chloramine T TS (1 in 10,000), mix immediately, and then proceed as directed for the test solution.

Procedure Measure the absorbances of both solutions at 590 nm using water as the reference. The absorbance of the test solution is not more than that of the control solution.

(3) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Zinc Not more than 70 μg/g as Zn.

Sample Solution Weigh 4.0 g of Crude Magnesium Chloride, and dissolve it in water to make 40 ml.

Procedure Measure 30 ml of the test solution, add 5 drops of acetic acid and 2 ml of potassium ferrocyanide solution (1 in 20), shake, and allow to stand for 10 minutes. The turbidity of this solution is not greater than that of the solution prepared as follows: To 14 ml of Zinc Standard Solution, add 10 ml of the test solution and water to make 30 ml, add 5 drops of acetic acid and 2 ml of potassium ferrocyanide solution (1 in 20), shake, and allow to stand for 10 minutes.

(5) Calcium Not more than 4.0% as Ca.

Measure exactly 20 ml of solution A prepared in the Assay, and add water to make 100 ml. Next, add 0.2 ml of tartaric acid solution (1 in 5), 10 ml of 2,2′,2″-nitrilotriethanol solution (3 in 10), and 10 ml of potassium hydroxide solution (1 in 10), and allow to stand for 5 minutes. Immediately titrate with 0.01 mol/L EDTA (indicator: NN indicator about 0.1 g), and express the amount of the EDTA solution consumed as b ml. The endpoint is when the red-purple color of the solution disappears completely and changes to blue. Calculate the content by the formula:

$$\text{Content(\%) of calcium (Ca)} = \frac{b \times 0.4008}{\text{Weight of the sample (g)}}$$

(6) Sodium Not more than 4.0% as Na.

Standard Solution Weigh 1.0 g of Crude Magnesium Chloride, and dissolve it in water to make 1,000 ml. Measure 10 ml of this solution, and add water to make 200 ml.

Control Solution Weigh 2.542 g of sodium chloride, previously dried at 130°C for 2 hours, and dissolve it in water to make exactly 1,000 ml. Measure exactly 2 ml of this solution, and add water to make exactly 1,000 ml.

Procedure Measure the absorbances of the test solution and the control solution as directed under Atomic Absorption Spectrophotometry according to the conditions given below. The absorbance of the test solution is not greater than that of the control solution.

Operating Conditions

Light source: Sodium hollow cathode lamp.

Analytical line (wavelength): 589.0 nm.

Supporting gas: Air.

Combustible gas: Acetylene.

(7) Potassium Not more than 6.0% as K.

Test Solution Use the test solution prepared in Purity (6).

Control Solution Weigh 1.907 g of potassium chloride, previously dried at 105°C for 2 hours, and dissolve it in water to make exactly 1,000 ml. Measure exactly 3 ml of this solution, and add water to make exactly 1,000 ml.

Procedure Measure the absorbances of the test solution and the control solution as directed under Atomic Absorption Spectrophotometry according to the conditions given below. The absorbance of the test solution is not greater than that of the control solution.

Operating Conditions

Light source: Potassium hollow cathode lamp.

Analytical line (wavelength): 766.5 nm.

Supporting gas: Air.

Combustible gas: Acetylene.

(8) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.5 g, Method 1, Apparatus B).

Assay Weigh accurately about 2 g of Crude Magnesium Chloride, add water to make exactly 200 ml, and refer to the solution obtained as solution A. Measure exactly 5 ml of solution A, add 50 ml of water and ammonia–ammonium chloride buffer (pH 10.7), titrate with 0.01 mol/L EDTA (indicator: eriochrome black T TS), and determine the amount of the EDTA solution consumed (a ml). The endpoint is when the color of the solution changes from red to blue. Calculate the content, using the consumed amount (b ml) obtained in Purity (5) and the following formula:

$$\text{Content (\%) of magnesium chloride } (MgCl_2) = \frac{(a - 0.25\,b) \times 3.803}{\text{Weight of the sample (g)}}$$

Cupric Sulfate

Copper Sulfate

硫酸銅

$CuSO_4 \cdot 5H_2O$ Mol. Wt. 249.69

Copper(II) sulfate pentahydrate [7758-99-8]

Content Cupric Sulfate contains 98.5–104.5% of cupric sulfate ($CuSO_4 \cdot 5H_2O$).

Description Cupric Sulfate occurs as blue crystals or granules or as a deep blue crystalline powder.

Identification Cupric Sulfate responds to all tests for Cu-

pric Salt and for Sulfate in the Qualitative Tests.

Purity

(1) Clarity of solution Almost clear.

Proceed as directed in Purity (1) for Copper Gluconate.

(2) Free acid Weigh 1.0 g of Cupric Sulfate, dissolve it in 20 ml of water, and add 2 drops of methyl orange TS. A green color develops.

(3) Alkali metals and alkali earth metals Not more than 0.30%.

Weigh 6.0 g of Cupric Sulfate, dissolve it in 150 ml of water, add 3 ml of sulfuric acid, and pass hydrogen sulfide through the solution while warming to about 70ºC until the solution is saturated. After cooling, add water to make 280 ml, and filter. To the filtrate, add water to make 300 ml. Measure 100 ml of this solution, evaporate to dryness on a sand bath, ignite at 450–550ºC to constant weight, and weigh the residue.

(4) Lead Not more than 10 µg/g as Pb.

Proceed as directed in Purity (2) for Copper Gluconate.

(5) Arsenic Not more than 4.0 µg/g as As_2O_3.

Test Solution Weigh 0.50 g of Cupric Sulfate, and dissolve it in 5 ml of water. Add 2 ml of acetic acid and 1.5 g of potassium iodide, allow to stand for 5 minutes, and add 0.2 g of L-ascorbic acid to dissolve.

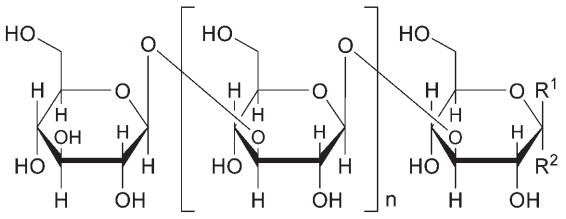
Apparatus Use Apparatus B.

Assay Weigh accurately about 0.7 g of Cupric Sulfate, and proceed as directed in the Assay for Copper Gluconate.

Each ml of 0.1 mol/L sodium thiosulfate = 24.97 mg of $CuSO_4 \cdot 5H_2O$

Curdlan

カードラン



R^1 = H, R^2 = OH
or R^1 = OH, R^2 = H

$(C_6H_{10}O_5)_n$

(3→1)-β-D-Glucopyranan [54724-00-4]

Definition Curdlan is obtained from the culture fluid of *Agrobacterium* biovar 1 or *Rhizobium radiobacter* and consists mainly of β-1,3-glucan.

Content Curdlan contains not less than 80.0% of curdlan.

Description Curdlan occurs as a white to light yellow-brown powder. It is odorless.

Identification

(1) To 0.2 g of Curdlan, add 5 ml of water, shake well, add 1 ml of sodium hydroxide solution (3 in 25), and shake well. It dissolves.

(2) Heat 10 ml of a 2% suspension of Curdlan in a water bath for 10 minutes. A gel is produced.

(3) To 10 ml of a 2% suspension of Curdlan, add 5 ml of sulfuric acid, heat in a water bath for 30 minutes, and cool. To 1 ml of the mixture, add 100 ml of water, and then neutralize with barium carbonate. Centrifuge at 900×g for 10 minutes. To 1 ml of the supernatant, add 5 ml of Fehling's TS, and heat in a water bath for 5 minutes. A red precipitate is produced.

Purity

(1) pH 6.0–7.5 (1% suspension).

(2) Lead Not more than 0.5 µg/g as Pb (20 g, Method 1).

(3) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50g, Method 3, Apparatus B).

(4) Total nitrogen Not more than 0.3%.

Weigh accurately about 0.5 g of Curdlan, and proceed as directed in the Semi-micro Kjeldahl Method under Nitrogen Determination.

Loss on Drying Not more than 10.0% (60°C, reduced pressure, 5 hours).

Residue on Ignition Not more than 6.0%.

Microbial Limits Proceed as directed in the Microbial Limit Tests. The total bacterial count is not more than 1,000/g, and *Escherichia coli* is negative.

Assay

Test Solution Weigh accurately about 0.1 g of Curdlan, and dissolve it in 0.1 mol/L sodium hydroxide with shaking to make exactly 100 ml. Measure exactly 5 ml of this solution, and add water to make exactly 100 ml. Measure exactly 1 ml of the second solution, add 1 ml of phenol solution (1 in 20) and 5 ml of sulfuric acid, shake well, and cool in ice water.

Standard Solution Proceed in the same manner as for the test solution, using 0.1 g of glucose, accurately weighed.

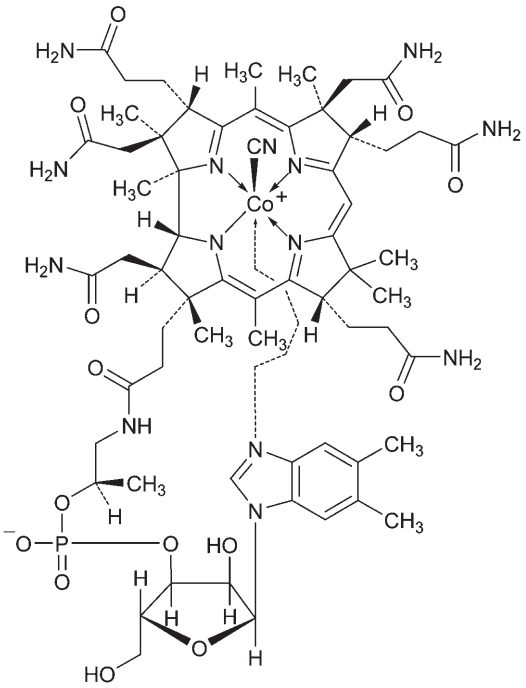
Procedure Measure the absorbances (A_T and A_S) of the test solution and the standard solution at 490 nm against the reference solution prepared as follows: Proceed as directed under Test Solution for Assay, using 0.1 ml of water instead of the sample. Calculate the content by the formula:

$$\text{Content (\%) of curdlan} = \frac{\text{Weight (g) of glucose}}{\text{Weight (g) of the sample}} \times \frac{A_T}{A_S} \times 0.900 \times 100$$

Cyanocobalamin

Vitamin B_{12}

シアノコバラミン



$C_{63}H_{88}CoN_{14}O_{14}P$ Mol. Wt. 1355.37

*Co*α-[α-(5,6-Dimethyl-1*H*-benzoimidazol-1-yl)]-*Co*β-cyanocobamide [68-19-9]

Definition Cyanocobalamin is obtained by isolation from the culture fluid of actinomycetes *Streptomyces* spp., or bacteria *Agrobacterium* spp., *Bacillus* spp., *Flavobacterium* spp., *Propionibacterium* spp., or *Rhizobium* spp. It consists of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$).

Content Cyanocobalamin, when calculated on the dried basis, contains 96.0–102.0% of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$).

Description Cyanocobalamin occurs as dark red crystals or powder.

Identification

(1) Determine the absorption spectra of the test solution and standard solution prepared in the Assay, given below, as directed under Ultraviolet-visible Spectrophotometry. The spectrum of the test solution exhibits similar intensities of absorption at the same wavelengths as the Reference Standard spectrum.

(2) Mix 1 mg of Cyanocobalamin with 0.05 g of potassium hydrogen sulfate, and ignite to melt. After cooling, crush the melt with a glass rod, add 3 ml of water, and boil to dissolve. Add 1 drop of phenolphthalein TS, and then add sodium hydroxide solution (1 in 20) dropwise until the solution is light red. To this solution, add 0.5 g of sodium acetate, 0.5 ml of diluted acetic acid (3 in 50), and 0.5 ml of a solution of disodium 1-nitroso-2-naphthol-3,6-disulfonate (1 in 500). A red to orange-red color develops immediately, and the color does not disappear on boiling for one minute with 0.5 ml of hydrochloric acid.

(3) Place 5 mg of Cyanocobalamin in a 50-ml distillation flask, dissolve it in 5 ml of water, and add 2.5 ml of hypophosphorous acid. Fit a short condenser to the flask, and immerse the lower end of the condenser into 1 ml of sodium hydroxide solution (1 in 50) in a test tube. Boil gently for 10 minutes, and keep distilling until 1 ml of distillate is obtained. To the solution in the test tube, add 4 drops of a saturated solution of ferrous ammonium sulfate, and shake gently. Add 0.03 g of sodium fluoride, heat to boil, and add diluted sulfuric acid (1 in 6) immediately until the solution becomes clear. On the addition of 3–5 drops of diluted sulfuric acid (1 in 6), a blue to blue-green color is produced.

Purity

(1) Clarity and color of solution Red in color and clear (0.020 g, water 10 ml).

(2) Pseudocyanocobalamin Dissolve 1.0 mg of Cyanocobalamin in 20 ml of water, transfer the solution to a separating funnel, add 5 ml of a 1:1 mixture of *m*-cresol/ carbon tetrachloride, and shake vigorously for 1 minute. Allow to stand, and then run the lower layer into another separating funnel. To the separated solution, add 5 ml of diluted sulfuric acid (1 in 7), shake vigorously, and allow to stand. If necessary, centrifuge the mixture. The supernatant is colorless or not darker in color than the following control solution prepared by diluting 0.6 ml of 0.02 mol/L potassium permanganate to 1,000 ml with water.

Loss on Drying Not more than 12.0% (0.050 g, not more than 0.67 kPa, desiccant phosphorus oxide(V), 100°C, 4 hours).

Assay

Test Solution Weigh accurately about 0.02 g of Cyanocobalamin, and dissolve it in water to make exactly 1,000 ml.

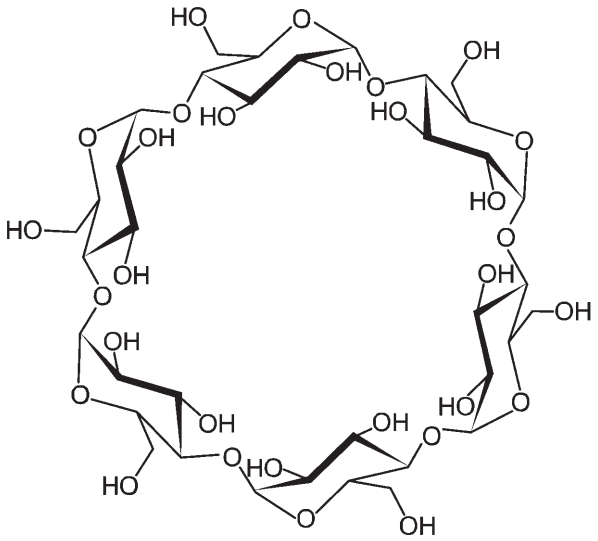
Standard Solution Weigh accurately about 0.02 g of Cyanocobalamin Reference Standard for which the loss on drying has been already measured, and dissolve it in water to make exactly 1,000 ml.

Procedure Measure the absorbances (A_T and A_S) of the test solution and the standard solution at a wavelength of 361 nm, using water as the reference. Calculate the content by the formula:

$$\text{Content (\%) of cyanocobalamin } (C_{63}H_{88}CoN_{14}O_{14}P) = \frac{\left(\begin{array}{c}\text{Dry basis weight (g) of}\\ \text{Cyanocobalamin Reference Standard}\end{array}\right)}{\text{Dry basis weight (g) of the sample}} \times \frac{A_T}{A_S} \times 100$$

α-Cyclodextrin

α-シクロデキストリン



$C_{36}H_{60}O_{30}$ Mol. Wt. 972.85

Cyclomaltohexaose [10016-20-3]

Definition α-Cyclodextrin* is a non-reducing cyclic dextrin obtained by enzymatic treatment of starch. It is a cyclic oligosaccharide consisting of six D-glucose units.

Content α-Cyclodextrin, when dried, contains not less than 98.0% of α-cyclodextrin ($C_{36}H_{60}O_{30}$).

Description α-Cyclodextrin occurs as white crystals or crystalline powder. It is odorless and has a slight sweet taste.

Identification To 0.2 g of α-Cyclodextrin, add 2 ml of iodine TS, dissolve by warming in a water bath, and allow to stand at room temperature. A blue-purple precipitate is formed.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +147 to +152°.

Weigh accurately about 1 g of α-Cyclodextrin, dried previously, and add water to make exactly 100 ml. Measure the angular rotation of the this solution within 30 minutes.

(2) Clarity and color of solution Colorless and clear (0.50 g, water 50 ml).

(3) Chloride Not more than 0.018% as Cl (0.50 g, Control solution 0.01 mol/L hydrochloric acid 0.25 ml).

(4) Heavy metals Not more than 5.0 μg/g as Pb (4.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(5) Lead Not more than 1.0 μg/g as Pb (10.0 g, Method 1).

(6) Arsenic Not more than 1.3 μg/g as As_2O_3 (1.5 g, Method 2, Apparatus B).

(7) Reducing substances Weigh exactly 1.0 g of α-Cyclodextrin, previously dried, dissolve it in 25 ml of water, add 40 ml of Fehling's TS, and boil gently for 3 minutes. After cooling, carefully filter the supernatant through a glass filter (1G4) leaving as much precipitate as possible in the flask. Wash the precipitate in the flask with warm water, filter the washings through the glass filter, and discard the filtrate. Repeat this washing and filtering process until the washings are free of alkali. Add 20 ml of ferric sulfate TS to the precipitate in the flask to dissolve, filter through the same glass filter, wash the inside of the flask and the glass filter with water, and combine the filtrate and washings. Heat to 80℃, and titrate with 0.02 mol/L potassium permanganate solution. The volume of potassium permanganate consumed is not more than 3.2 ml.

Loss on Drying Not more than 14.0% (105°C, not more than 0.67 kPa, 4 hours).

Residue on Ignition Not more than 0.10% (550°C).

Assay

Test Solution Weigh accurately about 0.5 g of α-Cyclodextrin, previously dried, dissolve it in about 35 ml of hot water, and cool. Add water to make exactly 50 ml.

Standard Solutions Prepare three standard solutions with different concentrations by the following procedure: Weigh accurately about 0.7 g of α-cyclodextrin for assay, previously dried, and dissolve it in 45 ml of hot water. After cooling, add water to make exactly 50 ml (standard solution 1). Transfer exactly 5 ml of this solution into each of 10-ml and 20-ml volumetric flasks, and add water exactly to volume (standards 2 and 3).

Procedure Analyze 10 μl portions of the test solution and the standard solutions by liquid chromatography using the operating conditions given below. Prepare a calibration curve by measuring the peak areas of α-cyclodextrin for the standard solutions. Determine the amount (g) of α-cyclodextrin in the test solution from the calibration curve and the peak area of α-cyclodextrin for the test solution. Calculate the content of α-cyclodextrin by the formula:

$$\text{Content (\%) of α-cyclodextrin } (C_{36}H_{60}O_{30}) = \frac{\left(\begin{array}{c}\text{Amount (g) of α-cyclodextrin}\\ \text{in the test solution}\end{array}\right)}{\text{Weight (g) of the sample}} \times 100$$

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube of 5–10 mm internal diameter and 20–50 cm length.

Column packing material: 9- to 10-μm strongly acidic cation exchange resin for liquid chromatography.

Column temperature: A constant temperature of 50–80℃.

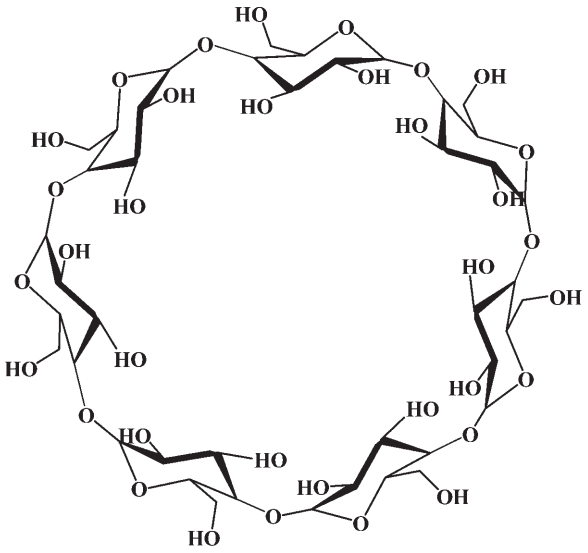
Mobile phase: Water.

Flow rate: A constant rate of 0.3–1.0 ml/min.

* α-Cyclodextrin belongs to the "Cyclodextrin" category contained in the List of Existing Food Additives.

β-Cyclodextrin

β-シクロデキストリン



$C_{42}H_{70}O_{35}$ Mol. Wt. 1134.98

Cyclomaltoheptaose [7585-39-9]

Definition β-Cyclodextrin* is a non-reducing cyclic dextrin obtained by enzymatic treatment of starch. It is a cyclic oligosaccharide consisting of seven D-glucose units.

Content β-Cyclodextrin, when dried, contains not less than 98.0% of β-cyclodextrin ($C_{42}H_{70}O_{35}$).

Description β-Cyclodextrin occurs as odorless white crystals or crystalline powder having a slight sweet taste.

Identification To 0.2 g of β-Cyclodextrin, add 2 ml of iodine TS, dissolve while warming in a water bath, and allow to stand at room temperature. A yellow-brown precipitate is formed.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +160 to +164°.

Weigh accurately about 1 g of β-Cyclodextrin, previously dried, add water to make exactly 100 ml. Measure the angular rotation of this solution within 30 minutes.

(2) Clarity of solution Colorless and clear (0.50 g, water 50 ml).

(3) Chloride Not more than 0.018% as Cl (0.50 g, Control solution 0.01 mol/L hydrochloric acid 0.25 ml).

(4) Heavy metals Not more than 5.0 μg/g as Pb (4.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(5) Lead Not more than 1.0 μg/g (10.0 g, Method 1).

(6) Arsenic Not more than 1.3 μg/g as As_2O_3 (1.5 g, Method 2, Apparatus B).

(7) Reducing substances Weigh exactly 1.0 g of β-Cyclodextrin, previously dried, dissolve it in 25 ml of water, add 40 ml of Fehling's TS, and boil gently for 3 minutes. After cooling, carefully filter the supernatant through a glass filter (1G4), leaving as much precipitate as possible in the flask. Next, wash the precipitate in the flask with warm water, carefully filter the washings through the glass filter, and discard the filtrate. Repeat this washing and filtering process until the washings are free of alkali. To the precipitate in the flask, add 20 ml of ferric sulfate TS to dissolve, and filter through the glass filter. Wash the inside of the flask and the glass filter with water, and combine the filtrate and washings. Heat the resulting solution to 80ºC, and titrate with 0.02 mol/L potassium permanganate. The volume of potassium permanganate solution consumed is not more than 3.2 ml.

Loss on drying Not more than 14.0% (105ºC, not more than 0.67 kPa, 4 hours).

Residue on ignition Not more than 0.10% (550ºC).

Assay

Test Solution Weigh accurately about 0.5 g of β-Cyclodextrin, previously dried, add about 35 ml of hot water to dissolve completely, and cool. Add water to make exactly 50 ml.

Standard Solutions Prepare 3 standard solutions with different concentrations by the following procedure: Weigh accurately about 0.7 g of β-cyclodextrin for assay, previously dried, add 45 ml of hot water to dissolve completely. After cooling, add water to make exactly 50 ml (standard solution 1). Transfer exactly 5 ml of this solution into 10-ml and 20-ml volumetric flasks, and add water exactly to volume (standard solutions 2 and 3).

Procedure Analyze 10 μl portions of the test solution and the standard solutions by liquid chromatography using the operating conditions given below. Prepare a calibration curve by measuring the peak areas of β-cyclodextrin for the standard solutions. Determine the amount of β-cyclodextrin (g) in the test solution, using the calibration curve and the peak area of β-cyclodextrin for the test solution. Calculate the content of β-cyclodextrin by the formula:

$$\text{Content (\%) of β - cyclodextr in } (C_{42}H_{70}O_{35}) = \frac{\left(\text{Amount (g) of β - cyclodextr in in the test solution}\right)}{\text{Weight (g) of the sample}} \times 100$$

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube of 10 mm internal diameter and 20–50 cm length.

Column packing material: 9- to 10-μm strongly acidic cation exchange resin for liquid chromatography.

Column temperature: A constant temperature of 50–80ºC.

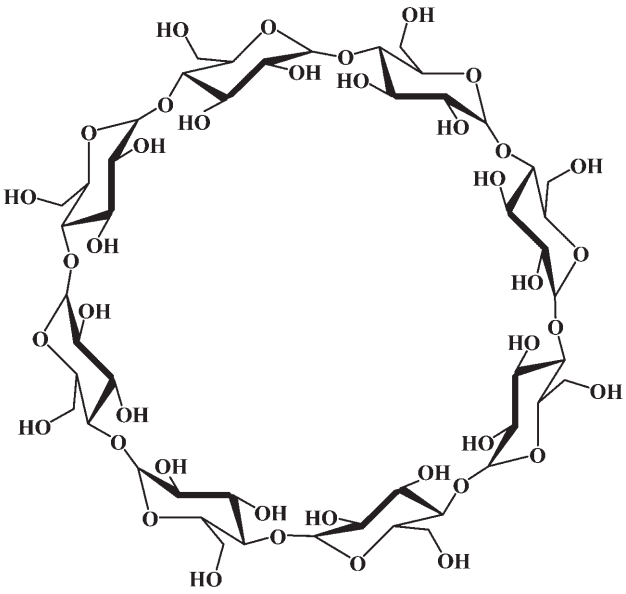
Mobile phase: Water.

Flow rate: A constant rate of 0.3–1.0 ml/min.

* β-Cyclodextrin belongs to the "Cyclodextrin" category contained in the List of Existing Food Additives.

γ-Cyclodextrin

γ-シクロデキストリン



$C_{48}H_{80}O_{40}$ Mol. Wt. 1297.14

Cyclomaltooctaose [17465-86-0]

Definition γ-Cyclodextrin* is a non-reducing cyclic dextrin obtained by enzymatic treatment of starch. It is a cyclic oligosaccharide consisting of eight D-glucose units.

Content γ-Cyclodextrin, when dried, contains not less than 98.0% of γ-cyclodextrin ($C_{48}H_{80}O_{40}$).

Description γ-Cyclodextrin occurs as white crystals or crystalline powder. It is odorless and has a slight sweet taste.

Identification To 0.2 g of γ-Cyclodextrin, add 2 ml of iodine TS, dissolve by warming in a water bath, and allow to stand at room temperature. A red-brown precipitate is formed.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +172 to +178°.

Weigh accurately about 1 g of γ-Cyclodextrin, dried previously, and add water to make exactly 100 ml. Measure the angular rotation of this solution within 30 minutes.

(2) Clarity and color of solution Colorless and clear (0.50 g, water 50 ml).

(3) Chloride Not more than 0.018% as Cl (0.50 g, Control solution 0.01 mol/L Hydrochloric acid 0.25 ml).

(4) Heavy metals Not more than 5.0 μg/g as Pb (4.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(5) Lead Not more than 1.0 μg/g as Pb (10.0 g, Method 1).

(6) Arsenic Not more than 1.3 μg/g as As_2O_3 (1.5 g, Method 2, Apparatus B).

(7) Reducing substances Weigh exactly 1.0 g of γ-Cyclodextrin, previously dried, dissolve it in 25 ml of water, add 40 ml of Fehling's TS, and boil gently for 3 minutes. After cooling, carefully filter the supernatant through a glass filter (1G4), leaving as much precipitate as possible in the flask. Wash the precipitate in the flask with warm water, filter the washings through the glass filter, and discard the filtrate. Repeat this washing and filtering process until the washings are free of alkali. To the precipitate in the flask, add 20 ml of ferric sulfate TS to dissolve, and filter through the glass filter. Wash the inside of the flask and glass filter with water, and combine the filtrate and washings. Heat to 80ºC, and titrate with 0.02 mol/L potassium permanganate solution. The volume of the potassium permanganate consumed is not more than 3.2 ml.

Loss on Drying Not more than 14.0% (105ºC, not more than 0.67 kPa, 4 hours).

Residue on Ignition Not more than 0.10% (550ºC).

Assay

Test Solution Weigh accurately about 0.5 g of γ-Cyclodextrin, previously dried, dissolve it in about 35 ml of hot water, and cool. Add water to make exactly 50 ml.

Standard Solutions Prepare three standard solutions with different concentrations by the following procedure: Weigh accurately about 0.7 g of γ-cyclodextrin for assay, previously dried, and dissolve it in 45 ml of hot water. After cooling, add water to make exactly 50 ml (standard solution 1). Transfer exactly 5 ml of this solution into 10-ml and 20-ml volumetric flasks, and add water exactly to volume (standard solutions 2 and 3).

Procedure Analyze 10 μl portions of the test solution and the standard solutions by liquid chromatography using the operating conditions given below. Prepare a calibration curve by measuring the peak areas of γ-cyclodextrin for the standard solutions. Determine the amount (g) of γ-cyclodextrin in the test solution from the calibration curve and the peak area of γ-cyclodextrin for the test solution. Calculate the content of γ-cyclodextrin by the formula:

$$\text{Content (\%) of γ - cyclodextrin } (C_{48}H_{80}O_{40}) = \frac{\left(\text{Amount (g) of γ - cyclodextrin in the test solution}\right)}{\text{Weight (g) of the sample}} \times 100$$

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube of 5–10 mm internal diameter and 20–50 cm length.

Column packing material: 9- to 10-μm strongly acid cation exchange resin for liquid chromatography.

Column temperature: A constant temperature of 50–80ºC.

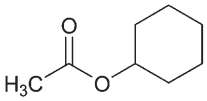
Mobile phase: Water.

Flow rate: A constant rate of 0.3–1.0 ml/min.

* γ-Cyclodextrin belongs to the "Cyclodextrin" category contained in the List of Existing Food Additives.

Cyclohexyl Acetate

酢酸シクロヘキシル



$C_8H_{14}O_2$ Mol. Wt. 142.20

Cyclohexyl acetate [622-45-7]

Content Cyclohexyl Acetate contains not less than 98.0%

of cyclohexyl acetate ($C_8H_{14}O_2$).

Description Cyclohexyl Acetate is a colorless or slightly yellowish, transparent liquid having a characteristic odor.

Identification

(1) Place about 2 ml of Cyclohexyl Acetate in an evaporating dish, add 1 ml of nitric acid, heat in a water bath for 20 minutes, and evaporate to dryness on a hot-plate, taking care not to carbonize it. After cooling, dissolve it in 4 ml of water and 0.5 ml of sodium hydroxide solution (1 in 25), add diluted nitric acid (1 in 10) to make the solution slightly acidic, transfer to a test tube, and add 1 ml of silver nitrate solution (1 in 50). A white precipitate is formed. Add diluted nitric acid (1 in 10) to make it strongly acidic. The precipitate dissolves.

(2) To 1 ml of Cyclohexyl Acetate, add 5 ml of ethanolic 10% potassium hydroxide TS, and heat under a reflux condenser in a water bath for 1 hour. The characteristic odor disappears. After cooling, and add 8 ml of water and 1 ml of diluted hydrochloric acid (1 in 4). The solution responds to test (3) for Acetate in the Qualitative Tests.

Purity

(1) Refractive index n_D^{20}: 1.439–1.442.

(2) Specific gravity 0.970–0.973.

(3) Clarity of solution Clear (2.0 ml, 70% (vol) ethanol 4.0 ml).

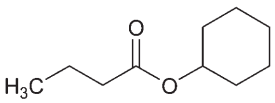
(4) Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Weigh accurately about 1 g of Cyclohexyl Acetate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 71.10 mg of $C_8H_{14}O_2$

Cyclohexyl Butyrate

酪酸シクロヘキシル



$C_{10}H_{18}O_2$ Mol. Wt. 170.25

Cyclohexyl butanoate [1551-44-6]

Content Cyclohexyl Butyrate contains not less than 98.0% of cyclohexyl butyrate ($C_{10}H_{18}O_2$).

Description Cyclohexyl Butyrate is a colorless or slightly yellowish, transparent liquid having a characteristic odor.

Identification

(1) To 1 ml of Cyclohexyl Butyrate, add 5 ml of ethanolic 10% potassium hydroxide TS, and heat under a reflux condenser in a water bath for 1 hour. The characteristic odor disappears. After cooling, add diluted sulfuric acid (1 in 20) to make it acidic, and shake in warm water. An odor of butyric acid is evolved.

(2) Place 0.2 ml of Cyclohexyl Butyrate in an evaporating dish, add 1 ml of nitric acid, heat in a water bath for 20 minutes, and evaporate to dryness on a hot-plate, taking care not to carbonize. After cooling, dissolve the residue by adding 4 ml of water and 0.5 ml of sodium hydroxide solution (1 in 25), and then add diluted nitric acid (1 in 10) to make it slightly acidic. Transfer the solution to a test tube, and add 1 ml of silver nitrate solution (1 in 50). A white precipitate is formed. When the solution is made strongly acidic with diluted nitric acid (1 in 10) the precipitate dissolves.

Purity

(1) Refractive index n_D^{20}: 1.441–1.444.

(2) Specific gravity 0.941–0.945.

(3) Clarity of solution Clear (1.0 ml, 70% (vol) ethanol 5.0 ml).

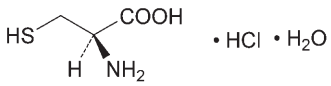
(4) Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Weigh accurately about 1 g of Cyclohexyl Butyrate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 85.12 mg of $C_{10}H_{18}O_2$

L-Cysteine Monohydrochloride

L-システイン塩酸塩



$C_3H_7NO_2S \cdot HCl \cdot H_2O$ Mol. Wt. 175.64

(2*R*)-2-Amino-3-sulfanylpropanoic acid monohydrochloride monohydrate [7048-04-6]

Content L-Cysteine Monohydrochloride, when calculated on the dried basis, contains 98.0–102.0% of L-cysteine monohydrochloride ($C_3H_7NO_2S \cdot HCl$ = 157.62).

Description L-Cysteine Monohydrochloride occurs as colorless to white crystals or as a white crystalline powder having a characteristic odor and taste.

Identification

(1) To 5 ml of a solution of L-Cysteine Monohydrochloride (1 in 1,000), add 0.5 ml of pyridine and 1 ml of ninhydrin solution (1 in 100), and heat for 5 minutes. A purple to purple-brown color develops.

(2) To 10 ml of a solution of L-Cysteine Monohydrochloride (1 in 1,000), add 2 ml of sodium hydroxide solution (1 in 25) and 2 drops of sodium nitroprusside solution (1 in 20). A purple-red color develops.

(3) To 10 ml of a solution of L-Cysteine Monohydrochloride (1 in 50), add 1 ml of hydrogen peroxide, and heat in a water bath for 10 minutes. The resulting solution responds to test (2) for Chloride in the Qualitative Tests.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +5.0 to +8.0° (4.0 g, hydrochloric acid (1 in 10), 50 ml, on the dried basis).

(2) Clarity and color of solution Colorless and almost clear (1.0 g, water 20 ml).

(3) Heavy metals Not more than 20 µg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 µg/g as As_2O_3.

Test Solution Weigh 0.50 g of L-Cysteine Monohydrochloride, transfer into a Kjeldahl flask, add 5 ml of sulfuric acid and 5 ml of nitric acid, and heat. Continue heating

while occasionally adding 2–3 ml of nitric acid until the solution is colorless to light yellow. After cooling, add 15 ml of a saturated solution of ammonium oxalate, and concentrate to 2–3 ml by heating until white fumes are evolved. After cooling, add water to make 10 ml. Use 5 ml of this solution as the test solution.

Standard Color Measure 2.0 ml of Arsenic Standard Solution, transfer into a Kjeldahl flask, add 5 ml of sulfuric acid and 5 ml of nitric acid, heat, and proceed in the same manner as the preparation of the test solution.

Apparatus Use Apparatus B.

(5) Cystine

Test Solution Weigh 0.20 g of L-Cysteine Monohydrochloride, dissolve it in *N*-ethylmaleimide solution (1 in 50) to make 100 ml. Measure 2 ml of this solution, add *N*-ethylmaleimide solution (1 in 50) to make 20 ml, and allow to stand for 30 minutes.

Procedure Analyze a 5 µl portion of the test solution by thin-layer chromatography, using a 2:1:1 mixture of 1-butanol/water/acetic acid as the developing solvent. No control solution is used. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110ºC for 1 hour. Stop the development when the solvent front has ascended to a point about 15 cm above the original line, and dry the plate at 80ºC for 30 minutes. Spray with a (1 in 100) solution of ninhydrin in a 97:3 mixture of methanol/acetic acid, and heat at 80ºC for 10 minutes to fix a color. Examine in daylight. Only one spot is observed.

Loss on Drying 8.0–12.0% (not more than 0.7 kPa, 24 hours).

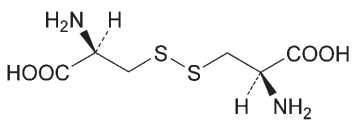
Residue on Ignition Not more than 0.20%.

Assay Weigh accurately about 0.25 g of L-Cysteine Monohydrochloride, dissolve it in 20 ml of water, and add 4 g of potassium iodide to dissolve. To this solution, add 5 ml of diluted hydrochloric acid (1 in 4) and exactly 25 ml of 0.05 mol/L iodine, and allow to stand in ice water for 20 minutes in a dark place. Titrate the excess iodine with 0.1 mol/L sodium thiosulfate (indicator: starch TS). Perform a blank test in the same manner.

Each ml of 0.05 mol/L iodine = 15.76 mg of $C_3H_7NO_2S{\cdot}HCl$

L-Cystine

L-シスチン



$C_6H_{12}N_2O_4S_2$ Mol. Wt. 240.30

(2*R*,2*R*′)-3,3′-Disulfanylbis[2-amino-3-sulfanylpropanoic acid] [56-89-3]

Content L-Cystine, when calculated on the dried basis, contains 98.0–120.0% of L-cystine ($C_6H_{12}N_2O_4S_2$).

Description L-Cystine occurs as white crystals or crystalline powder. It has a very slight characteristic odor, and it is tasteless or has a very slight characteristic taste.

Identification

(1) To 5 ml of a saturated solution of L-Cystine, add 1 ml of ninhydrin solution (1 in 50), and heat for 3 minutes in a water bath. A purple color develops.

(2) To 3 ml of a solution of L-Cystine in 2 mol/L hydrochloric acid (1 in 30), add 0.04 g of zinc powder, and heat for 10 minutes in a water bath. After cooling, filter if necessary. Add 10 ml of sodium hydroxide solution (1 in 20), shake, and add 1 drop of sodium nitroprusside solution (1 in 20). A reddish purple color develops.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: –215 to –230°.

Weigh accurately about 2 g of L-Cystine, and dissolve it in 1 mol/L hydrochloric acid to make exactly 100 ml. Measure the angular rotation of this solution and calculate on the dried basis.

(2) Clarity and color of solution Colorless and clear (1.0 g, 1 mol/L hydrochloric acid 20 ml).

(3) pH 5.0–6.5 (saturated solution).

(4) Chloride Not more than 0.1% as Cl (0.07 g, Control solution 0.01 mol/L hydrochloric acid 0.20 ml).

(5) Heavy metals Not more than 20 µg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(6) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Loss on Drying Not more than 0.30% (105ºC, 3 hours).

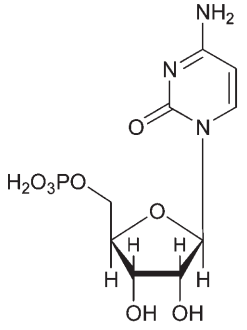
Residue on Ignition Not more than 0.10%.

Assay Weigh accurately about 0.3 g of L-Cystine, proceed as directed in the Kjeldahl Method under Nitrogen Determination, and calculate on the dried basis. In decomposing the sample, use 0.2 g of selenium dioxide as a decomposition-promoting agent, and heat for 4 hours.

Each ml of 0.05 mol/L sulfuric acid = 12.02 mg of $C_6H_{12}N_2O_4S_2$

5′-Cytidylic Acid

5′-シチジル酸



$C_9H_{14}N_3O_8P$ Mol. Wt. 323.20

Cytidine 5′-monophosphoric acid [63-37-6]

Definition 5′-Cytidylic Acid is obtained by enzymatic hydrolysis of the nucleic acids that are water extracted from the yeast *Candida utilis* in the presence of salt, followed by isolation. It consists of 5′-cytidylic acid ($C_9H_{14}N_3O_8P$).

Content 5′-Cytidylic Acid, when calculated on the dried basis, contains 98.0–102.0% of 5′-cytidylic acid ($C_9H_{14}N_3O_8P$).
Description 5′-Cytidylic Acid occurs as colorless or white crystals or crystalline powder.
Identification
(1) Dissolve 0.010 g of 5′-Cytidylic Acid in 1,000 ml of diluted hydrochloric acid (1 in 1,000). This solution exhibits an absorption maximum at a wavelength of 277–281 nm.
(2) Dissolve 0.25 g of 5′-Cytidylic Acid in 1 ml of sodium hydroxide TS, add 5 ml of water, and then add 2 ml of magnesia TS. No precipitate is formed. To this solution, add 7 ml of nitric acid, and boil for 10 minutes. It responds to test (2) for Phosphate as described in the Quantitative Tests.
Purity
(1) Clarity and color of solution Colorless and almost clear.
Test Solution Weigh 0.50 g of 5′-Cytidylic Acid, dissolve it in 2 ml of sodium hydroxide TS, and add water to make 20 ml.
(2) Heavy metals Not more than 10 μg/g as Pb.
Test Solution Weigh 2.0 g of 5′-Cytidylic Acid, and add 8 ml of sodium hydroxide TS and 30 ml of water to dissolve. Neutralize the solution with diluted acetic acid (1 in 20) or ammonia TS, and then add 2 ml of the acetic acid (1 in 20) and water to make 50 ml.
Control Solution Measure exactly 2 ml of Lead Standard Solution, and add 2 ml of the acetic acid (1 in 20) and water to make 50 ml.
(3) Arsenic Not more than 4.0 μg/g as As_2O_3.
Test Solution Weigh 0.50 g of 5′-Cytidylic Acid, and dissolve it in 5 ml of diluted hydrochloric acid (1 in 4). Use apparatus B.
(4) Absorbance ratio Weigh 0.010 g of 5′-Cytidylic Acid, dissolve it in diluted hydrochloric acid (1 in 1,000), and make 1,000 ml. When the absorbances of the solution at 250 nm, 260 nm, and 280 nm are expressed as A_1, A_2, and A_3, respectively, A_1/A_2 is 0.40–0.52 and A_3/A_2 is 1.85–2.20.
(5) Other nucleic acid decomposition products
Test Solution Weigh 0.010 g of 5′-Cytidylic Acid, dissolve it in 0.5 ml of sodium hydroxide TS, and add water to make 20 ml.
Procedure Analyze a 1-μl portion of the test solution by thin-layer chromatography using a 6:5:2 mixture of 1-propanol/ammonia TS/acetone as the developing solvent. No control solution is used. Use a thin-layer plate coated with fluorescent silica gel for thin-layer chromatography and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the original line, air-dry the plate, and examine under ultraviolet light (around 250 nm) in a dark place. Only one spot is observed.
Loss on Drying Not more than 6.0% (120°C, 4 hours).
Assay Weigh accurately about 0.2 g of 5′-Cytidylic Acid, dissolve it in 1 ml of sodium hydroxide TS, add water to make exactly 200 ml. Measure exactly 2 ml of this solution, and add diluted hydrochloric acid (1 in 1,000) to make exactly 100 ml. Measure the absorbance (A) of the prepared solution at 280 nm, and calculate the content by the formula:

$$\text{Content (\%) of 5'-cytidylic acid } (C_9H_{14}N_3O_8P) = \frac{0.2 \times 1.224 \times A}{\text{Dry basis weight (g) of the sample}} \times 100$$

Dammar Resin

ダンマル樹脂

Definition Dammar Resin is obtained from the exudate of trees of *Shorea* spp., *Hopea* spp., or *Agathis* spp. and consists mainly of resin and polysaccharides.
Description There are two types of Dammar Resin: Crude Dammar Resin and Purified Dammar Resin. Crude Dammar Resin occurs as a white to yellow or brown, irregular shaped powder, flakes, or lumps. Purified Dammar Resin occurs as a white to light yellow powder, flakes, or lumps.
Identification
(1) To 1 g of powdered Dammar Resin, add 100 ml of water. It does not dissolve. To 1 g of powdered Dammar Resin, add 9 ml of toluene. It almost dissolves.
(2) Prepare a test solution by dissolving powdered Dammar Resin in toluene (1 in 10). Analyze a 2 μl portion of the test solution by thin-layer chromatography, using a 6:5 mixture of diethyl ether/heptane as the developing solvent. No control solution is used. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 105°C for 2 hours. Stop the development when the solvent front has ascended to a point about 10 cm above the original line, and air-dry the plate. Spray with sulfuric acid, and heat for 10 minutes at 105°C. Spots are observed at R_f values of about 0.7 and 0.8.
Purity
(1) Acid value 20–40.
Weigh accurately about 1.0 g of powdered Dammar Resin, and proceed as directed in the Acid Value Test in the Fats and Related Substances Tests.
(2) Softening point 86–100°C.
(i) Apparatus Use the apparatus outlined in Fig. 1 to Fig. 5.
A: Steel ball (9.5 mm in diameter, 3.5 g in weight)
B: Ring (made of brass, outlined in Fig. 2)
C: Ring holder (made of metal, outlined in Fig. 3)
D: Bottom plate (with 40 circulating holes (J), outlined in Fig. 4)
E: Holding plate (outlined in Fig. 5)
F: Thermometer No. 1 (set the center of the mercury bulb at the same level as the lower surface of ring holder C)
G: Glass container (not less than 85 mm in inner diameter, not less than 127 mm in height)
H: Holding hole
I: Hole to enter the mercury bulb of thermometer
J: Circulating hole (about 4 mm in diameter)

(ii) Procedure Melt Dammar Resin rapidly at as low a temperature as possible. Fill the melted sample into the rings horizontally placed on a flat metal plate, taking care not to allow air bubbles to form in the ring. After cooling, cut off the excess sample over the upper end of the ring by a knife slightly heated previously. Place the holder in glass container G, pour previously boiled and cooled water to not less than 90 mm of depth. Then, immerse in the water ball A and each of the rings filled with the sample, avoiding direct contact of the ball and the ring, and keep for 15 minutes at 20°C. Then place the ball on the center of the sample in the ring, and place the ring with the ball at the fixed place on the

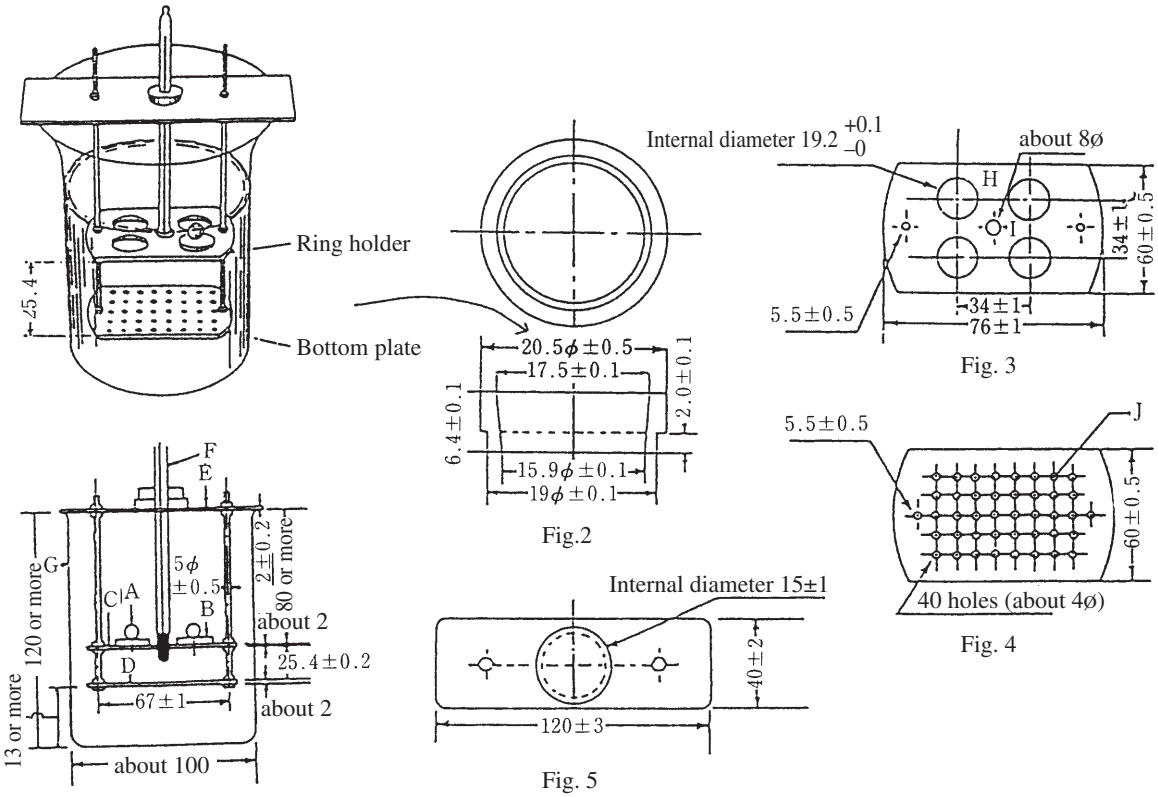


Fig.1 Fig.2 Fig. 3 Fig. 4 Fig. 5

(Unit: mm Fig.1 – Fig. 5)

holder. Keep the top of ring 50 mm below the water surface, and place a thermometer so that the center of its mercury bulb comes to the same height as the center of ring. Heat the container by a Bunsen burner so that the flame comes to between the brim and center of the bottom of the container. When the temperature of the water reaches 40°C, raise at a rate of 5.0±0.5°C per minute. Measure the temperature when the softened sample flows off from the ring and reaches the bottom plate. Determine the average of two measurements or more as the softening point.

(3) Iodine value 10–40.

Weigh accurately about 1 g of powdered Dammar Resin into a glass container. Place the glass container into a 500-ml flask with a ground-glass stopper, and add 10 ml of toluene to dissolve. Add exactly 25 ml of Wijs TS, and stir vigorously. Add toluene again if the solution is not clear to make the clear solution. Proceed as directed in the Iodine Value Test in the Fats and Related Substances Tests.

(4) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(5) Lead Not more than 10 μg/g as Pb (1.0 g, Method 1).

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

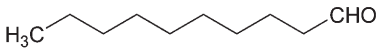
Loss on Drying Not more than 6.0% (105°C, 18 hours).

Ash Not more than 0.5%.

Decanal

Decyl Aldehyde

デカナール



$C_{10}H_{20}O$ Mol. Wt. 156.27

Decanal [112-31-2]

Content Decanal contains not less than 93.0% of decanal ($C_{10}H_{20}O$).

Description Decanal is a colorless to light yellow, transparent liquid having a characteristic odor.

Identification To 1 ml of Decanal, add 3 ml of sodium hydrogen sulfite TS, and shake. The mixture evolves heat immediately and forms crystalline lumps.

Purity

(1) Refractive index n_D^{20}: 1.427–1.435.

(2) Specific gravity 0.826–0.835.

(3) Clarity of solution Clear (2.0 ml, 70% (vol) ethanol 6.0 ml).

(4) Acid value Not more than 10.0 (Flavoring Substances Tests).

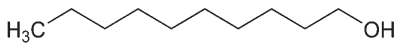
Assay Weigh accurately about 1 g of Decanal, and proceed as directed in Method 2 in the Aldehyde and Ketone Content Test in the Flavoring Substances Tests. Allow the mixture to stand for 15 minutes before titrating.

Each ml of 0.5 mol/L hydrochloric acid = 78.13 mg of $C_{10}H_{20}O$

Decanol

Decan-1-ol
Decyl Alcohol

デカノール



$C_{10}H_{22}O$ Mol. Wt. 158.28
Decan-1-ol [112-30-1]
Content Decanol contains not less than 98.0% of decanol ($C_{10}H_{22}O$).
Description Decanol is a colorless or slightly yellowish, transparent liquid having a characteristic odor.
Identification To 2–3 drops of Decanol, add 5 ml of potassium permanganate solution (1 in 20) and 1 ml of diluted sulfuric acid (1 in 20), and shake. An odor of decanal is evolved.
Purity
(1) Congealing point Not less than 5°C.
(2) Refractive index n_D^{20}: 1.435–1.438.
(3) Specific gravity 0.826–0.831.
(4) Clarity of solution Clear (2.0 ml, 70% (vol) ethanol 4.0 ml).
(5) Acid value Not more than 1.0 (Flavoring Substances Tests).
Assay Proceed as directed in Method 1 in the Alcohol Content Test in the Flavoring Substances Tests, using about 1 g of acetylated oil.

Dextran

デキストラン

Definition Dextran is obtained by isolation from the culture fluid of the Gram-positive bacterium *Leuconostoc mesenteroides* or *Streptococcus equinus*. It consists of dextran.
Description Dextran occurs as a white to light yellow powder or granules having no odor.
Identification To 1 ml of a solution of dextran (1 in 3,000), add 2 ml of anthrone TS. A blue-green color develops, and it gradually changes to dark blue-green. The color does not change any more on the addition of 1 ml of diluted sulfuric acid (1 in 2) or acetic acid.
Purity
(1) Heavy metals Not more than 40 μg/g as Pb (0.50 g, Method 1, Control solution Lead Standard Solution 2.0 ml).
(2) Lead Not more than 10 μg/g as Pb (1.0 g, Method 1).
(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).
(4) Total nitrogen Not more than 1.0%.
Wight accurately about 0.5 g of Dextran, and proceed as directed in the Semi-micro Kjeldahl Method under Nitrogen Determination.
Loss on Drying Not more than 10.0 % (105°C, 6 hours).
Residue on Ignition Not more than 2.0%.
Microbial Limits Proceed as directed in the Microbial Limit Tests. The total bacterial count is not more than 1,000/g, and *Escherichia coli* is negative.

Diammonium Hydrogen Phosphate

Ammonium Phosphate, Dibasic

リン酸水素二アンモニウム

$(NH_4)_2HPO_4$ Mol. Wt. 132.06
Diammonium hydrogenphosphate [7783-28-0]
Content Diammonium Hydrogen Phosphate contains 96.0–102.0% of diammonium hydrogen phosphate ($(NH_4)_2HPO_4$).
Description Diammonium Hydrogen Phosphate occurs as colorless to white crystals or as a white crystalline powder having an odor of ammonia.
Identification Diammonium Hydrogen Phosphate responds to all tests for Ammonium Salt and for Phosphate in the Qualitative Tests.
Purity
(1) Clarity and color of solution Colorless and almost clear (1.0 g, water 20 ml).
(2) pH 7.6–8.4 (1.0 g, water 100 ml).
(3) Chloride Not more than 0.035% as Cl (0.50 g, Control solution 0.01 mol/L hydrochloric acid 0.50 ml).
(4) Sulfate Not more than 0.038% as SO_4 (0.50 g, Control solution 0.005 mol/L sulfuric acid 0.40 ml).
(5) Heavy metals Not more than 20 μg/g as Pb.
Test Solution Weigh 1.0 g of Diammonium Hydrogen Phosphate, dissolve it in about 25 ml of water, neutralize with diluted acetic acid (1 in 20), and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.
Control Solution To 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.
(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).
Assay Weigh accurately about 2 g of Diammonium Hydrogen Phosphate, dissolve it in 50 ml of water, keep at about 15°C, and titrate with 1 mol/L hydrochloric acid (indicator: 3–4 drops of methyl orange–xylene cyanol FF TS).
Each ml of 1 mol/L hydrochloric acid = 132.1 mg of $(NH_4)_2HPO_4$

Diatomaceous Earth

ケイソウ土

Definition Diatomaceous Earth is silicon dioxide derived from diatom and consists mainly of silicon dioxide. There are three types of products: dried, burned, and flux burned. They are called Diatomaceous Earth (dried), Diatomaceous Earth (burned), and Diatomaceous Earth (flux burned), re-

spectively.

Burned products are obtained by burning diatomaceous earth at 800–1,200ºC, and flux burned products are obtained by burning diatomaceous earth at 800–1,200°C with a small amount of alkali salt of carbonic acid. Acid-treated flux burned products should be followed by the directions for burned products.

Description Diatomaceous Earth (dried) occurs as a whitish or light gray powder; Diatomaceous Earth (burned) occurs as a light yellow to light orange or pink to light brown powder; Diatomaceous Earth (flux burned) occurs as a white or light red-brown powder.

Identification

(1) Place 0.2 g of Diatomaceous Earth in a platinum crucible, dissolve it in 5 ml of hydrofluoric acid, and heat. Almost all of it evaporates.

(2) Observe Diatomaceous Earth, magnified 100–200 times under a microscope. The characteristic porous diatom skeleton is observed.

Purity

(1) pH

Dried products and burned products: 5.0–10.0.

Flux burned products: 8.0–11.0.

Weigh 10.0 g of Diatomaceous Earth, previously dried, into a flask, add 100 ml of water, and boil gently for 2 hours while stirring with a stirrer and replenishing the lost water. Cool, and filter with suction, using a filter holder equipped with a 47 mm-diameter membrane filter (0.45 μm in pore diameter). If the filtrate is turbid, filter with suction again through the same filter. Wash the flask and the residue on the filter with water, combine the filtrate and the washings, and add water to make 100 ml. Refer to this solution as solution A. Perform the test on solution A.

(2) Water-soluble substances Not more than 0.50%.

Measure 50 ml of solution A prepared in Purity (1), evaporate to dryness, dry the residue at 105°C for 2 hours, and weigh.

(3) Hydrochloric acid-soluble substances Not more than 2.5%.

Weigh 2.0 g of Diatomaceous Earth, previous dried, into a flask, add 50 ml of diluted hydrochloric acid (1 in 4), and warm at 50°C for 15 minutes with occasional shaking. Cool and filter the mixture, and wash the flask and the residue on the filter paper with 3 ml of diluted by hydrochloric acid (1 in 4). Combine the filtrate and the washings. Add 5 ml of diluted sulfuric acid (1 in 20) to this solution, evaporate to dryness, ignite at 450 –550°C to constant weight, and weigh.

(4) Heavy metals Not more than 50 μg/g as Pb.

Test Solution Weigh 2.0 g of Diatomaceous Earth into a flask, add 50 ml of dilute hydrochloric acid (1 in 4), cover with a watch glass, and heat at 70°C for 15 minutes while stirring. After cooling, filter the supernatant through a filter paper for a quantitative analysis (5C). Wash the residues in the flask three times with 10 ml of warm water each time, filter through the same filter paper, and wash the filter paper and the residues on the paper with 15 ml of water. Combine the filtrate and the washings, add water to make 100 ml, and refer to this solution as solution B. Measure 20 ml of solution B, evaporate to dryness on a water bath, dissolve the residue by adding 2 ml of diluted acetic acid (1 in 20) and 20 ml of water, filter if necessary, and add water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(5) Lead Not more than 10 μg/g as Pb.

Test Solution Measure 25 ml of solution B prepared in Purity (4), evaporate to dryness on a water bath, and dissolve the residue in diluted hydrochloric acid (1 in 10) to make 10 ml.

Control Solution To 1.0 ml of Lead Standard Solution, add diluted hydrochloric acid (1 in 10) to make 20 ml.

Procedure Proceed as directed in Method 1 in the Lead Limit Test.

(6) Arsenic Not more than 10 μg/g as As_2O_3.

Test Solution Measure 10 ml of solution B prepared in Purity (4).

Apparatus Use Apparatus B.

Loss on Drying

Dried products: Not more than 10.0% (105°C, 2 hours).

Burned products and flux burned products: Not more than 3.0% (105°C, 2 hours).

Loss on Ignition Dry Diatomaceous Earth at 105°C for 2 hours, and immediately perform the test, using it as the sample.

Dried products: Not more than 7.0% (1,000°C, 30 minutes).

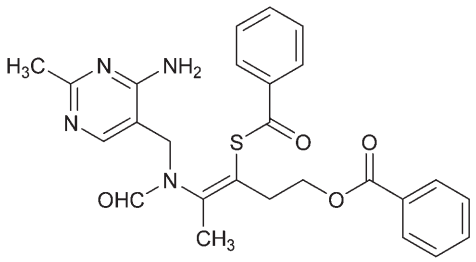
Burned products and flux burned products: Not more than 2.0% (1,000°C, 30 minutes).

Hydrofluoric Acid Residue Not more than 25.0%.

Ignite a platinum crucible at 1,000°C for 30 minutes, allow to cool in a desiccator, and weigh accurately. Weigh accurately about 0.2 g of Diatomaceous Earth, transfer into the platinum crucible, and weigh accurately. Add 5 ml of hydrofluoric acid and 2 drops of diluted sulfuric acid (1 in 2), evaporate almost completely to dryness on a water bath, and cool. To the residue, add 5 ml of hydrofluoric acid, evaporate to dryness, and heat at 550°C for 1 hour. Raise the temperature gradually, ignite at 1,000°C for 30 minutes, allow to cool in a desiccator, and weigh accurately.

Dibenzoyl Thiamine

ジベンゾイルチアミン



$C_{26}H_{26}N_4O_4S$ Mol. Wt. 490.58

4-[*N*-[(4-Amino-2-methylpyrimidin-5-ylmethyl)formamido]-3-(benzoylsulfanyl)pent-3-en-1-yl benzoate [299-88-7]

Content Dibenzoyl Thiamine, when dried, contains not less than 97.0% of dibenzoyl thiamine ($C_{26}H_{26}N_4O_4S$).

Description Dibenzoyl Thiamine occurs as a white crystalline powder. It is odorless.

Identification

(1) To 0.03 g of Dibenzoyl Thiamine, add 7 ml of diluted hydrochloric acid (1 in 100), and dissolve while heating in a water bath. To this solution, add 2 ml of a 1:1 mixture of hydroxylamine hydrochloride solution (3 in 20)/sodium hydroxide solution (3 in 20), shake for 1 minute, and add 0.8 ml of hydrochloric acid and 0.5 ml of iron(III) chloride solution (1 in 10). A purple color develops.

(2) To 5 mg of Dibenzoyl Thiamine, add 1 ml of methanol, and dissolve while warming. Add 2 ml of water, 2 ml of cysteine hydrochloride solution (1 in 100), and 2 ml of phosphate buffer (pH 7), shake, and allow to stand for 30 minutes. To this solution, add 1 ml of freshly prepared potassium ferricyanide solution (1 in 10), 5 ml of sodium hydroxide solution (1 in 50) and 5 ml of 2-methyl-1-propanol, shake vigorously for 2 minutes, and allow to stand to separate the solution into two layers. Expose the liquid to ultraviolet light from above, and examine the top of the upper-layer solution from perpendicular direction and from the irradiation direction. A blue-purple fluorescence is observed. This fluorescence disappears when the solution is made acidic, and reappears when it is made alkaline.

Purity

(1) Melting point 163–174°C (decomposition).

(2) Chloride Not more than 0.053% as Cl.

Test Solution Weigh 0.40 g of Dibenzoyl Thiamine, dissolve it in 20 ml of methanol, and add 6 ml of diluted nitric acid (1 in 10) and water to make 50 ml.

Control Solution To 0.60 ml of 0.01 mol/L hydrochloric acid, add 20 ml of methanol, 6 ml of diluted nitric acid (1 in 10) and water to make 50 ml.

(3) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

Loss on Drying Not more than 3.0% (105°C, 2 hours).

Residue on Ignition Not more than 0.20%.

Assay

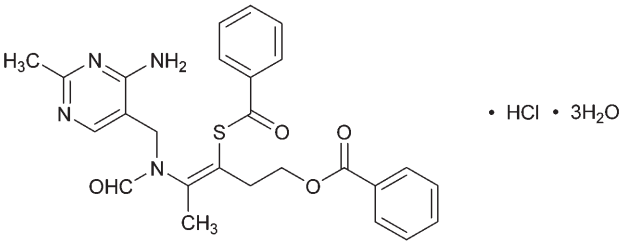
Test Solution Weigh accurately about 0.4 g of Dibenzoyl Thiamine, previously dried, add 40 ml of methanol and 40 ml of diluted hydrochloric acid (1 in 100) to dissolve, and add water to make exactly 1,000 ml. Measure exactly 5 ml of this solution, and add diluted hydrochloric acid (1 in 100) to make exactly 250 ml.

Procedure Measure the absorbance (A) of the test solution at a wavelength of 237 nm, using water as the reference. Perform a blank test in the same manner, take the absorbance as A_0, and calculate the content by the formula:

$$\text{Content (\%) of dibenzoyl thiamine } (C_{26}H_{26}N_4O_4S) = \frac{(A - A_0) \times 0.4}{\text{Weight (g) of the sample} \times 0.452} \times 100$$

Dibenzoyl Thiamine Hydrochloride

ジベンゾイルチアミン塩酸塩



$C_{26}H_{26}N_4O_4S \cdot HCl \cdot 3H_2O$ Mol. Wt. 581.08

4-[*N*-[(4-Amino-2-methylpyrimidin-5-ylmethyl)formamido]-3-(benzoylsulfanyl)pent-3-en-1-yl benzoate monohydrochloride trihydrate [35660-60-7]

Content Dibenzoyl Thiamine Hydrochloride, when dried, contains not less than 97.0% of dibenzoyl thiamine hydrochloride ($C_{26}H_{26}N_4O_4S \cdot HCl$ = 527.04).

Description Dibenzoyl Thiamine Hydrochloride occurs as a white crystalline powder. It is odorless.

Identification

(1) Proceed as directed in Identification (1) and (2) for Dibenzoyl Thiamine.

(2) Dissolve 0.1 g of Dibenzoyl Thiamine Hydrochloride in 10 ml of methanol, add 1 ml of diluted nitric acid (1 in 10), and add 1 ml of silver nitrate solution (1 in 50).
A white precipitate is formed.

Purity

(1) Clarity of solution Almost clear.

Test Solution Weigh 1.0 g of Dibenzoyl Thiamine Hydrochloride, add 10 ml of water, and dissolve while heating in a water bath.

(2) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

Loss on Drying Not more than 11.0% (reduced pressure, 24 hours).

Residue on Ignition Not more than 0.20%.

Assay Weigh accurately about 0.4 g of Dibenzoyl Thiamine Hydrochloride, previously dried, proceed as directed in the Assay for Dibenzoyl Thiamine, and calculate the content by the formula:

$$\text{Content (\%) of dibenzoyl thiamine hydrochloride } (C_{26}H_{26}N_4O_4S \cdot HCl) = \frac{(A - A_0) \times 0.4}{\text{Weight (g) of the sample} \times 0.421} \times 100$$

Diluted Benzoyl Peroxide

希釈過酸化ベンゾイル

[Benzoyl peroxide 94-36-0]

Definition Diluted Benzoyl Peroxide is produced by diluting benzoyl peroxide with one or more of the following

food additives and food: “Aluminum Potassium Sulfate,” “Calcium Salts of Phosphate,” “Calcium Sulfate,” “Calcium Carbonate,” “Magnesium Carbonate,” and starch.

Content Diluted Benzoyl Peroxide contains 19.0–22.0% of benzoyl peroxide ($C_{14}H_{10}O_4$ = 242.23).

Description Diluted Benzoyl Peroxide occurs as a white powder.

Identification Place 0.2 g of Diluted Benzoyl Peroxide into a test tube, add 7 ml of chloroform, shake well, and allow to stand. A white insoluble substance remains on the bottom of the test tube. Add 2.0 ml of 4,4′-diaminodiphenylamine TS. The color of the solution and the insoluble substance changes to a blue-green color.

Purity

(1) Fineness Weigh 5.0 g of Diluted Benzoyl Peroxide, transfer into a dried 53-μm standard sieve, shake vigorously in all directions for 2 minutes, occasionally tapping the bottom of the receiver, and allow to stand for 1 minute. After the fine powder has settled, weigh the residue on top of the sieve. It is not more than 1.0 g.

(2) Spread of fire Weigh 1.0 g of Diluted Benzoyl Peroxide, transfer on a glass plate so that it is 3 mm high and 10 mm wide, and light one end. The flame does not spread to the other end.

(3) Hydrochloric acid-insoluble substances Weigh 0.20 g of Diluted Benzoyl Peroxide, add 10 ml of diluted hydrochloric acid (1 in 4), shake well, heat gradually, and boil for about 1 minute. Cool, add about 8 ml of diethyl ether, shake well, and allow to stand. Both liquid layers are clear, and no flocculent substances exist at the interface.

(4) pH 6.0–9.0.

Test Solution Weigh 3.0 g of Diluted Benzoyl Peroxide, add 30 ml of water, shake for 3 minutes, and filter.

(5) Ammonium salt Weigh 0.20 g of Diluted Benzoyl Peroxide, add 3 ml of sodium hydroxide solution (2 in 5), and boil. The evolved gas does not change the color of red litmus paper moistened with water to blue.

(6) Heavy metals Not more than 40 μg/g as Pb.

Test Solution Weigh 1.0 g of Diluted Benzoyl Peroxide, add 7 ml of diluted hydrochloric acid (1 in 4) and 10 ml of water, shake well, and boil gently. Cool, and add water to make 50 ml. Filter, measure 25 ml of the filtrate, adjust the pH to 4.0–4.5 with ammonia TS, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution To 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(7) Barium Weigh 2.0 g of Diluted Benzoyl Peroxide, add 15 ml of diluted nitric acid (1 in 10), shake, and filter. Wash with water, combine the filtrate and the washings, and add water to make 40 ml. Adjust the pH to 2.4–2.8 with ammonia TS, add water to make 50 ml, add 1 ml of diluted sulfuric acid (1 in 20), and allow to stand for 10 minutes. The solution is not turbid.

(8) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.50 g of Diluted Benzoyl Peroxide, add 5 ml of diluted hydrochloric acid (1 in 4), heat gently, cool quickly in ice water, and filter. Wash the residue with 15 ml of water, combine the filtrate and the washings, and add water to make 40 ml. Use 20 ml of this solution as the test solution.

Apparatus Use Apparatus B.

Procedure Proceed as directed in the Arsenic Limit Test, but skip the procedure in which the test solution is neutralized with ammonia solution or ammonia TS.

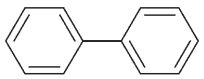
Assay Weigh accurately about 1 g of Diluted Benzoyl Peroxide, transfer into a flask with a ground-glass stopper, add 50 ml of a 1:1 mixture of methanol/chloroform, and shake. Add 0.5 ml of a solution of citric acid in methanol (1 in 10) and 2 ml of potassium iodide solution (1 in 2), immediately stopper tightly, allow to stand in a dark place for 15 minutes with occasional shaking. Titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: starch TS). Perform a blank test in the same manner, and make any necessary correction.

Each ml of 0.1 mol/L sodium thiosulfate = 12.11 mg of $C_{14}H_{10}O_4$

Diphenyl

Biphenyl

ジフェニル



$C_{12}H_{10}$ Mol. Wt. 154.21

Biphenyl [92-52-4]

Content Diphenyl contains 98.0–102.0% of diphenyl ($C_{12}H_{10}$).

Description Diphenyl occurs as colorless to white crystals, crystalline powder, or crystalline lumps having a characteristic odor.

Identification

(1) To 2 drops of a solution of Diphenyl in ethyl acetate (1 in 100), add 0.5 ml of acetic acid and 1 ml of nitric acid, and heat at 70°C for 30 minutes. Cool, add 5 ml of water and 10 ml of ethyl acetate, and shake. Measure 5 ml of the ethyl acetate layer, and evaporate the ethyl acetate. Dissolve the residue in 1 ml of ethanol, add 2 ml of diluted hydrochloric acid (1 in 2) and 0.2 g of zinc dust, and heat in a water bath for 10 minutes. Cool and filter the mixture. To the filtrate, add 50 ml of water, then add 1 ml of sodium nitrite solution (1 in 100), and shake. Allow to stand for 10 minutes, add 1 ml of ammonium sulfamate (1 in 40), and allow to stand for 5 minutes. Add 2 ml of a solution prepared by dissolving 1 g of *N*-1-naphthylethylenediamine dihydrochloride in 100 ml of diluted hydrochloric acid (1 in 4), shake well, and allow to stand for 20 minutes. A purple color develops.

(2) On the surface of 1 ml of a solution of Diphenyl in ethyl acetate (1 in 100), place 1 ml of formalin–sulfuric acid TS. The lower layer shows blue to green-blue color.

Purity

(1) Melting point 69–71°C.

(2) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of powdered Diphenyl, transfer to a quartz or porcelain crucible, moisten with 1 ml of sulfuric acid, and heat gradually to almost completely incinerates at the lowest possible temperature. Allow to cool, add another 1 ml of sulfuric acid, and heat gradually. When the

white fumes are almost no longer evolved, ignite at 450–550°C until the residue incinerates, and cool. To the residue, add 1 ml of hydrochloric acid and 0.2 ml of nitric acid, and evaporate to dryness on a water bath. To the residue, add 1 ml of diluted hydrochloric acid (1 in 4) and 15 ml of water, dissolve while heating, cool, and add 1 drop of phenolphthalein TS. Add, dropwise, ammonia TS until the color of the solution changes to a slightly pink color, then add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(3) Naphthalene and its derivatives

Test Solution Weigh 2.5 g of Diphenyl, dissolve it in 50 ml of chloroform, add 2.0 ml of a solution of methyl salicylate in chloroform (1 in 50), and add chloroform to make 100 ml.

Control Solution Measure 5 ml of a solution of naphthalene in chloroform (1 in 1,000), add 2.0 ml of a solution of methyl salicylate in chloroform (1 in 50), and add chloroform to make 100 ml.

Procedure Analyze equal portions of the test solution and the control solution by gas chromatography, using the conditions given below. The ratio (A/A_S) of the sum (A) of the peak area of the naphthalene and the peak areas of all peaks appearing between the peaks of methyl salicylate and diphenyl for the test solution to the peak area (A_S) of the methyl salicylate does not exceed the ratio (A'/A'_S) of the peak area (A') of the naphthalene to the peak area (A'_S) of the methyl salicylate for the control solution.

Operating Conditions

Detector: Hydrogen flame ionization detector.

Column: A stainless steel or glass tube of 2–3 m length and 3–4 mm internal diameter.

Column packing material

Liquid phase: 10% Polyethylene glycol 6,000 of the amount of support.

Support: 177- to 250-μm diatomaceous earth for gas chromatography.

Column temperature: A constant temperature of 160–180°C.

Carrier gas: Use nitrogen.

Flow rate: Adjust so that the peak of the methyl salicylate appears about 5 minutes after the injection.

Assay Weigh accurately about 0.1 g of Diphenyl, and dissolve it in methanol to make exactly 1,000 ml. Measure exactly 10 ml of this solution, and add methanol to make exactly 200 ml. Measure the absorbance (A) of the second solution at a wavelength of 248 nm, using methanol for reference, and calculate the content by the formula:

$$\text{Content (\%) of diphenyl } (C_{12}O_{10}) = \frac{A}{1{,}118} \times \frac{20 \times 10}{\text{Weight (g) of the sample}} \times 100$$

Dipotassium Hydrogen Phosphate

Potassium Phosphate, Dibasic

リン酸水素二カリウム

K_2HPO_4 Mol. Wt. 174.18

Dipotassium hydrogenphosphate [7758-11-4]

Content Dipotassium Hydrogen Phosphate, when dried, contains not less than 98.0% of dipotassium hydrogen phosphate (K_2HPO_4).

Description Dipotassium Hydrogen Phosphate occurs as white crystals, powder, or lumps.

Identification A solution of Dipotassium Hydrogen Phosphate (1 in 20) responds to all tests for Potassium Salt and for Phosphate in the Qualitative Tests.

Purity

(1) Clarity and color of solution Colorless, very slightly turbid (1.0 g, water 20 ml).

(2) pH 8.7–9.3 (1.0 g, water 100 ml).

(3) Chloride Not more than 0.011% as Cl (1.0 g, Control solution 0.01 mol/L hydrochloric acid 0.30 ml).

(4) Sulfate Not more than 0.019% as SO_4 (1.0 g, Control solution 0.005 mol/L sulfuric acid 0.40 ml).

(5) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of Dipotassium Hydrogen Phosphate, dissolve it in 30 ml of water, neutralize with diluted acetic acid (1 in 20), and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Drying Not more than 5.0% (105°C, 4 hours).

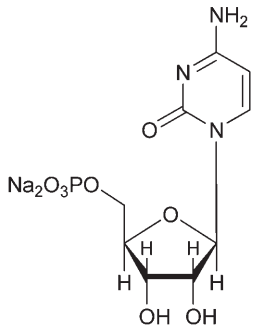
Assay Weigh accurately about 3 g of Dipotassium Hydrogen Phosphate, previously dried, dissolve it in 50 ml of water, keep at about 15°C, and titrate with 1 mol/L hydrochloric acid (indicator: 2–3 drops of methyl orange–indigo carmine TS).

Each ml of 1 mol/L hydrochloric acid = 174.2 mg of K_2HPO_4

Disodium 5′-Cytidylate

Sodium 5′-Cytidylate

5′-シチジル酸二ナトリウム



$C_9H_{12}N_3Na_2O_8P$ Mol. Wt. 367.16
Disodium cytidine 5′-monophosphate [6757-06-8]

Content Disodium 5′-Cytidylate, when calculated on the anhydrous basis, contains 97.0–102.0% of disodium 5′-cytidylate ($C_9H_{12}N_3Na_2O_8P$).

Description Disodium 5′-Cytidylate occurs as colorless to white crystals or as a white crystalline powder having a slight characteristic taste.

Identification

(1) To 3 ml of a solution of Disodium 5′-Cytidylate (3 in 10,000), add 1 ml of hydrochloric acid and 1 ml of bromine TS, heat in a water bath for 30 minutes, and remove the bromine by blowing with air. Add 0.2 ml of a solution of orcinol in ethanol (1 in 10), then add 3 ml of a solution of ferric ammonium sulfate in hydrochloric acid (1 in 1,000), and heat in a water bath for 20 minutes. A green color develops.

(2) To 5 ml of a solution of Disodium 5′-Cytidylate solution (1 in 20), add 2 ml of magnesia TS. No precipitate is formed. Then add 7 ml of nitric acid, boil for 10 minutes, and neutralize with sodium hydroxide solution (1 in 25). The solution responds to test (2) for Phosphate in the Qualitative Tests.

(3) Dissolve 0.02 g of Disodium 5′-Cytidylate in 1,000 ml of diluted hydrochloric acid (1 in 1,000). The solution exhibits an absorption maximum at a wavelength of 277–281 nm.

(4) Disodium 5′-Cytidylate responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Clarity and color of solution Colorless and almost clear (0.50 g, water 10 ml).

(2) pH 8.0–9.5 (1.0 g, water 20 ml).

(3) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

(5) Absorbance ratio Weigh 0.020 g of Disodium 5′-Cytidylate, and dissolve it in diluted hydrochloric acid (1 in 1,000) to make 1,000 ml. Measure the absorbances (A_1, A_2, and A_3) of this solution at wavelengths of 250 nm, 260 nm, and 280 nm, respectively. A_1/A_2 is 0.40–0.52, and A_3/A_2 is 1.85–2.20.

(6) Other decomposed substances of ribonucleic acids

Proceed as directed in Purity (6) for Disodium 5′-Inosinate.

Water Content Not more than 26.0% (0.15 g, Back Titration).

Before performing the titration, add water determination TS in excess, and stir for 20 minutes.

Assay

Test Solution Weigh accurately about 0.5 g of Disodium 5′-Cytidylate, and dissolve it in diluted hydrochloric acid (1 in 1,000) to make exactly 1,000 ml. Measure exactly 10 ml of this solution, and add diluted hydrochloric acid (1 in 1,000) to make exactly 250 ml.

Procedure Measure the absorbance (A) of the test solution at a wavelength of 280 nm, and calculate the content by the formula:

$$\text{Content (\%) of disodium 5'- cytidylate } (C_9H_{12}N_3Na_2O_8P) = \frac{0.5 \times 1.446 \times A}{\text{Anhydrous basis weight (g) of the sample}} \times 100$$

Disodium Dihydrogen Pyrophosphate

Acid Sodium Pyrophosphate
Sodium Acid Pyrophosphate
Disodium Diphosphate

ピロリン酸二水素二ナトリウム

$Na_2H_2P_2O_7$ Mol. Wt. 221.94
Sodium dihydrogendiphosphate [7758-16-9]

Content Disodium Dihydrogen Pyrophosphate, when dried, contains not less than 95.0% of disodium dihydrogen pyrophosphate ($Na_2H_2P_2O_7$).

Description Disodium Dihydrogen Pyrophosphate occurs as a white crystalline powder.

Identification

(1) To 10 ml of a solution of Disodium Dihydrogen Pyrophosphate (1 in 100), add 1 ml of silver nitrate solution (1 in 50). A white precipitate is formed.

(2) Disodium Dihydrogen Pyrophosphate responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Water-insoluble substances Not more than 0.80%.

Weigh accurately a glass filter (1G4), previously dried at 110°C for 30 minutes and allowed to cool in a desiccator. Weigh 5.0 g of Disodium Dihydrogen Pyrophosphate, dissolve it in 100 ml of water, and allow to stand for 1 hour with occasional shaking. Collect the insoluble substances by filtration through the glass filter, wash with 30 ml of water, dry at 110°C for 2 hours together with the glass filter, allow to cool in the desiccator, and weigh accurately the glass filter with residue.

(2) pH 3.8–4.5 (1.0 g, water 100 ml).

(3) Chloride Not more than 0.057% as Cl (0.25 g, Control solution 0.01 mol/L hydrochloric acid 0.40 ml).

(4) Orthophosphate Weigh 1.0 g of Disodium Dihydrogen Pyrophosphate, and add dropwise 2–3 drops of silver nitrate solution (1 in 50). No obvious yellow color develops.

(5) Sulfate Not more than 0.038% as SO_4 (0.50 g, Control

solution 0.005 mol/L sulfuric acid 0.40 ml).

(6) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of Disodium Dihydrogen Pyrophosphate, dissolve by adding 2 ml of diluted acetic acid (1 in 20) and 30 ml of water, filter if necessary, and add water to make 50 ml.

Control Solution To 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(7) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Drying Not more than 5.0% (110°C, 4 hours).

Assay

Test Solution Weigh accurately about 0.2 g of Disodium Dihydrogen Pyrophosphate, previously dried, add 5 ml of nitric acid and 25 ml of water, boil for 30 minutes while replenishing the lost water, and cool. Add water to make exactly 500 ml, and if necessary, filter through a dry filter paper.

Procedure Measure exactly 5 ml of the test solution, add 20 ml of vanadic acid–molybdic acid TS and water to make exactly 100 ml. Shake well, allow to stand for 30 minutes, and measure the absorbance at a wavelength of 400 nm against a reference solution prepared using 5 ml of water instead of the test solution.

Measure accurately 10 ml of Monopotassium Phosphate Standard Solution, and add 20 ml of diluted nitric acid (1 in 25) and water to make exactly 250 ml. Measure exactly 10 ml, 15 ml, and 20 ml of this solution, and proceed in the same manner as for the test solution. Measure the absorbance for each solution, and prepare a calibration curve.

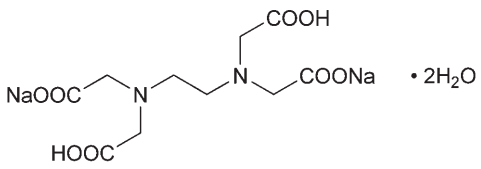
Determine the weight (mg) of phosphorus (P) in 5 ml of the test solution from the calibration curve and the absorbance of the test solution, and calculate the content by the formula:

$$\text{Content (\%) of disodium dihydrogen pyrophosphate } (Na_2H_2P_2O_7) = \frac{\left(\text{Weight (g) of phosphorus (P) in 5 ml of the test solution}\right) \times 3.583 \times 100}{\text{Weight (g) of the sample}} \times 100$$

Disodium Ethylenediaminetetraacetate

Disodium EDTA

エチレンジアミン四酢酸二ナトリウム



$C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ Mol. Wt. 372.24

Disodium dihydrogen ethylenediaminetetraacetate dihydrate [6381-92-6]

Content Disodium Ethylenediaminetetraacetate contains not less than 99.0% of disodium ethylenediaminetetraacetate ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$).

Description Disodium Ethylenediaminetetraacetate occurs as a white to whitish crystalline powder. It is odorless.

Identification

(1) A solution of Disodium Ethylenediaminetetraacetate (1 in 20) responds to all tests for Sodium Salt in the Qualitative Tests.

(2) Proceed as directed in Identification (2) for Calcium Disodium Ethylenediaminetetraacetate.

Purity

(1) pH 4.3–4.7.

Weigh 1.0 g of Disodium Ethylenediaminetetraacetate, dissolve it in water to make 100 ml, and measure.

(2) Heavy metals Not more than 20 μg/g as Pb.

Proceed as directed in Purity (2) for Calcium Disodium Ethylenediaminetetraacetate.

(3) Arsenic Not more than 4.0 μg/g as As_2O_3.

Proceed as directed in Purity (3) for Calcium Disodium Ethylenediaminetetraacetate.

(4) Cyanide Not more than 1.0 μg/g as CN.

Test Solution Weigh 1.0 g of Disodium Ethylenediaminetetraacetate into a round-bottom flask, dissolve it in 100 ml of water, add 10 ml of phosphoric acid, and distill. Use a 100-ml measuring cylinder containing 15 ml of sodium hydroxide solution (1 in 50) as the receiver, immerse the end of the condenser in it, and distill until the total amount becomes 100 ml.

Control Solution Measure 1.0 ml of Cyanide Standard Solution, and add 15 ml of sodium hydroxide solution (1 in 50) and water to make 1,000 ml.

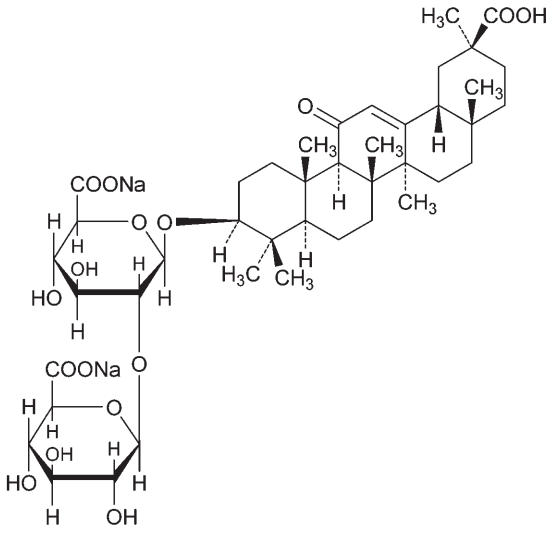
Procedure Measure 20 ml each of the test solution and the control solution, and separately transfer them to test tubes with a ground-glass stopper. Add 1 drop of phenolphthalein TS to each, neutralize with diluted acetic acid (1 in 20), add 5 ml of phosphate buffer (pH 6.8) and 1 ml of chloramine T solution (1 in 500), and immediately stopper. Mix gently, allow to stand for 2–3 minutes, add 5 ml of pyridine–pyrazolone TS, mix thoroughly, and allow to stand at 20–30°C for 50 minutes. The color of the solution is not darker than that of the control solution.

Assay Weigh accurately about 0.4 g of Disodium Ethylenediaminetetraacetate, dissolve it in 20 ml of water, add 10 ml of ammonia–ammonium chloride buffer (pH 10.7), and titrate with 0.05 mol/L zinc chloride (indicator: 2 drops of eriochrome black T TS) until the blue color of the solution changes to red.

Each ml of 0.05 mol/L zinc chloride = 18.61 mg of $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$

Disodium Glycyrrhizinate

グリチルリチン酸二ナトリウム



$C_{42}H_{60}Na_2O_{16}$ Mol. Wt. 866.90

20β-Carboxy-11-oxo-30-norolean-12-en-3β-yl (sodium β-D-glucopyranosyluronate)-(1→2)-(sodium α-D-glucopyranosiduronate)

Content Disodium Glycyrrhizinate, when calculated on the anhydrous basis, contains 95.0–102.0% of disodium glycyrrhizinate ($C_{42}H_{60}Na_2O_{16}$).

Description Disodium Glycyrrhizinate occurs as a white to light yellow powder having an extremely sweet taste.

Identification

(1) To 0.5 g of Disodium Glycyrrhizinate, add 10 ml of diluted hydrochloric acid (1 in 10), boil gently for 10 minutes, cool, and filter. Wash the residue on the filter paper with water thoroughly, and dry at 105°C for 1 hour. To 1 ml of a solution of the dried substance in ethanol (1 in 1,000), add 0.5 ml of a solution of butylated hydroxytoluene in ethanol (1 in 100) and 1 ml of sodium hydroxide solution (1 in 5), and heat in a water bath for 30 minutes, allowing the ethanol to vaporize. Red-purple to purple suspended solids are produced in the residual solution.

(2) To 1 ml of the filtrate obtained in Identification (1), add 0.010 g of naphthoresorcinol and 5 drops of hydrochloric acid, boil gently for 1 minute, allow to stand for 5 minutes, and immediately cool. To this solution, add 3 ml of toluene, and shake. The toluene layer turns red-purple.

(3) The residue on ignition of Disodium Glycyrrhizinate responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Clarity and color of solution Weigh 0.50 g of Disodium Glycyrrhizinate, and dissolve it in 5 ml of water. The solution is clear, and its color is not darker than that of Matching Fluid I.

(2) pH 5.5–6.5 (1.0 g, water 20 ml).

(3) Chloride Not more than 0.014% as Cl.

Test Solution Weigh 0.50g of Disodium Glycyrrhizinate, add 6 ml of diluted nitric acid (1 in 10) and 10 ml of water, boil gently for 10 minutes, and filter. Wash the residue on the filter paper twice with a small amount of water, and combine the filtrate and the washings. If the solution is colored, add 1 ml of hydrogen peroxide, and heat on a water bath for 10 minutes. Cool, filter the deposit, and wash the residue on the filter paper twice with a small amount of water. Combine the filtrate and the washings, and add water to make 50 ml.

Control Solution Measure 0.20 ml of 0.01 mol/L hydrochloric acid, add 6 ml of diluted nitric acid (1 in 10), and then add water to make 50 ml.

(4) Sulfate Not more than 0.029% as SO_4.

Test Solution Weigh 0.50 g of Disodium Glycyrrhizinate, add 5 ml of diluted hydrochloric acid (1 in 4) and 10 ml of water, boil gently for 10 minutes, and filter. Wash the residue on the filter paper twice with a small amount of water, combine the filtrate and the washings, and neutralize with ammonia TS. If the solution is colored, add 1 ml of hydrogen peroxide, and heat on a water bath for 10 minutes. After cooling, filter if necessary, wash the residue on the filter paper twice with a small amount of water, combine the filtrate and the washings, and add water to make 50 ml.

Control Solution Measure 0.30 ml of 0.005 mol/L sulfuric acid, and add 1 ml of diluted hydrochloric acid (1 in 4) and water to make 50 ml.

(5) Heavy metals Not more than 30 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 3.0 ml).

(6) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 2.0 g of Disodium Glycyrrhizinate, transfer into a Kjeldahl flask, add 10 ml of sulfuric acid and 10 ml of nitric acid, and heat until white fumes are evolved. If the solution still remains brown, cool, add 2 ml of nitric acid, and heat. Repeat until the solution becomes colorless to light yellow. After cooling, add 15 ml of ammonium oxalate solution (1 in 25), and heat until white fumes are evolved again. Cool, and add water to make 25 ml. Perform the test, using 10 ml of this solution as the test solution.

Standard Color Measure 8.0 ml of Arsenic Standard Solution, transfer into a Kjeldahl flask, add 10 ml of sulfuric acid and 10 ml of nitric acid, and proceed in the same manner as for the sample.

Apparatus Use Apparatus B.

Water Content Not more than 13.0% (0.2 g, Back Titration).

Residue on Ignition 15.0–18.0% (calculated on the anhydrous basis).

Assay

Test Solution Weigh accurately about 0.1 g of Disodium Glycyrrhizinate, and dissolve it in water to make exactly 1,000 ml. Measure exactly 10 ml of this solution, and add water to make exactly 25 ml.

Standard Solution Weigh accurately about 0.05 g of Nicotinamide Reference Standard, previously dried for 4 hours in a vacuum desiccator, and dissolve it in water to make exactly 1,000 ml. Measure exactly 10 ml of this solution, and add water to make exactly 25 ml.

Procedure Measure the absorbance (A_T) of the test solution at a wavelength of 259 nm, using water as the reference solution. Measure the absorbance (A_S) of the standard solution at a wavelength of 261 nm, using water as the reference solution and calculate the content by the formula:

Content (%) of disodium glycyrrhizinate ($C_{42}H_{60}Na_2O_{16}$)

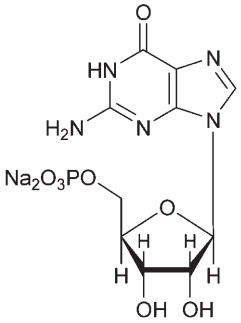
$$= \frac{\text{Weight (g) of nicotinamide}}{\text{Anhydrous basis weight (g) of the sample}} \times \frac{2A_T}{A_S \times F} \times 100$$

F = 1.093.

Disodium 5′-Guanylate

Disodium Guanylate
Sodium 5′-Guanylate

5′-グアニル酸二ナトリウム



$C_{10}H_{12}N_5Na_2O_8P$ Mol. Wt. 407.18
Disodium guanosine 5′-monophosphate [5550-12-9]
Content Disodium 5′-Guanylate, when dried, contains 97.0 –102.0% of disodium 5′-guanylate ($C_{10}H_{12}N_5Na_2O_8P$).
Description Disodium 5′-Guanylate occurs as colorless to white crystals or powder having a characteristic taste.
Identification
(1) To 3 ml of a solution of Disodium 5′-Guanylate (3 in 10,000), add 0.2 ml of a solution of orcinol in ethanol (1 in 10), then add 3 ml of a solution of ferric ammonium sulfate in hydrochloric acid (1 in 1,000), and heat in a water bath for 10 minutes. A green color develops.
(2) To 5 ml of a solution of Disodium 5′-Guanylate (1 in 100), add 2 ml of magnesia TS. No precipitate is formed. Then add 7 ml of nitric acid, boil for 10 minutes, and neutralize with sodium hydroxide solution (1 in 25). The solution responds to test (2) for Phosphate in the Qualitative Tests.
(3) Dissolve 0.02 g of Disodium 5′-Guanylate in 1,000 ml of diluted hydrochloric acid (1 in 1,000). The solution exhibits an absorption maximum at a wavelength of 254–258 nm.
(4) Disodium 5′-Guanylate responds to all tests for Sodium Salt in the Qualitative Tests.
Purity
(1) Clarity and color of solution Colorless and almost clear (1.0 g, water 10 ml).
(2) pH 7.0–8.5 (1.0 g, water 20 ml).
(3) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).
(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).
(5) Absorbance ratio Weigh 0.020 g of Disodium 5′-Guanylate, and dissolve it in diluted hydrochloric acid (1 in 1,000) to make 1,000 ml. Measure the absorbances (A_1, A_2, and A_3) of this solution at wavelengths of 250 nm, 260 nm, and 280 nm, respectively. A_1/A_2 is 0.95–1.03, and A_3/A_2 is 0.63–0.71.
(6) Other decomposed substances of nucleic acid Proceed as directed in Purity (6) for Disodium 5′-Inosinate.
Loss on Drying Not more than 25.0% (120°C, 4 hours).
Assay Weigh accurately about 0.5 g of Disodium 5′-Guanylate, and dissolve it in diluted hydrochloric acid (1 in 1,000) to make exactly 1,000 ml. Measure exactly 10 ml of this solution, and add diluted hydrochloric acid (1 in 1,000) to make exactly 250 ml. Use the second solution as the test solution. Measure the absorbance (A) of the test solution at a wavelength of 260 nm, and calculate the content by the formula:

Content (%) of disodium 5'-guanylate ($C_{10}H_{12}N_5Na_2O_8P$)

$$= \frac{250}{\text{Dry basis weight (g) of the sample}} \times \frac{A}{289.8} \times 100$$

Disodium Hydrogen Phosphate

Disodium Phosphate
Sodium Phosphate, Dibasic

リン酸水素二ナトリウム

$Na_2HPO_4 \cdot nH_2O$ (n=12, 10, 8, 7, 5, 2, or 0)
Mol. Wt. dodecahydrate 358.14
anhydrous 141.96
Disodium hydrogenphosphate dodecahydrate [10039-32-4]
Disodium hydrogenphosphate decahydrate
Disodium hydrogenphosphate octahydrate
Disodium hydrogenphosphate heptahydrate [7782-85-6]
Disodium hydrogenphosphate pentahydrate
Disodium hydrogenphosphate dihydrate [10028-24-7]
Disodium hydrogenphosphate [7558-79-4]
Definition Disodium Hydrogen Phosphate occurs in two forms: the crystalline form (dodeca-, deca-, octa-, hepta-, penta-, or dihydrate) called Disodium Hydrogen Phosphate (crystal) and the anhydrous form called Disodium Hydrogen Phosphate (anhydrous).
Content Disodium Hydrogen Phosphate, when dried, contains not less than 98.0% of disodium hydrogen phosphate (Na_2HPO_4).
Description Disodium Hydrogen Phosphate (crystal) occurs as colorless to white crystals or crystalline lumps. Disodium Hydrogen Phosphate (anhydrous) occurs as a white powder.
Identification A solution of Disodium Hydrogen Phosphate (1 in 20) responds to all tests for Sodium Salt and for Phosphate in the Qualitative Tests.
Purity For Disodium Hydrogen Phosphate (crystal), dry the sample before performing the tests.
(1) Clarity and color of solution Colorless and almost clear (0.50 g, water 20 ml).
(2) pH 9.0 – 9.6 (1.0 g, water 100 ml).
(3) Chloride Not more than 0.21% as Cl (0.10 g, Control

solution 0.01 mol/L hydrochloric acid 0.60 ml).

(4) Sulfate Not more than 0.038% as SO_4 (0.50 g, Control solution 0.005 mol/L sulfuric acid 0.40 ml).

(5) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of Disodium Hydrogen Phosphate, dissolve it in 30 ml of water, neutralize with diluted acetic acid (1 in 20), and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Drying

Crystal: Not more than 61.0% (40°C, 3 hours, then 120°C, 4 hours).

Anhydrous: Not more than 2.0% (120°C, 4 hours).

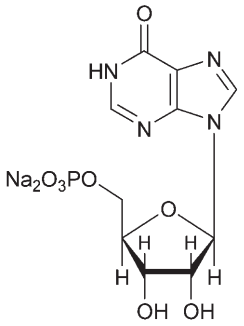
Assay Weigh accurately about 3 g of Disodium Hydrogen Phosphate, previously dried, dissolve it in 50 ml of water, keep at about 15°C, and titrate with 1 mol/L hydrochloric acid (indicator: 2–3 drops of methyl orange–indigo carmine TS).

Each ml of 1 mol/L hydrochloric acid = 142.0 mg of Na_2HPO_4

Disodium 5′-Inosinate

Disodium Inosinate
Sodium 5′-Inosinate

5′-イノシン酸二ナトリウム



$C_{10}H_{11}N_4Na_2O_8P$ Mol. Wt. 392.17

Disodium inosine 5′-monophosphate [4691-65-0]

Content Disodium 5′-Inosinate, when calculated on the anhydrous basis, contains 97.0–102.0% of disodium 5′-inosinate ($C_{10}H_{11}N_4Na_2O_8P$).

Description Disodium 5′-Inosinate occurs as colorless to white crystals or as a white crystalline powder having a characteristic taste.

Identification

(1) To 3 ml of a solution of Disodium 5′-Inosinate (3 in 10,000), add 0.2 ml of a solution of orcinol in ethanol (1 in 10), then add 3 ml of a solution of ferric ammonium sulfate in hydrochloric acid (1 in 1,000), and heat in a water bath for 10 minutes. A green color develops.

(2) To 5 ml of a solution of Disodium 5′-Inosinate (1 in 20), add 2 ml of magnesia TS. No precipitate is formed. Then add 7 ml of nitric acid, boil for 10 minutes, and neutralize with sodium hydroxide solution (1 in 25). The solution responds to test (2) for Phosphate in the Qualitative Tests.

(3) Dissolve 0.02 g of Disodium 5′-Inosinate in 1,000 ml of diluted hydrochloric acid (1 in 1,000). The solution exhibits an absorption maximum at a wavelength of 248–252 nm.

(4) Disodium 5′-Inosinate responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Clarity and color of solution Colorless and almost clear (0.50 g, water 10ml).

(2) pH 7.0–8.5 (1.0 g, water 20 ml).

(3) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

(5) Absorbance ratio Weigh 0.020 g of Disodium 5′-Inosinate, and dissolve it in diluted hydrochloric acid (1 in 1,000) to make 1,000 ml. Measure the absorbances (A_1, A_2, and A_3) of this solution at wavelengths of 250 nm, 260 nm, and 280 nm, respectively. A_1/A_2 is 1.55–1.65, and A_3/A_2 is 0.20–0.30.

(6) Other decomposed substances of nucleic acid

Test Solution Weigh 0.10 g of Disodium 5′-Inosinate and dissolve it in water to make 20 ml.

Procedure Analyze a 1-μl portion of the test solution by thin-layer chromatography, using a 6:5:2 mixture of 1-propanol/ammonia TS/acetone as the developing solvent. No control solution is used. Use a thin-layer plate that is coated with fluorescent silica gel for thin-layer chromatography as the support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the original line, and air-dry the plate. Examine under ultraviolet light (around 250 nm) in a dark place. Only one spot is observed.

Water Content Not more than 29.0% (0.15 g, Back Titration).

Before titrating, add water determination TS in excess and stir for 20 minutes.

Assay Weigh accurately about 0.5 g of Disodium 5′-Inosinate, and dissolve it in diluted hydrochloric acid (1 in 1,000) to make exactly 1,000 ml. Measure exactly 10 ml of this solution, and add diluted hydrochloric acid (1 in 1,000) to make exactly 250 ml. Use the second solution as the test solution. Measure the absorbance (A) of the test solution at a wavelength of 250 nm and calculate the content by the formula:

$$\text{Content (\%) of disodium 5′-inosinate } (C_{10}H_{11}N_4Na_2O_8P) = \frac{250 \times A}{\text{Anhydrous basis weight (g) of the sample} \times 310.0} \times 100$$

Disodium 5′-Ribonucleotide

Sodium 5′-Ribonucleotide

5′-リボヌクレオチド二ナトリウム

Definition Disodium 5′-Ribonucleotide is a mixture of disodium 5′-inosinate, disodium 5′-guanylate, disodium 5′-cytidylate, and disodium 5′-uridylate, or a mixture of disodium 5′-inosinate and disodium 5′-guanylate.

Content Disodium 5′-Ribonucleotide, when calculated on the anhydrous basis, contains 97.0–102.0% of disodium 5′-ribonucleotide, of which not less than 95.0% consists of disodium 5′-inosinate and disodium 5′-guanylate.

Description Disodium 5′-Ribonucleotide occurs as white to whitish crystals or powder. It is odorless and has a characteristic taste.

Identification

(1) To 1 ml of a solution of Disodium 5′-Ribonucleotide (1 in 2,000), add 0.2 ml of a solution of orcinol in ethanol (1 in 10), then add 3 ml of a solution of ferric ammonium sulfate in hydrochloric acid (1 in 1,000), and heat in a water bath for 10 minutes. A green color develops.

(2) To 1 ml of a solution of Disodium 5′-Ribonucleotide (1 in 1,000), add 2 ml of diluted hydrochloric acid (1 in 4) and 0.1 g of zinc dust, heat in a water bath for 10 minutes, and filter. Cool the filtrate in ice water, add 1 ml of sodium nitrite solution (3 in 1,000), shake, and allow to stand for 10 minutes. Add 1 ml of ammonium sulfamate solution (1 in 200), shake well, and allow to stand for 5 minutes. To the mixture, add 1 ml of *N*-1-naphthylethylenediamine dihydrochloride solution (1 in 500). A purple-red color develops.

(3) To 1 ml of a solution of Disodium 5′-Ribonucleotide (1 in 5,000), add 1 ml of diluted hydrochloric acid (1 in 4), heat in a water bath for 10 minutes, and cool. Add 0.5 ml of Folin's TS and 2 ml of sodium carbonate saturated solution. A blue color develops.

(4) To 5 ml of a solution of Disodium 5′-Ribonucleotide (1 in 20), add 2 ml of magnesia TS. No precipitate is formed. Add 7 ml of nitric acid, boil for 10 minutes, and neutralize with sodium hydroxide solution (1 in 25). The solution responds to test (2) for Phosphate in the Qualitative Tests.

(5) A solution of Disodium 5′-Ribonucleotide (1 in 10) responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) pH 7.0–8.5 (1.0 g, water 20 ml).

(2) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Water Content Not more than 27.0% (0.15 g, Back Titration).

Before titrating, add water determination TS in excess, and stir for 20 minutes.

Assay Calculate the content of disodium 5′-ribonucleotide and the total content of disodium 5′-inosinate ($C_{10}H_{11}N_4Na_2O_8P$) and disodium 5′-guanylate ($C_{10}H_{12}N_5Na_2O_8P$), using the following formulae from the values (I, G, and P) determined as directed in (1), (2), and (3).

$$\text{Content (\%) of disodioum 5′-ribonucleotide} = \frac{I + G + P}{100 - \text{Water content (\%)}} \times 100$$

$$\text{Content (\%) of disodium 5′-inosinate } (C_{10}H_{11}N_4Na_2O_8P) \text{ and disodium 5′-guanylate } (C_{10}H_{12}N_5Na_2O_8P) = \frac{I + G}{100 - \text{Water content (\%)}} \times 100$$

(1) Disodium 5′-inosinate

Test Solution Weigh accurately about 0.65 g of Disodium 5′-Ribonucleotide, dissolve it in water to make exactly 500 ml, and use this solution as the sample solution. Measure exactly 1 ml of the sample solution, and add 4 ml of diluted hydrochloric acid (1 in 2) and water to make exactly 10 ml. Heat the mixture in a water bath for 40 minutes, and then cool. Add 0.4 g of zinc dust, allow to stand for 50 minutes with occasional vigorous shaking, add water to make exactly 20 ml, and filter. Measure exactly 10 ml of the filtrate, add 1 ml of diluted hydrochloric acid (1 in 2), add 1 ml of sodium nitrite solution (3 in 1,000) while cooling in ice, shake well, and allow to stand for 10 minutes. Add 1 ml of ammonium sulfamate solution (1 in 200), shake well, and allow to stand for 5 minutes. To this solution, add 1 ml of *N*-1-naphthylethylenediamine dihydrochloride solution (1 in 500), shake well, allow to stand for 15 minutes, and add water to make exactly 20 ml.

Standard Solutions and Calibration Weigh accurately about 0.03 g each of disodium 5′-inosinate and disodium 5′-guanylate, dissolve separately in diluted hydrochloric acid (1 in 1,000) to prepare two standard solutions of exactly 1,000 ml each, and measure the absorbance of each solution. Use a wavelength of 250 nm for disodium 5′-inosinate and a wavelength of 260 nm for disodium 5′-guanylate. Calculate the molecular extinction coefficients (E_I and E_G) from each absorbance measured, and determine the contents of disodium 5′-inosinate and disodium 5′-guanylate using the following formulae:

$$\text{Content (\%) of disodium 5'-inosinate } (C_{10}H_{11}N_4Na_2O_8P) = \frac{E_I}{12{,}160} \times 100$$

$$\text{Content (\%) of disodium 5'-guanylate } (C_{10}H_{12}N_5Na_2O_8P) = \frac{E_G}{11{,}800} \times 100$$

Based on each content above, weigh accurately an amount equivalent to about 0.05 g each of disodium 5′-inosinate and disodium 5′-guanylate, combine them, and dissolve in water to make a standard stock solution of exactly 200 ml. Measure exactly 1 ml, 2 ml, and 3 ml of the standard stock solution, and add 4 ml of diluted hydrochloric acid (1 in 2) and water to each to make exactly 10 ml. Proceed as directed for the test solution to prepare standard solutions. Measure the absorbance of each standard solution at a wavelength of 515 nm, using the same reference solution as used for the test solution under Procedure to prepare a calibration curve.

Procedure Measure the absorbance of the test solution at a wavelength of 515 nm against a reference solution prepared in the same manner as the preparation of the test solution, using 1 ml of water instead of the sample solution. From the calibration curve and the absorbance of the test so-

lution, calculate the content (I (%)) of disodium 5′-inosinate ($C_{10}H_{11}N_4Na_2O_8P$) in the sample.

(2) Disodium 5′-guanylate

Test Solution Measure exactly 1 ml of the sample solution obtained in (1), and add 4 ml of diluted hydrochloric acid (1 in 6) and water to make exactly 10 ml. Heat the mixture in a water bath for 30 minutes, and then cool. Add 2 ml of Folin's TS and 5 ml of a saturated solution of sodium carbonate, allow to stand for 15 minutes, and add water to make exactly 50 ml. Centrifuge the solution if necessary.

Standard Solutions and Calibration Transfer exactly 1 ml, 2 ml, and 3 ml of the standard stock solution obtained in (1) into separate 10-ml volumetric flasks, add 4 ml of diluted hydrochloric acid (1 in 6) to each, and add water to volume. Using these solutions, proceed as directed in the preparation of the test solution.

Measure each absorbance at a wavelength of 750 nm using the same reference solution as used for the test solution to prepare a calibration curve.

Procedure Measure the absorbance of the test solution at a wavelength of 750 nm against a reference solution prepared as directed for the test solution using 1 ml of water instead of the sample solution. From the calibration curve and the absorbance of the test solution, calculate the content (G (%)) of disodium 5′-guanylate ($C_{10}H_{12}N_5Na_2O_8P$) in the sample.

(3) Disodium 5′-cytidylate and disodium 5′-uridylate

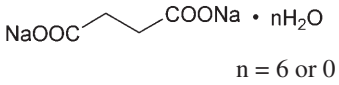
Test Solution Weigh accurately about 1.5 g of Disodium 5′-Ribonucleotide, add water to make exactly 50 ml, and use this solution as the sample solution. Measure exactly 1 ml of the sample solution, add 2 ml of hydrazine (hydrate), heat in a water bath for 1 hour, and then cool. Next, add diluted hydrochloric acid (1 in 10) to make the solution slightly acidic, and add diluted hydrochloric acid (1 in 1,000) to make exactly 100 ml. Measure exactly 10 ml of this solution, and add diluted hydrochloric acid (1 in 1,000) to make exactly 100 ml.

Procedure Measure the absorbances (A_{260} and A_{280}) of the test solution at wavelengths of 260 nm and 280 nm, respectively, using a reference solution prepared as directed for the test solution with 1 ml of water instead of the sample solution. Separately, measure exactly 1 ml of the sample solution, and add diluted hydrochloric acid (1 in 1,000) to make exactly 100 ml. Measure exactly 10 ml of the second solution, and add diluted hydrochloric acid (1 in 1,000) to make exactly 100 ml. Measure the absorbances (A'_{260} and A'_{280}) of the last solution at wavelengths of 260 nm and 280 nm, respectively, and calculate the total content (P (%)) of disodium 5′-cytidylate ($C_9H_{12}N_3Na_2O_8P$) and disodium 5′-uridylate ($C_9H_{11}N_2Na_2O_9P$) in the sample by the formula:

$$\text{P (\%)} = \frac{170.5 \times (A'_{260} - A_{260}) + 68.6 \times (A'_{280} - A_{280})}{\text{Weight (g) of the sample}}$$

Disodium Succinate

コハク酸二ナトリウム



n = 6 or 0

$C_4H_4Na_2O_4 \cdot nH_2O$ (n=6 or 0)

Mol. Wt. hexahydrate 270.14
anhydrous 162.05

Disodium butanedioate hexahydrate
Disodium butanedioate [150-90-3]

Definition Disodium Succinate occurs in two forms: the crystalline form (hexahydrate) called Disodium Succinate (crystal) and the anhydrous form called Disodium Succinate (anhydrous).

Content Disodium Succinate, when dried, contains 98.0–101.0% of disodium succinate ($C_4H_4Na_2O_4$).

Description Disodium Succinate occurs as colorless to white crystals or as a white powder. It is odorless and has a characteristic taste.

Identification Disodium Succinate responds to all tests for Sodium Salt and for Succinate in the Qualitative Tests.

Purity

(1) pH 7.0–9.0 (1.0 g, water 20 ml).

(2) Sulfate Not more than 0.019% as SO_4.

Test Solution Weigh 1.0 g of Disodium Succinate, dissolve it in 30 ml of water, and neutralize with diluted hydrochloric acid (1 in 40).

Control Solution Use 0.40 ml of 0.005 mol/L sulfuric acid.

(3) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of Disodium Succinate, dissolve it in 20 ml of water, neutralize with diluted hydrochloric acid (1 in 40), add 2 ml of diluted acetic acid (1 in 20), and add water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

(5) Readily oxidizable substances Weigh 2.0 g of Disodium Succinate, dissolve it in 20 ml of water and 30 ml of diluted sulfuric acid (1 in 20), and add 4.0 ml of 0.02 mol/L potassium permanganate. The pink color of the solution does not disappear within 3 minutes.

Loss on Drying

Crystal: 37.0–41.0% (120°C, 2 hours).

Anhydrous: Not more than 2.0% (120°C, 2 hours).

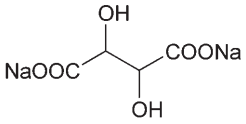
Assay Weigh accurately about 0.15 g of Disodium Succinate, previously dried, dissolve it in 30 ml of acetic acid for nonaqueous titration, and titrate with 0.1 mol/L perchloric acid (indicator: 1 ml of crystal violet–acetic acid TS) until the color of the solution changes from purple through blue to green. Perform a blank test in the same manner, and make any necessary correction.

Each ml of 0.1 mol/L perchloric acid = 8.103 mg of $C_4H_4Na_2O_4$

Disodium DL-Tartrate

Disodium *dl*-Tartrate

DL-酒石酸ナトリウム

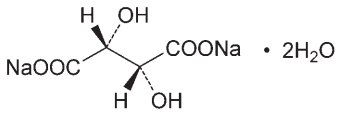


$C_4H_4Na_2O_6$ Mol. Wt. 194.05
Disodium 2,3-dihydroxybutanedioate
Content Disodium DL-Tartrate, when dried, contains not less than 98.5% of disodium DL-tartrate ($C_4H_4Na_2O_6$).
Description Disodium DL-Tartrate occurs as colorless crystals or as a white crystalline powder.
Identification
(1) A solution of Disodium DL-Tartrate solution (1 in 10) has no optical rotation.
(2) Disodium DL-Tartrate responds to all tests for Sodium Salt and for Tartrate in the Qualitative Tests.
Purity
(1) Clarity of solution Almost clear (1.0 g, water 20 ml).
(2) pH 7.0–9.0 (1.0 g, water 20 ml).
(3) Sulfate Not more than 0.019% as SO_4 (1.0 g, Control solution 0.005 mol/L sulfuric acid 0.40 ml).
(4) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).
(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).
(6) Readily oxidizable substances Weigh 2.0 g of Disodium DL-Tartrate, add 20 ml of water and 30 ml of diluted sulfuric acid (1 in 20) to dissolve, and then add 4.0 ml of 0.1 mol/L potassium permanganate while keeping at 20°C. The pink color of the solution does not disappear within 3 minutes.
Loss on Drying Not more than 0.5% (105°C, 4 hours).
Assay Weigh accurately about 0.2 g of Disodium DL-Tartrate, previously dried, add 3 ml of formic acid, dissolve by warming, add 50 ml of acetic acid for nonaqueous titration, and titrate with 0.1 mol/L perchloric acid. The endpoint is usually confirmed using a potentiometer. When crystal violet–acetic acid TS (1 ml) is used as the indicator, the endpoint is when the color of the solution changes from purple through blue to green. Perform a blank test in the same manner, and make any necessary correction.
Each ml of 0.1 mol/L perchloric acid = 9.703 mg of $C_4H_4Na_2O_6$

Disodium L-Tartrate

Disodium *d*-Tartrate
Disodium Tartrate

L-酒石酸ナトリウム

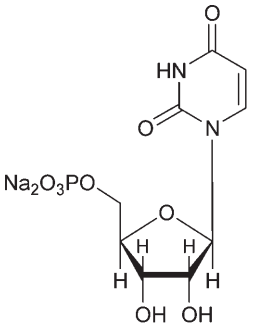


$C_4H_4Na_2O_6 \cdot 2H_2O$ Mol. Wt. 230.08
Disodium (2*R*,3*R*)-2,3-dihydroxybutanedioate dihydrate
[6106-24-7]
Content Disodium L-Tartrate, when dried, contains not less than 98.5% of disodium L-tartrate ($C_4H_4Na_2O_6$ = 194.05).
Description Disodium L-Tartrate occurs as colorless crystals or as a white crystalline powder.
Identification
(1) A solution of Disodium L-Tartrate (1 in 10) is dextrorotary.
(2) Disodium L-Tartrate responds to all tests for Sodium Salt and for Tartrate in the Qualitative Tests.
Purity
(1) Specific rotation $[\alpha]_D^{20}$: +25.0 to +27.5° (5 g, water, 50 ml).
(2) Clarity of solution Almost clear.
Proceed as directed in Purity (1) for Disodium DL-Tartrate.
(3) pH 7.0–9.0.
Proceed as directed in Purity (2) for Disodium DL-Tartrate.
(4) Sulfate Not more than 0.019% as SO_4.
Proceed as directed in Purity (3) for Disodium DL-Tartrate.
(5) Heavy metals Not more than 10 μg/g as Pb.
Proceed as directed in Purity (4) for Disodium DL-Tartrate.
(6) Arsenic Not more than 4.0 μg/g as As_2O_3.
Proceed as directed in Purity (5) for Disodium DL-Tartrate.
(7) Oxalate Weigh 1.0 g of Disodium L-Tartrate, dissolve it in 10 ml of water, and add 2 ml of calcium chloride solution (2 in 25). The solution is not turbid.
Loss on Drying 14.0–17.0% (150°C, 3 hours).
Assay Proceed as directed in the Assay for Disodium DL-Tartrate.
Each ml of 0.1 mol/L perchloric acid = 9.703 mg of $C_4H_4Na_2O_6$

Disodium 5′-Uridylate

Sodium 5′-Uridylate

5′-ウリジル酸二ナトリウム



$C_9H_{11}N_2Na_2O_9P$ Mol. Wt. 368.15
Disodium uridine 5′-monophosphate [3387-36-8]

Content Disodium 5′-Uridylate, when calculated on the anhydrous basis, contains 97.0–102.0% of disodium 5′-uridylate ($C_9H_{11}N_2Na_2O_9P$).

Description Disodium 5′-Uridylate occurs as colorless to white crystals or as a white crystalline powder having a slight, characteristic taste.

Identification

(1) To 3 ml of a solution of Disodium 5′-Uridylate (3 in 10,000), add 1 ml of hydrochloric acid and 1 ml of bromine TS, heat on a water bath for 30 minutes, remove the bromine by blowing with air, and add 0.2 ml of a solution of orcinol in ethanol (1 in 10). To this solution, add 3 ml of a solution of ferric ammonium sulfate in hydrochloric acid (1 in 1,000), and heat in a water bath for 20 minutes. A green color develops.

(2) To 5 ml of a solution of Disodium 5′-Uridylate (1 in 20), add 2 ml of magnesia TS. No precipitate is formed. Then add 7 ml of nitric acid, boil for 10 minutes, and neutralize with sodium hydroxide solution (1 in 25). The solution responds to test (2) for Phosphate in the Qualitative Tests.

(3) Dissolve 0.02 g of Disodium 5′-Uridylate in 1,000 ml of diluted hydrochloric acid (1 in 1,000). The resulting solution exhibits an absorption maximum at a wavelength of 260–264 nm.

(4) Disodium 5′-Uridylate responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Clarity and color of solution Colorless and almost clear (0.50 g, water 10 ml).

(2) pH 7.0–8.5 (1.0 g, water 20 ml).

(3) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

(5) Absorbance ratio Weigh 0.020 g of Disodium 5′-Uridylate, and dissolve it in diluted hydrochloric acid (1 in 1,000) to make 1,000 ml. Measure the absorbances (A_1, A_2, and A_3) of this solution at wavelengths of 250 nm, 260 nm, and 280 nm, respectively. A_1/A_2 is 0.7–0.78, and A_3/A_2 is 0.34–0.42.

(6) Other decomposed substances of ribonucleic acids

Weigh 0.10 g of Disodium 5′-Uridylate, dissolve it in water to make 10 ml, and use this solution as the test solution. Analyze a 1 μl portion of the test solution by thin-layer chromatography, using a 2:2:1 mixture of ethanol/ethylene glycol monomethyl ether/diluted hydrochloric acid (1 in 10) as the developing solvent. No control solution is used. Use a thin-layer plate coated with microcrystalline cellulose for thin-layer chromatography as the solid support and then dried at 60–80°C for 20 minutes. Stop the development when the solvent front has ascended to a point 10 cm above the original line, and air-dry the plate. Examine under ultraviolet light (around 250 nm) in a dark place. Only one spot is observed.

Water Content Not more than 26.0% (0.15 g, Back Titration).

Before titrating, add water determination TS in excess, and stir for 20 minutes.

Assay Weigh accurately about 0.5 g of Disodium 5′-Uridylate, and dissolve it in diluted hydrochloric acid (1 in 1,000) to make exactly 1,000 ml. Measure exactly 10 ml of this solution, and add diluted hydrochloric acid (1 in 1,000) to make exactly 250 ml. Use the second solution as the test solution. Measure the absorbance (A) of the test solution at a wavelength of 260 nm, and calculate the content by the formula:

$$\text{Content (\%) of disodium 5′-uridylate } (C_9H_{11}N_2Na_2O_9P) = \frac{0.5 \times 1.859 \times A}{\text{Anhydrous basis weight (g) of the sample}} \times 100$$

Dry Formed Vitamin A

粉末ビタミンA

Definition Dry Formed Vitamin A is produced by powdering vitamin A esters of fatty acids or vitamin A in oil.

Content Dry Formed Vitamin A contains 90–120% of the labeled value of vitamin A.

Description Dry Formed Vitamin A occurs as a light yellow to light red-brown powder.

Identification Weigh an amount of Dry Formed Vitamin A equivalent to 1,500 units of vitamine A, and grind in a mortar. Add 10 ml of warm water, stir thoroughly to make a milky emulsion, and add 10 ml of ethanol to dissolve the emulsion. Transfer it to a flask, add 20 ml of hexane, shake well, and let it separate into two layers either by allowing to stand or by centrifuging. Collect the hexane layer, wash with 20 ml of water by shaking well, separate the aqueous layer, and evaporate the hexane layer to dryness under reduced pressure. Dissolve the residue in 5 ml of petroleum ether, and use as the test solution. Proceed as directed in Identification (1) for Vitamin Esters of Fatty Acids.

Purity

(1) Decay Dry Formed Vitamin A has no unpleasant odor.

(2) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 2.0 g of Dry Formed Vitamin A,

transfer into a Kjeldahl flask, add 20 ml of nitric acid, and heat weakly until the contents is flowable. After cooling, add 5 ml of sulfuric acid, and heat until white fumes are evolved. If the solution is still brown, add another 5 ml of nitric acid after cooling, and heat. Repeat this procedure until the solution is colorless or light yellow. After cooling, add 15 ml of ammonium oxalate solution (1 in 25), heat until white fumes are evolved again, and cool. Add water to make 25 ml, and use 10 ml of the resulting solution as the test solution.

Standard Color Measure 8.0 ml of Arsenic Standard Solution, transfer into a Kjeldahl flask, and proceed as in the same manner as for the test solution.

Apparatus Use Apparatus B.

Loss on Drying Not more than 5.0% (reduced pressure, 4 hours).

Residue on Ignition Not more than 5.0%.

Assay Weigh accurately about 5 g of Dry Formed Vitamin A, add a small amount of warm water, shake thoroughly to obtain milky emulsion, transfer into a flask, and proceed as directed in the Assay for Vitamin A in Oil.

Storage Standards Store in a hermetic, light-resistant container.

Dunaliella Carotene

デュナリエラカロテン

Definition Dunaliella Carotene is obtained from the entire part of the alga *Dunaliella bardawil* or *Dunaliella salina* and consists mainly of β-carotene. It may contain edible fats or oils.

Content (Color Value) Dunaliella Carotene contains the equivalent of not less than 10% of β-carotene ($C_{40}H_{56}$ = 536.88) and the equivalent of 95–115% of the labeled content; or its Color Value ($E_{1cm}^{10\%}$) is not less than 2,500 and in the range of 95–115% of the labeled value.

Description Dunaliella Carotene is a dark orange to red-brown, suspended oily substance having a slightly characteristic odor.

Identification

(1) Weigh the equivalent of 0.05 g of Dunaliella Carotene with a Color Value 2,500, dissolve it in 5 ml of a 1:1 mixture of acetone/cyclohexane. An orange color develops.

(2) Prepare a solution of Dunaliella Carotene in a 1:1 mixture of acetone/cyclohexane at the concentration equivalent to either about 1 mg β-Carotene per ml or about a Color Value 1 per ml, calculated from the labeled value. To 1 ml of the solution, add acetone to make 5 ml. To the resulting solution, add 1 ml of 5% sodium nitrite solution and 1 ml of 0.5 mol/L sulfuric acid. The solution immediately discolors.

(3) A solution of Dunaliella Carotene in cyclohexane exhibits an absorption maximum at a wavelength of either 446–457 nm or 472–486 nm or absorption maxima at both 446–457 nm and 472–486 nm.

Purity

(1) Heavy metals Not more than 20 μg/g as Pb (1.0g, Method 2, Control solution Lead standard solution 2.0 ml).

(2) Lead Not more than 10 μg/g as Pb (1.0g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50g, Method 4, Apparatus B).

Assay (Color Value Test) Proceed as directed in the Color Value Test, using the conditions below. Obtain the color value or determine the content of β-carotene by dividing color value by 250.

Operating Conditions

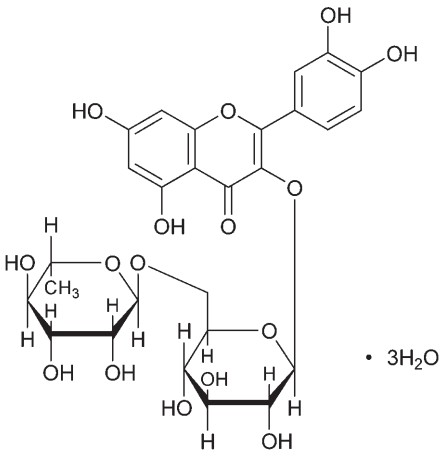
Solvent: Cyclohexane.

Wavelength: Maximum absorption wavelength of 446–457nm.

Enju Extract

Japanese Pagoda Tree Extract

エンジュ抽出物



$C_{27}H_{30}O_{16}{\cdot}3H_2O$ Mol. Wt. 664.56

5,7-Dihydroxy-2-(3,4-dihydroxyphenyl)-4-oxo-4*H*-chromen-7-yl α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside trihydrate [rutin, anhydrous 153-18-4]

Definition Enju Extract* is obtained from the buds or flowers of the plant *Sophora japonica* Linné by extraction with water, ethanol, or methanol and then removal of the solvent used. It consists mainly of rutin.

Content Enju Extract, when dried, contains 95.0–105.0% of rutin ($C_{27}H_{30}O_{16}$ = 610.52).

Description Enju Extract occurs as a light yellow to light yellow-green crystalline powder. It is odorless or has a slight characteristic odor.

Identification

(1) Dissolve 0.02 g of Enju Extract in 10 ml of ethanol. A yellow color develops, and on the addition of 1 to 2 drops of iron (III) chloride solution (1 in 50), the color changes to greenish brown.

(2) Dissolve 0.02 g of Enju Extract in 5 ml of ethanol by warming. A yellow color develops, and on the addition of 2 ml of hydrochloric acid and 0.05 g of magnesium powder,

*Enju Extract is one of the substances belonging to the "Rutin (extract)" category. For the definition of the Rutin (extract), see Enzymatically Decomposed Rutin.

the color gradually changes to red.

(3) Dissolve 0.01 g of Enju Extract in 100 ml of ethanol. The solution exhibits absorption maxima at wavelengths of approximately 257 nm and 361 nm.

Purity

(1) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 5.0 μg/g as Pb (2.0 g, Method 1).

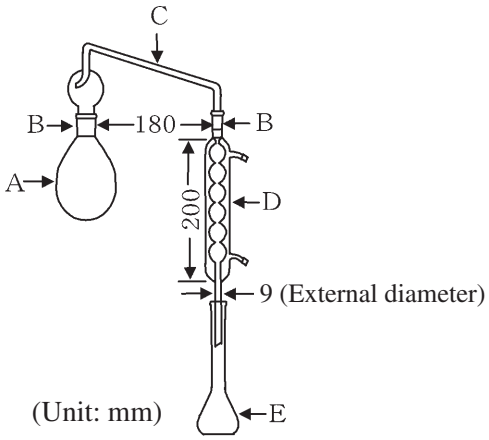
(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(4) Methanol Not more than 0.015%.

(i) Apparatus

Use apparatus as illustrated below.

A: Eggplant-shaped flask (200 ml)
B: Ground-glass joints
C: Delivery tube with a spray trap
D: Condenser
E: Measuring flask (50 ml)



(ii) Method

Test Solution Weigh accurately about 5 g of Enju Extract in eggplant-shaped flask A, add 100 ml of boric acid-sodium hydroxide buffer, mix well, and add 2–3 boiling chips. Place exactly 2 ml of the internal standard solution in measuring flask E. Assemble the apparatus, and moisten the ground-glass joints with water. Distill at a rate of 2–3 ml/minute until about 45 ml of distillate is obtained. To the distillate, add water to make exactly 50 ml. Use this solution as the test solution. Use tert-butanol solution (1 in 1,000) as the internal standard.

Standard Solution Weigh accurately about 0.5 g of methanol, and add water to make exactly 100 ml. Measure exactly 5 ml of this solution, and add water to make exactly 100 ml. Next, measure exactly 3 ml of the second solution and exactly 2 ml of the internal standard solution, and add water to make exactly 50 ml.

Procedure Analyze 2.0 μl portions of the test solution and the standard solution by gas chromatography using the operating conditions below. Determine the peak area ratios (Q_T and Q_S) of methanol to *tert*-butanol for the test solution and the standard solution, and calculate the methanol content by the formula:

$$\text{Content (\%) of methanol} = \frac{\text{Weight (g) of methanol}}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 0.15$$

Operating Conditions

Detector: Flame-ionization detector.
Column: A glass tube of 3 mm internal diameter and 2 m length.
Column packing material: 180- to 250-μm styrene-divinylbenzene porous polymer for gas chromatography.
Column temperature: A constant temperature at about 120°C.
Injection port: A constant temperature at about 200°C.
Injection: Full filling.
Carrier gas: Nitrogen or helium.
Flow rate: Adjust so that the retention time of methanol is about 2 minutes.

Loss on Drying Not more than 9.0 % (135°C, 2 hours).

Residue on Ignition Not more than 0.30 % (550°C, 4 hours).

Assay

Test Solution and Standard Solution Weigh accurately about 0.05 g each of Enju Extract and rutin for assay, previously dried at 135°C for 2 hours, and separately dissolve them in methanol to make 2 solutions of exactly 50 ml each. Measure exactly 5 ml of each solution, and add an 800:200:1 mixture of water/acetonitrile/phosphoric acid to each to make exactly 50 ml. Use these solutions as the test solution and the standard solution, respectively.

Procedure Analyze 20 μl portions of the test solution and the standard solution by liquid chromatography using the operating conditions below. Measure the peak areas (A_T and A_S) of the test solution and the standard solution, and calculate the rutin content by the formula:

$$\text{Content (\%) of rutin } (C_{27}H_{30}O_{16}) = \frac{\text{Weight (g) of rutin for assay}}{\text{Weight (g) of the sample}} \times \frac{A_T}{A_S} \times 100$$

Operating Conditions

Detector: Ultraviolet absorption spectrophotometer (determination wavelength: 254 nm).
Column: A stainless steel tube of 3–6 mm internal diameter and 15–25 cm length.
Column packing material: 5- to 10-μm octadecylsilanized silica gel for liquid chromatography.
Column temperature: 40°C.
Mobile phase: An 800:200:1 mixture of water/acetonitrile/phosphoric acid.
Flow rate: Adjust the flow rate so that the retention time of rutin is about 8–12 minutes.

Enzymatically Decomposed Lecithin

酵素分解レシチン

Definition Enzymatically Decomposed Lecithin is obtained from vegetable lecithin, which is derived from the seeds of the rape plant *Brassica rapa* Linné or *Brassica napus* Linné or the soybean plant *Glycine max* Merrill, or obtained from yolk lecithin, which is derived from egg yolk. It consists mainly of phosphatidic acid and lysolecithin. There are two types of commercial products: enzymatically decomposed

vegetable lecithin and enzymatically decomposed yolk lecithin.

Description Enzymatically Decomposed Lecithin occurs as a white to brown powder, granules, or lumps or as a light yellow to dark brown viscous liquid. It has a characteristic odor.

Identification

(1) Place 1 g of Enzymatically Decomposed Lecithin in a decomposition flask, add 5 g of powdered potassium sulfate, 0.5 g of cupric sulfate, and 20 ml of sulfuric acid. Heat gently with a tilt of about 45° until effervescence almost stops. Raise the temperature, and boil until the content becomes a clear blue liquid. Heat for an additional 1 to 2 hours. After cooling, add an equal volume of water. To 5 ml of this solution, add 10 ml of ammonium molybdate solution (1 in 5), and heat. A yellow precipitate is formed.

(2) Fatty acids To 1 g of Enzymatically Decomposed Lecithin, add 25 ml of ethanolic potassium hydroxide TS, reflux for 1 hour, and cool with ice. A precipitate or turbidity of potassium soap is formed.

Purity

(1) Acid value Not more than 65.

Test Solution For vegetable lecithin, dissolve about 2 g of the sample, accurately weighed, in 50 ml of toluene. For yolk lecithin, to about 2 g of the sample, accurately weighed, add 50 ml of methanol, and dissolve by warming in a water bath at 60°C or less.

Procedure Proceed as directed in the Acid Value Test in the Fats and Related Substances Tests.

(2) Acetone-soluble substances Not more than 60%.

Weigh accurately about 2 g of Enzymatically Decomposed Lecithin into a 50-ml graduated centrifuge tube with a stopper, dissolve it in 3 ml of toluene for vegetable lecithin or in 3 ml of methanol for yolk lecithin, and warm in a water bath at 60°C or less if necessary. To this solution, add 15 ml of acetone, stir well, and allow to stand in icy water for 15 minutes. Add acetone, previously cooled to 0–5°C, to make 50 ml, stir well, and allow to stand in icy water for 15 minutes. Centrifuge the solution obtained at about 3,000 rpm for 10 minutes, and transfer the upper layer solution into a flask. To the residue in the centrifuge tube, add acetone cooled to a temperature of 0–5°C to make 50 ml, and stir well while cooling in icy water. Centrifuge in the same manner, and transfer the upper layer solution into the flask to combine them. Distill the combined solution on a water bath, dry the residue at 105°C for 1 hour, and weigh accurately.

(3) Peroxide value Not more than 10.

Weigh accurately about 5 g of Enzymatically Decomposed Lecithin into a 250-ml Erlenmeyer flask, add 35 ml of a 2:1 mixture of chloroform/acetic acid, and shake gently to uniformly disperse or dissolve. Replace the air in the flask with clean nitrogen, and add exactly 1 ml of potassium iodine TS under nitrogen. Stop the nitrogen flow, immediately stopper the flask, shake for 1 minute, and allow to stand for 5 minutes in a dark place. To this solution, add 15 ml of water, stopper, and shake vigorously. Titrate with 0.01 mol/L sodium thiosulfate, using starch TS as the indicator. Calculate the peroxide value, using the following formula. Conduct a blank test to make any necessary correction.

$$\text{Peroxide value} = \frac{\left(\begin{array}{c}\text{Volume (ml) of}\\ \text{0.01 mol/L sodium thiosulfate consumed}\end{array}\right)}{\text{Weight (g) of the sample}} \times 10$$

(4) Heavy metals Not more than 40 μg/g as Pb (0.50 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(5) Lead Not more than 10 μg/g as Pb (1.0 g, Method 1).

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Loss on Drying Not more than 4.0% (105°C, 1 hour).

If the sample is a powder, proceed as directed under Loss on Drying in the General Tests. If the sample consists of granules, lumps, or a viscous liquid, proceed as directed in the following procedure. Place about 3 g of the sample in a weighing bottle, along with about 15 g of sea sand, weighed accurately, and a small glass rod, weighed accurately. Accurately weigh the bottle containing the sand and rod. Promptly grind the sample into particles of 2 mm or less using the rod if it is solid, or mix well if it is liquid. Finally, heat the bottle with the glass rod, and determine the loss.

Enzymatically Decomposed Rutin

ルチン酵素分解物

Definition Enzymatically Decomposed Rutin is obtained by enzymatically treating and purifying rutin (extract).* Its principal constituent is isoquercitrin.

Content Enzymatically Decomposed Rutin, when dried, contains 91.0–103.0% of isoquercitrin ($C_{21}H_{20}O_{12}$ = 464.38).

Description Enzymatically Decomposed Rutin occurs as a light yellow to yellow powder, lumps, or paste having a slight characteristic odor.

Identification

(1) Dissolve 5 mg of Enzymatically Decomposed Rutin in 10 ml of ethanol. A yellow color develops, and when 1 to 2 drops of iron(III) chloride solution (1 in 50) is added, the color changes to greenish brown.

(2) Dissolve 5 mg of Enzymatically Decomposed Rutin in 5 ml of ethanol. A yellow color develops, and when 2 ml of hydrochloric acid and 0.05 g of magnesium powder are added, it gradually changes to red.

(3) Dissolve 0.01 g of Enzymatically Decomposed Rutin in 500 ml of ethanol. The solution exhibits absorption maxima at wavelengths of approximately 258 nm and 362 nm.

(4) Prepare a test solution by dissolving 1.0 g of Enzymatically Decomposed Rutin in 20 ml of methanol. If necessary, filter before testing. Analyze a 2-μl portion of the test solution by thin-layer chromatography, using a 2-μl portion of a solution of rutin for assay in methanol (1 in 20) as the control solution and a 4:2:1 mixture of 1-butanol/acetic acid/water as the developing solvent. Use a thin-layer plate

*Rutin (extract)" is defined in the List of Existing Food Additives as a substance that is obtained from the entire part of the *azuki* plant *Vigna angularis* Ohwi et H. Ohashi, the buds or flowers of the plant *Sophora japonica* Linné, or the entire part of the buckwheat plant *Fagopyrum esculentum* Moench and that consists mainly of rutin.

coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 15 cm above the original line, and air-dry the plate. Spray with iron(III) chloride–hydrochloric acid TS. A brown main spot with an R_f value greater than that of the main spot of rutin for assay is observed.

Purity

(1) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 5.0 μg/g as Pb (2.0 g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Loss on Drying Not more than 50.0% (135°C, 2 hours).

Assay

Test Solution Weigh accurately about 0.05 g of Enzymatically Decomposed Rutin, previously dried, and dissolve it in methanol to make exactly 100 ml. If necessary, filter the solution obtained. Measure exactly 4 ml of the solution, and add diluted phosphoric acid (1 in 1,000) to make exactly 100 ml.

Standard Solution Weigh accurately about 0.05 g of rutin for assay, dried at 135°C for 2 hours, and dissolve it in methanol to make exactly 100 ml. Measure exactly 4 ml of this solution, and add diluted phosphoric acid (1 in 1,000) to make exactly 100 ml.

Procedure Measure the absorbances (A_T and A_S) of the test solution and the standard solution as directed under Ultraviolet-Visible Spectrophotometry at 351 nm. Use diluted phosphoric acid (1 in 1,000) as the reference. Calculate the content by the formula:

$$\text{Content (\%) of isoquercitrin}(C_{21}H_{20}O_{12}) = \frac{\text{Weight (g) of the rutin for assay} \times 0.761}{\text{Weight (g) of the sample}} \times \frac{A_T}{A_S} \times 100$$

Enzymatically Modified Hesperidin

酵素処理ヘスペリジン

Definition Enzymatically Modified Hesperidin is obtained by glucosylating hesperidin, which is extracted from the peels, juice, or seeds of citrus fruits with an alkaline aqueous solution, using cyclodextrin glucosyltransferase.

Content Enzymatically Modified Hesperidin, when dried, contains the equivalent of not less than 30.0% of total hesperetin glycosides.

Description Enzymatically Modified Hesperidin occurs as a pale yellow to yellow-brown powder having a slight characteristic odor.

Identification

(1) Dissolve 5 mg of Enzymatically Modified Hesperidin in 10 ml of water, and add 1–2 drops of dilute iron(III) chloride TS. A brown color develops.

(2) Dissolve 0.5 g of Enzymatically Modified Hesperidin in 100 ml of an 80:20:0.01 mixture of water/acetonitrile/acetic acid, and use this solution as the test solution. Separately, dissolve 0.05 g of monoglucosyl hesperidin for assay in 250 ml of an 80:20:0.01 mixture of water/acetonitrile/acetic acid, and use the solution obtained as the standard solution. Analyze 10 μl portions of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Enzymatically Modified Hesperidin exhibits a peak at the position corresponding to monoglucosyl hesperidin, having an absorption maximum at a wavelength of 280–286 nm.

Operating Conditions

Detector: Photodiode array detector (determination wavelength: 280 nm, 200–400 nm).

Column: A stainless steel tube of 3.9–4.6 mm internal diameter and 15–30 cm length.

Column packing material: 5- to 10-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 40°C.

Mobile phase: An 80:20:0.01 mixture of water/acetonitrile/acetic acid.

Flow rate: Adjust so that the retention time of monoglucosyl hesperidin is about 15 minutes.

Purity

(1) Clarity Clear (0.5 g, water 100 ml).

(2) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(3) Lead Not more than 10 μg/g as Pb (1.0 g, Method 1).

(4) Arsenic Not more than 2.0 μg/g as As_2O_3 (1.0 g, Method 3, Apparatus B).

Loss on Drying Not more than 6.0% (Not more than 2.7 kPa, 120°C, 2 hours).

Assay The total content of hesperetin glycosides is obtained as the sum of the contents of hesperidin, monoglucosyl hesperidin, and α-glucosyl residues released by glucoamylase treatment.

(i) Hesperidin and monoglucosyl hesperidin

Test Solution Weigh accurately about 1 g of Enzymatically Modified Hesperidin, previously dried, and dissolve it in 100 ml of water. Pour this solution into a glass tube (25 mm internal diameter) packed with 50 ml of acrylic acid ester resin, allow it to flow through at a rate of 2.5 ml/minute or less, and wash the resin with 250 ml of water. Next, allow 200 ml of 50% (vol) ethanol to flow through at a rate of 2.5 ml/minute or less to elute the absorbed fraction. Evaporate the eluate obtained to about 40 ml, add 10,000 units of glucoamylase, and allow the mixture to stand at 55°C for exactly 30 minutes. Heat it at 95°C for 30 minutes, cool to room temperature, and add water to make exactly 50 ml. Refer to this as solution A. Measure exactly 3 ml of solution A, and add an 80:20:0.01 mixture of water/acetonitrile/acetic acid to make exactly 50 ml.

Standard Solution Weigh accurately about 0.05 g of monoglucosyl hesperidin for assay, previously dried, and dissolve it in an 80:20:0.01 mixture of water/acetonitrile/acetic acid to make exactly 250 ml.

Procedure Analyze 10 μl portions of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Measure the peak areas (A_{TH}, A_{TM}) of hesperidin and monoglucosyl hesperidin for the test solution and the peak area (A_S) of monoglucosyl hesperidin for the standard solution. Calculate the contents of hesperidin and monoglucosyl hesperidin using the following formulas. The relative retention time of hesperidin to monoglucosyl hesperidin is about 1.1.

$$\text{Content (\%) of hesperidin} = \frac{\left(\begin{array}{c}\text{Weight (g) of}\\ \text{dried monoglucosyl hesperidin for assay}\end{array}\right)}{\text{Weight (g) of the dried sample}} \times \frac{A_{TH}}{A_S} \times \frac{10}{3} \times 0.790 \times 100$$

$$\text{Content (\%) of monoglucosyl hesperidin} = \frac{\left(\begin{array}{c}\text{Weight (g) of}\\ \text{dried monoglucosyl hesperidin for assay}\end{array}\right)}{\text{Weight (g) of the dried sample}} \times \frac{A_{TM}}{A_S} \times \frac{10}{3} \times 100$$

Operating Conditions

Detector: Ultraviolet absorption photometry (Determination wavelength: 280 nm).

Column: A stainless steel tube of 3.9–4.6 mm internal diameter and 15–30 cm length.

Column packing material: 5- to 10-µm octadecylsilanized silica gel.

Column temperature: 40°C.

Mobile phase: An 80:20:0.01 mixture of water/acetonitrile/acetic acid.

Flow rate: Adjust the flow rate so that the retention time of monoglucosyl hesperidin is about 15 minutes.

(ii) α-Glucosyl residues released by glucoamylase treatment

Test Solution Use solution A prepared in section (i) above.

Blank Test Solution Add 10,000 units of glucoamylase to about 40 ml of water, allow to stand at 55°C for 30 minutes, heat at 95°C for about 30 minutes, and cool to room temperature. Add water to make exactly 50 ml.

Standard Solutions Weigh accurately about 1g of glucose, and dissolve it in water to make exactly 100 ml. Transfer exactly 5 ml, 10 ml, 20 ml, and 30 ml of this solution into separate 100-ml volumetric flasks, and dilute each with water to volume.

Procedure Measure 20 µl of the test solution, add exactly 3 ml of color fixing TS for D-glucose determination, and shake. Allow the solution to stand at exactly 37°C for 5 minutes, and then cool to room temperature. Measure the absorbance at a wavelength of 505 nm against a reference solution prepared as directed for the test solution, using 20 µl of water instead of the test solution. Perform a blank test by measuring the absorbance of the blank test solution in the same manner as for the test solution, and make any necessary correction. Prepare a calibration curve by measuring the absorbances of the standard solutions in the same manner as for the test solution. Determine the concentration of D-glucose in the test solution from the calibration curve and the corrected absorbance of the test solution, and calculate the content of α-glucosyl residue released by glucoamylase treatment content by the formula:

$$\text{Content (\%) of α-glucosyl residue} = \frac{\left(\begin{array}{c}\text{Concentration (mg/ml) of}\\ \text{D-glucose in the test solution}\end{array}\right) \times 50}{\text{Weight (g) of the dried sample} \times 1{,}000} \times 0.900 \times 100$$

(iii) Total content of hesperetin glycosides (dry matter)

Calculate the total content of hesperetin glycosides by the formula:

Total content (%) of hesperetin glucosides
= Content (%) of hesperidin
+ Content (%) monoglucosyl hesperidin
+ Content (%) of α-glucosyl residue

Enzymatically Modified Isoquercitrin

酵素処理イソクエルシトリン

Definition Enzymatically Modified Isoquercitrin is obtained by glucosylating a mixture of "Enzymatically Decomposed Rutin" and starch or dextrin with cyclodextrin glucosyltransferase. It consists mainly of α-glucosylisoquercitrin.

Content Enzymatically Modified Isoquercitrin, when dried, contains α-glucosylisoquercitrin, equivalent to not less than 60.0% of rutin ($C_{27}H_{30}O_{16}$ = 610.52).

Description Enzymatically Modified Isoquercitrin occurs as a yellow to yellow-orange powder, lumps, or paste. It is a slight characteristic odor.

Identification

(1) Dissolve 5 mg of Enzymatically Modified Isoquercitrin in 10 ml of water. A yellow to yellow-orange color develops, and on the addition of 1 to 2 drops of iron(III) chloride solution (1 in 50), the color changes to blackish-brown.

(2) Dissolve 5 mg of Enzymatically Modified Isoquercitrin in 5 ml of water. A yellow to yellow-orange color is produced, and on the addition of 2 ml of hydrochloric acid and 0.05 g of magnesium powder, the color gradually changes to orange to red.

(3) Dissolve 0.1 g of Enzymatically Modified Isoquercitrin in 100 ml of 0.5 mol/L sulfuric acid, boil for 2 hours, and cool. A yellow precipitate is formed.

(4) Dissolve 0.01 g of Enzymatically Modified Isoquercitrin in 500 ml of diluted phosphoric acid solution (1 in 1,000). The solution obtained has absorption maxima at wavelengths of approximately 255 nm and 350 nm.

(5) Dissolve 0.1 g of Enzymatically Modified Isoquercitrin in 20 ml of water, and use the resulting solution as the test solution. Analyze a 5-µl portion of the test solution by thin-layer chromatography, using a 2-µl portion of a solution of rutin for assay in methanol (1 in 20) as the control solution and a 4:2:1 mixture of 1-butanol/acetic acid/water as the developing solvent. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 15 cm above the original line, air-dry the plate, and spray with iron(III) chloride–hydrochloric acid TS. Several brown spots are observed: one having an R_f value greater than that of the main spot of rutin for assay and others having R_f values the same as or smaller than that of the main spot of rutin for assay.

Purity

(1) Heavy metals Not more than 10 µg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 5.0 µg/g as Pb (2.0 g, Method 1).

(3) Arsenic Not more than 2.0 µg/g as As_2O_3 (1.0 g, Meth-

od 3, Apparatus B).

Loss on Drying Not more than 50.0% (135°C, 2 hours).

Assay

Test Solution Weigh accurately about 0.05 g of Enzymatically Modified Isoquercitrin, previously dried, and dissolve it in water to make exactly 100 ml. Filter if necessary. Measure exactly 4 ml of this solution, add diluted phosphoric acid (1 in 1,000), and make exactly 100 ml.

Standard Solution Weigh accurately about 0.05 g of rutin for assay, dried at 135°C for 2 hours, and dissolve it in methanol to make exactly 100 ml. Measure 4 ml of this solution, and add diluted phosphoric acid (1 in 1,000) to make exactly 100 ml.

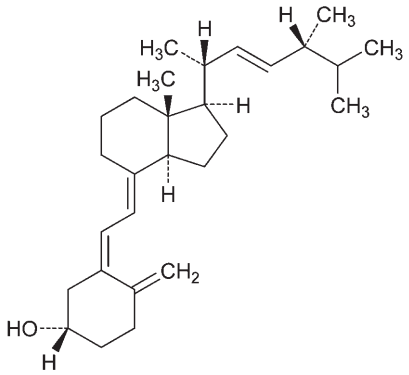
Procedure Measure the absorbances (A_T and A_S) of the test solution and the standard solution at 351 nm as directed under Spectrophotometry, using diluted phosphoric acid (1 in 1,000) as the reference solution. Calculate the α-glucosylisoquercitrin content as rutin ($C_{27}H_{30}O_{16}$) by the formula:

$$\text{Content (\%) of α-glucosylisoquercitrin} = \frac{\text{Weight (g) of the rutin for assay}}{\text{Weight (g) of the sample}} \times \frac{A_T}{A_S} \times 100$$

Ergocalciferol

Calciferol
Vitamin D2

エルゴカルシフェロール



$C_{28}H_{44}O$ Mol. Wt. 396.65
(3*S*,5*Z*,7*E*,22*E*)-9,10-Secoergosta-5,7,10(19),22-tetraen-3-ol [50-14-6]

Description Ergocalciferol occurs as white crystals. It is odorless.

Identification

(1) Dissolve 0.5 mg of Ergocalciferol in 5 ml of toluene, and add 0.3 ml of acetic anhydride and 0.1 ml of sulfuric acid, and shake. A red color develops, which immediately changes through purple and blue eventually to green.

(2) Dissolve 0.05 g of Ergocalciferol in 1 ml of dehydrated pyridine, add a solution of 0.05 g of 3,5-dinitrobenzoyl chloride in 1 ml of dehydrated pyridine, heat under a reflux condenser on a water bath for 10 minutes, and cool to room temperature. Transfer the solution to a separating funnel, and extract with 15 ml of diluted hydrochloric acid (1 in 10) and 30 ml of diethyl ether by shaking. Wash the diethyl ether extract three times with 15 ml of diluted hydrochloric acid (1 in 10) each time, and wash with 30 ml of water. Add 5 g of anhydrous sodium sulfate, allow to stand for 20 minutes, filter through absorbent cotton, and wash with a small amount of diethyl ether. Combine the filtrate and the washings, and evaporate the diethyl ether under reduced pressure. Recrystallize the residue twice from acetone, and dry in a desiccator for 2 hours (under reduced pressure). The melting point is 147–149°C.

Purity

(1) Specific absorbance $E_{1cm}^{1\%}$ (265 nm): 445–485.

Weigh accurately about 0.1 g of Ergocalciferol, and dissolve it in ethanol to make exactly 200 ml. Measure exactly 2 ml of this solution, and add ethanol to make exactly 100 ml. Measure the absorbance of the second solution.

(2) Specific rotation $[\alpha]_D^{20}$: +102.0 to +107.0° (0.3 g, ethanol, 20 ml).

(3) Melting point 115–118°C.

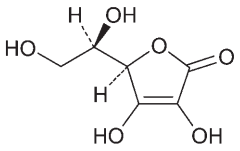
(4) Ergosterol Weigh 0.010 g of Ergocalciferol, and add 2 ml of 90% (vol) ethanol to dissolve. To the resulting solution, add a solution prepared by dissolving 0.020 g of digitonin in 2 ml of 90% (vol) ethanol, and allow to stand for 18 hours. No precipitate is formed.

Storage Standards Store in a cold place in a hermetic, light-resistant container under inert gas.

Erythorbic Acid

Isoascorbic Acid

エリソルビン酸



$C_6H_8O_6$ Mol. Wt. 176.12
(5*R*)-3,4-Dihydroxy-5-[(1*R*)-1,2-dihydroxyethyl]furan-2(5*H*)-one [89-65-6]

Content Erythorbic Acid, when dried, contains not less than 99.0% of erythorbic acid ($C_6H_8O_6$).

Description Erythorbic Acid occurs as white to yellowish white crystals or crystalline powder. It is odorless and has an acid taste.

Identification

(1) Dissolve 0.1 g of Erythorbic Acid in 100 ml of metaphosphoric acid solution (1 in 50). To 5 ml of this solution, add iodine TS dropwise until a slightly yellow color develops. To the solution, add 1 drop of cupric sulfate solution (1 in 1,000) and 1 drop of pyrrole, and warm in a water bath at 50–60°C for 5 minutes. A blue to blue–green color develops.

(2) To 10 ml of a solution of Erythorbic Acid (1 in 100), add 1 ml of potassium permanganate solution (1 in 300). A

pink color develops, which disappears immediately.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: −16.2 to −18.2° (previously dried, 1 g, freshly boiled and cooled water, 10 ml).

(2) Melting point 166–172°C (decomposition).

(3) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Drying Not more than 0.40% (reduced pressure, 3 hours).

Residue on Ignition Not more than 0.30%.

Assay Weigh accurately about 0.4 g of Erythorbic Acid, dried previously, dissolve it in metaphosphoric acid solution (1 in 50) to make exactly 100 ml. Measure exactly 50 ml of this solution, and titrate with 0.05 mol/L iodine (indicator: starch TS).

Each ml of 0.05 mol/L iodine = 8.806 mg of $C_6H_8O_6$

Ester Gum

エステルガム

Definition Ester Gum is esters of rosins or their derivatives, such as rosin polymers. There are several types of ester gums, according to the alcohols used, including glycerol ester gum, pentaerythritol ester gum, and methanol ester gum.

Description Ester Gum occurs as a white to yellow-whitish powder, as light yellow to light brown glassy lumps, or as a clear, viscous liquid. It is odorless or has a slight, characteristic odor.

Identification

(1) Dissolve 0.1 g of Ester Gum in 10 ml of acetic anhydride while heating in a water bath, cool, and add 1 drop of sulfuric acid. A purple-red color develops.

(2) To 1 g of Ester Gum, add 5 ml of sodium hydroxide solution (1 in 25) and 5 ml of water, and shake vigorously. Light yellow turbidity appears, and persistent foams are produced.

(3) In the case of glycerol ester gum compounds or pentaerythritol ester gum compounds

Test Solution Weigh about 5 g of Ester Gum, place into a 100-ml flask, add 40 ml of a solution of potassium hydroxide in 1-hexanol (1 in 10), and reflux under a condenser for 2 hours. Add 40 ml of diethyl ether and 40 ml of water, mix, and transfer to a separating funnel. Adjust the pH to 1.0 to 1.5 with hydrochloric acid (1 in 4), and allow to stand. After the solution is separated into two layers, collect the aqueous layer (lower layer), remove water by heating under reduced pressure, and evaporate to dryness. To about 0.1 g of the residue, add 1 ml of silylation TS, and silylate by heating at 70°C for 20 minutes.

Standard Solution Weigh about 0.05 g of glycerol for glycerol ester gum or of pentaerythritol for pentaerythritol ester gum, add 1 ml of silylation TS, and silylate in the same manner as for the test solution.

Procedure Analyze equal portions of the test solution and the standard solution by gas chromatography using the operating conditions given below. The retention time of the main peak from the test solution corresponds to that of the peak of silylated glycerol or silylated pentaerythritol from the standard solution. The peak of the solvent should be excluded.

Operating Conditions

Detector: Flame ionization detector.

Column: A glass or stainless steel tube of 2 mm internal diameter and 2 m length.

Column packing material

Liquid phase: 5 % Methyl silicon polymer of the amount of support.

Support: 149- to 177- μm diatomaceous earth for gas chromatography.

Column temperature: A constant temperature at around 150°C.

Carrier gas: Nitrogen.

Flow rate: About 50 ml/minute.

(4) In the case of methanol ester gum compounds

Test Solution Weigh about 5 g of Ester Gum, place into a 100-ml flask, add 40 ml of a solution of potassium hydroxide in 1-hexanol (1 in 10), and reflux under a condenser for 2 hours. Distil under reduced pressure (15 kPa), and take the distillate at 50°C. Add 5 g of 1-hexanol to the distillate.

Standard Solution Prepare a solution of methanol in 1-hexanol (1 in 10), and use as the standard solution.

Procedure Analyze equal portions of the test solution and the standard solution by gas chromatography using the operating conditions. The retention time of the main peak from the test solution corresponds to that of the peak of methanol from the standard solution. The peak of the solvent should be excluded.

Operating Conditions

Detector: Flame ionization detector.

Column: A glass or stainless steel tube of 2 mm internal diameter and 2 m length.

Column packing material

Liquid phase: 5% Methyl silicon polymer of the amount of support.

Support: 149- to 177- μm diatomaceous earth for gas chromatography.

Column temperature: A constant temperature at around 50°C.

Carrier gas: Nitrogen.

Flow rate: About 50 ml/minute.

Purity

(1) Clarity of solution Clear.

Test Solution Weigh 10 g of Ester Gum, add 10 ml of toluene, dissolve by warming to 70–75°C, filter while warm, and allow to stand for 24 hours.

(2) Acid value Not more than 8.0.

Glycerol ester gum compounds: Not more than 8.0.

Pentaerythritol ester gum compounds: Not more than 18.0.

Methanol ester gum compounds: Not more than 8.0.

Test Solution Weigh accurately about 3 g of Ester Gum, dissolve it in 50 ml of a 2:1 mixture of toluene/ethanol.

Procedure Proceed as directed in the Acid Value Test in the Fats and Related Substances Tests.

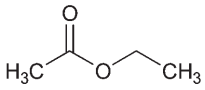
(3) Heavy metals Not more than 40 μg/g as Pb (0.50 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Residue on Ignition Not more that 0.10%.

Ethyl Acetate

酢酸エチル



$C_4H_8O_2$ Mol. Wt. 88.11

Ethyl acetate [141-78-6]

Content Ethyl Acetate contains not less than 98.0% of ethyl acetate ($C_4H_8O_2$).

Description Ethyl Acetate is a colorless, transparent liquid having a fruity odor.

Identification

(1) To 1 ml of Ethyl Acetate, add 25 ml of sodium hydroxide solution (1 in 25), heat in a water bath for 5 minutes. After cooling, neutralize with diluted hydrochloric acid (1 in 4) and add 5 drops of iron(III) chloride solution (1 in 10). A deep red color develops.

(2) To 1 ml of Ethyl Acetate, add 5 ml of sodium hydroxide solution (1 in 5), and heat in a water bath while shaking. The fruity odor disappears. Acidify this solution with diluted sulfuric acid (1 in 20), and heat again in a water bath while shaking. An odor of acetic acid is evolved.

Purity

(1) Refractive index n_D^{20}: 1.370–1.375.

(2) Specific gravity 0.900–0.904.

(3) Acid value Not more than 0.1 (Flavoring Substances Tests).

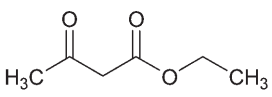
Weigh 20 g of Ethyl Acetate, and proceed as directed in the Acid Value Test in the Flavoring Substances Tests.

Assay Place 10 ml of ethanol into a 100-ml flask, and weigh the flask with ethanol accurately. To the flask, add about 1 g of Ethyl Acetate, and weigh it accurately. Add exactly 40 ml of 0.5 mol/L ethanolic potassium hydroxide, and heat under a reflux condenser in a water bath at 78–82°C for 20 minutes. After cooling, titrate the excess alkali with 0.5 mol/L hydrochloric acid (indicator: 2–3 drops of phenolphthalein TS). Perform a blank test in the same manner.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 44.05 mg of $C_4H_8O_2$

Ethyl Acetoacetate

アセト酢酸エチル



$C_6H_{10}O_3$ Mol. Wt. 130.14

Ethyl 3-oxobutanoate [141-97-9]

Content Ethyl Acetoacetate contains 98.0–102.0% of ethyl acetoacetate ($C_6H_{10}O_3$).

Description Ethyl Acetoacetate is a colorless, transparent liquid having a characteristic odor.

Identification Determine the absorption spectrum of Ethyl Acetoacetate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit absorptions having almost the same intensity at the same wavenumbers.

Purity

(1) Refractive index n_D^{20}: 1.418–1.421.

(2) Specific gravity 1.027–1.032.

(3) Clarity of solution Clear (1.0 ml, 30% (vol) ethanol 3.0 ml).

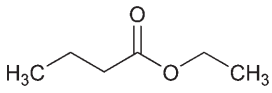
(4) Free acid Measure 15 ml of Ethyl Acetoacetate, add 15 ml of freshly boiled and cooled water, shake for 2 minutes, and allow to stand. Measure 10 ml of the water layer, and add 2 drops of phenolphthalein TS and 3.4 ml of 0.1 mol/L potassium hydroxide. A pink color develops.

Assay Weigh accurately about 0.8 g of Ethyl Acetoacetate, and proceed as directed in Method 2 in the Aldehyde and Ketone Content Test in the Flavoring Substances Tests. In the test, allow the mixture to stand for 15 minutes before titrating.

Each ml of 0.5 mol/L hydrochloric acid = 65.07 mg of $C_6H_{10}O_3$

Ethyl Butyrate

酪酸エチル



$C_6H_{12}O_2$ Mol. Wt. 116.16

Ethyl butanoate [105-54-4]

Content Ethyl Butyrate contains not less than 98.0% of ethyl butyrate ($C_6H_{12}O_2$).

Description Ethyl Butyrate is a colorless to light yellow, transparent liquid having a fruity odor.

Identification To 1 ml of Ethyl Butyrate, add 5 ml of ethanolic 10% potassium hydroxide TS. Heat in a water bath while shaking. The fruity odor disappears. After cooling, acidify with diluted sulfuric acid (1 in 20). An odor of butyric acid is evolved.

Purity

(1) Refractive index n_D^{20}: 1.390–1.394.

(2) Specific gravity 0.875–0.882.

(3) Clarity of solution Clear (2.0 ml, 70% (vol) ethanol 4.0 ml).

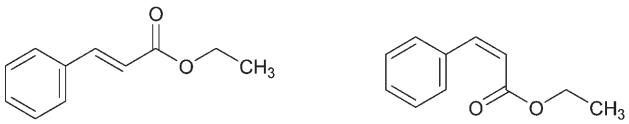
(4) Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Weigh accurately about 0.5 g of Ethyl Butyrate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 58.08 mg of $C_6H_{12}O_2$

Ethyl Cinnamate

ケイ皮酸エチル

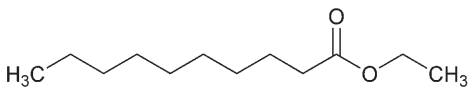


$C_{11}H_{12}O_2$ Mol. Wt. 176.21
Ethyl 3-phenylprop-2-enoate [103-36-6]
Content Ethyl Cinnamate contains not less than 99.0% of ethyl cinnamate ($C_{11}H_{12}O_2$).
Description Ethyl Cinnamate is a colorless to light yellow liquid having a characteristic odor.
Identification To 1 ml of Ethyl Cinnamate, add 10 ml of ethanolic 10% potassium hydroxide TS, and heat in a water bath. Ethyl Cinnamate dissolves, a white precipitate is formed, and the characteristic odor disappears. Add 10 ml of water while warm. The precipitate dissolves. When the solution is made acidic with diluted sulfuric acid (1 in 20), a white crystalline precipitate is formed.
Purity
(1) Refractive index n_D^{20}: 1.559–1.561.
(2) Specific gravity 1.049–1.052.
(3) Clarity of solution Clear (1.0 ml, 70% (vol) ethanol 5.0 ml).
(4) Acid value Not more than 1.0 (Flavoring Substances Tests).
Assay Weigh accurately about 1 g of Ethyl Cinnamate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests. Add 5 ml of water before heating.
Each ml of 0.5 mol/L ethanolic potassium hydroxide = 88.11 mg of $C_{11}H_{12}O_2$

Ethyl Decanoate

Ethyl Caprate

デカン酸エチル



$C_{12}H_{24}O_2$ Mol. Wt. 200.32
Ethyl decanoate [110-38-3]
Content Ethyl Decanoate contains not less than 98.0% of ethyl decanoate ($C_{12}H_{24}O_2$).
Description Ethyl Decanoate is a colorless, transparent liquid having a brandy-like odor.
Identification Determine the absorption spectrum of Ethyl Decanoate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit absorptions having almost the same intensity at the same wavenumbers.
Purity
(1) Refractive index n_D^{20}: 1.424–1.427.
(2) Specific gravity 0.864–0.867.
(3) Clarity of solution Clear (1.0 ml, 80% (vol) ethanol 4.0 ml).
(4) Acid value Not more than 1.0 (Flavoring Substances Tests).
Assay Weigh accurately about 1 g of Ethyl Decanoate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.
Each ml of 0.5 mol/L ethanolic potassium hydroxide = 100.2 mg of $C_{12}H_{24}O_2$

2-Ethyl-3,(5 or 6)-dimethylpyrazine

2-Ethyl-3,5(6)-dimethylpyrazine

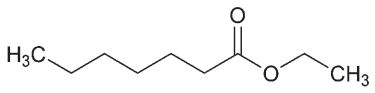
2-エチル-3,5-ジメチルピラジン及び
2-エチル-3,6-ジメチルピラジンの混合物

$C_8H_{12}N_2$ Mol. Wt.136.20
Mixture of 2-ethyl-3,5-dimethylpyrazine and
2-ethyl-3,6-dimethylpyrazine [55031-15-7]
Content 2-Ethyl-3,(5 or 6)-dimethylpyrazine contains not less than 95.0% of a mixture of 2-ethyl-3,5-dimethylpyrazine and 2-ethyl-3,6-dimethylpyrazine ($C_8H_{12}N_2$).
Description 2-Ethyl-3,(5 or 6)-dimethylpyrazine is a colorless to slightly yellow, transparent liquid. It has a characteristic odor
Identification Determine the absorption spectrum of 2-Ethyl-3,(5 or 6)-dimethylpyrazine as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.
Purity
(1) Refractive index n_D^{20}: 1.496–1.506.
(2) Specific gravity 0.950–0.980.
Assay Proceed as directed in Method 1 in the Gas Chromatographic Assay in the Flavor Substance Tests, using operating conditions (1).

Ethyl Heptanoate

Ethyl Enanthate

ヘプタン酸エチル

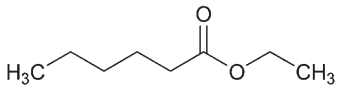


$C_9H_{18}O_2$ Mol. Wt. 158.24
Ethyl heptanoate [106-30-9]
Content Ethyl Heptanoate contains not less than 98.0% of ethyl heptanoate ($C_9H_{18}O_2$).
Description Ethyl Heptanoate is a colorless to light yellow, transparent liquid having a wine-like odor.
Identification Determine the absorption spectrum of Ethyl Heptanoate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum of Ethyl heptanoate. Both spectra exhibit similar intensities of absorption at the same wavenumbers.
Purity
(1) Refractive index n_D^{20}: 1.411–1.416.
(2) Specific gravity 0.869–0.874.
(3) Clarity of solution Clear (1.0 ml, 70% (vol) ethanol 5.0 ml).
(4) Acid value Not more than 1.0 (Flavoring Substances Tests).
Assay Weigh accurately about 0.8 g of Ethyl Heptanoate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.
Each ml of 0.5 mol/L ethanolic potassium hydroxide = 79.12 mg of $C_9H_{18}O_2$

Ethyl Hexanoate

Ethyl Caproate

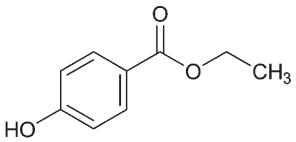
ヘキサン酸エチル



$C_8H_{16}O_2$ Mol. Wt. 144.21
Ethyl hexanoate [123-66-0]
Content Ethyl Hexanoate contains not less than 98.0% of ethyl hexanoate ($C_8H_{16}O_2$).
Description Ethyl Hexanoate is a colorless to light yellow, transparent liquid having a characteristic odor.
Identification To 1 ml of Ethyl Hexanoate, add 5 ml of ethanolic 10% potassium hydroxide TS, and heat in a water bath while shaking. The characteristic odor disappears. After cooling, acidify with diluted sulfuric acid (1 in 20). An odor of hexanoic acid is evolved.
Purity
(1) Refractive index n_D^{20}: 1.406–1.409.
(2) Specific gravity 0.871–0.875.
(3) Clarity of solution Clear (1.0 ml, 70% (vol) ethanol 4.0 ml).
(4) Acid value Not more than 1.0 (Flavoring Substances Tests).
Assay Weigh accurately about 0.7 g of Ethyl Hexanoate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.
Each ml of 0.5 mol/L ethanolic potassium hydroxide = 72.11 mg of $C_8H_{16}O_2$

Ethyl *p*-Hydroxybenzoate

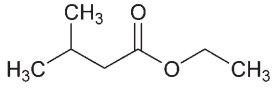
パラオキシ安息香酸エチル



$C_9H_{10}O_3$ Mol. Wt. 166.17
Ethyl 4-hydroxybenzoate [120-47-8]
Content Ethyl *p*-Hydroxybenzoate, when dried, contains not less than 99.0% of ethyl *p*-hydroxybenzoate ($C_9H_{10}O_3$).
Description Ethyl *p*-Hydroxybenzoate occurs as colorless crystals or as a white crystalline powder. It is odorless.
Identification
(1) Proceed as directed in Identification (1) for Butyl *p*-Hydroxybenzoate.
(2) To 0.05 g of Ethyl *p*-Hydroxybenzoate, add 2 drops of acetic acid and 5 drops of sulfuric acid, and warm for 5 minutes. An odor of ethyl acetate is evolved.
Purity
(1) Melting point 115–118°C.
(2) Free acid Not more than 0.55% as *p*-hydroxybenzoic acid.
Proceed as directed in Purity (2) for Butyl *p*-Hydroxybenzoate.
(3) Sulfate Not more than 0.024% as SO_4.
Proceed as directed in Purity (3) for Butyl *p*-Hydroxybenzoate.
(4) Heavy metals Not more than 10 μg/g as Pb.
Proceed as directed in Purity (4) for Butyl *p*-Hydroxybenzoate.
(5) Arsenic Not more than 4.0 μg/g as As_2O_3.
Proceed as directed in Purity (5) for Butyl *p*-Hydroxybenzoate.
Loss on Drying Not more than 0.50% (80°C, 2 hours).
Residue on Ignition Not more than 0.05% (5 g).
Assay Proceed as directed in the Assay for Butyl *p*-Hydroxybenzoate.
Each ml of 1 mol/L sodium hydroxide = 166.2 mg of $C_9H_{10}O_3$

Ethyl Isovalerate

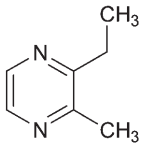
イソ吉草酸エチル



$C_7H_{14}O_2$ Mol. Wt. 130.18
Ethyl 3-methylbutanoate [108-64-5]
Content Ethyl Isovalerate contains not less than 98.0% of ethyl isovalerate ($C_7H_{14}O_2$).
Description Ethyl Isovalerate is a colorless to light yellow, transparent liquid having a fruity odor.
Identification To 1 ml of Ethyl Isovalerate, add 5 ml of ethanolic 10% potassium hydroxide TS, and heat in a water bath while shaking. The fruity odor disappears. After cooling, acidify with diluted sulfuric acid (1 in 20). An odor of isovaleric acid develops.
Purity
(1) Refractive index n_D^{20}: 1.393–1.399.
(2) Specific gravity 0.865–0.869.
(3) Clarity of solution Clear (2.0 ml, 70% (vol) ethanol 6.0 ml).
(4) Acid value Not more than 1.0 (Flavoring Substances Tests).
Assay Weigh accurately about 0.7 g of Ethyl Isovalerate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.
Each ml of 0.5 mol/L ethanolic potassium hydroxide = 65.09 mg of $C_7H_{14}O_2$

2-Ethyl-3-methylpyrazine

2-エチル-3-メチルピラジン

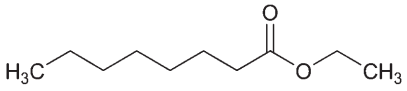


$C_7H_{10}N_2$ Mol. Wt. 122.17
2-Ethyl-3-methylpyrazine [15707-23-0]
Content 2-Ethyl-3-methylpyrazine contains not less than 98.0% of 2-Ethyl-3-methylpyrazine ($C_7H_{10}N_2$).
Description 2-Ethyl-3-methylpyrazine is a colorless or yellow liquid having a characteristic odor.
Identification Determine the absorption spectrum of 2-Ethyl-3-methylpyrazine, as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit absorptions having about the same intensity at the same wavenumbers.
Purity
(1) Refractive index n_D^{20}: 1.502–1.505.
(2) Specific gravity d_{25}^{25}: 0.978–0.988.
Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavor Substance Tests. Use operating conditions (1).

Ethyl Octanoate

Ethyl Caprylate

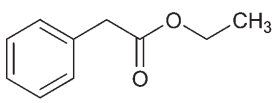
オクタン酸エチル



$C_{10}H_{20}O_2$ Mol. Wt. 172.26
Ethyl octanoate [106-32-1]
Content Ethyl Octanoate contains not less than 98.0% of ethyl octanoate ($C_{10}H_{20}O_2$).
Description Ethyl Octanoate is a colorless or slightly yellowish, transparent liquid having a brandy-like odor.
Identification Determine the absorption spectrum of Ethyl Octanoate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit absorptions having almost the same intensity at the same wavenumbers.
Purity
(1) Refractive index n_D^{20}: 1.417–1.419.
(2) Specific gravity 0.867–0.871.
(3) Clarity of solution Clear (1.0 ml, 70% (vol) ethanol 8 ml).
(4) Acid value Not more than 1.0 (Flavoring Substances Tests).
Assay Weigh accurately about 1 g of Ethyl Octanoate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.
Each ml of 0.5 mol/L ethanolic potassium hydroxide = 86.13 mg of $C_{10}H_{20}O_2$

Ethyl Phenylacetate

フェニル酢酸エチル



$C_{10}H_{12}O_2$ Mol. Wt. 164.20
Ethyl 2-phenylacetate [101-97-3]
Content Ethyl Phenylacetate contains not less than 98.0% of ethyl phenylacetate ($C_{10}H_{12}O_2$).
Description Ethyl Phenylacetate is a colorless, transparent liquid having a characteristic odor.

Identification Determine the absorption spectrum of Ethyl Phenylacetate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit absorptions having almost the same intensity at the same wavenumbers.

Purity

(1) Refractive index n_D^{20}: 1.496–1.500.

(2) Specific gravity 1.031–1.036.

(3) Clarity of solution Clear (1.0 ml, 70% (vol) ethanol 3.0 ml).

(4) Acid value Not more than 1.0 (Flavoring Substances Tests).

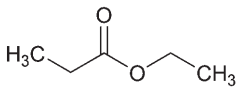
(5) Halogenated compounds Proceed as directed for Halogenated Compounds in the Flavoring Substances Tests.

Assay Weigh accurately about 1.5 g of Ethyl Phenylacetate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 82.10 mg of $C_{10}H_{12}O_2$

Ethyl Propionate

プロピオン酸エチル



$C_5H_{10}O_2$ Mol. Wt. 102.13

Ethyl propanoate [105-37-3]

Content Ethyl Propionate contains not less than 98.0% of ethyl propionate ($C_5H_{10}O_2$).

Description Ethyl Propionate is a colorless, transparent liquid having a characteristic odor.

Identification To 1 ml of Ethyl Propionate, add 5 ml of ethanolic 10% potassium hydroxide TS, and warm in warm water. The characteristic odor disappears. After cooling, acidify with diluted sulfuric acid (1 in 20). An odor of propionic acid is evolved.

Purity

(1) Refractive index n_D^{20}: 1.383–1.385.

(2) Specific gravity 0.890–0.893.

(3) Clarity of solution Clear (1.0 ml 50% (vol) ethanol 3.0 ml).

(4) Acid value Not more than 1.0 (Flavoring Substances Tests).

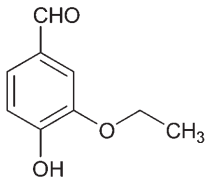
Assay Weigh accurately about 1 g of Ethyl Propionate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 51.07 mg of $C_5H_{10}O_2$

Ethylvanillin

Ethyl Vanillin

エチルバニリン



$C_9H_{10}O_3$ Mol. Wt. 166.17

3-Ethoxy-4-hydroxybenzaldehyde [121-32-4]

Content Ethylvanillin contains not less than 98.0% of ethylvanillin ($C_9H_{10}O_3$).

Description Ethylvanillin occurs as white to light yellow, flaky crystals or crystalline powder having a vanilla-like odor and taste.

Identification Determine the absorption spectrum of Ethylvanillin as directed in the Paste Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit absorptions having almost the same intensity at the same wavenumbers.

Purity

(1) Melting point 76–78°C.

(2) Clarity of solution Clear (1.0 g, 60% (vol) ethanol 10 ml).

(3) Heavy metals Not more than 10 µg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 4, Apparatus B).

Loss on Drying Not more than 0.5% (4 hours).

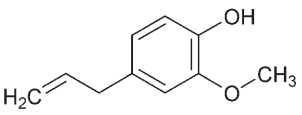
Residue on Ignition Not more than 0.05%.

Assay Weigh accurately about 1 g of Ethylvanillin, and proceed as directed in Method 2 in the Aldehyde and Ketone Content Test in the Flavoring Substances Tests. In the test, allow the mixture to stand for 15 minutes before titrating.

Each ml of 0.5 mol/L hydrochloric acid = 83.09 mg of $C_9H_{10}O_3$

Eugenol

オイゲノール



$C_{10}H_{12}O_2$ Mol. Wt. 164.20

4-Allyl-2-methoxyphenol [97-53-0]

Content Eugenol contains not less than 98.0% (vol) of eugenol ($C_{10}H_{12}O_2$).

Description Eugenol is a colorless to light yellow-brown, transparent liquid having a clove-like odor.
Identification Determine the absorption spectrum of Eugenol as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit absorptions having almost the same intensity at the same wavenumbers.
Purity
(1) Refractive index n_D^{20}: 1.539–1.542.
(2) Specific gravity 1.065–1.071.
(3) Clarity of solution Clear (2.0 ml, 70% (vol) ethanol 4.0 ml).
Assay Proceed as directed in the Phenol Content Test in the Flavoring Substances Tests. Instead of allowing to stand for 30 minutes, heat in a water bath for 30 minutes, and allow to cool to room temperature.

Ferric Ammonium Citrate

Ferric Ammonium Citrate, Brown
Ferric Ammonium Citrate, Green

クエン酸鉄アンモニウム

Ammonium iron(III) salt of 2-hydroxypropane-1,2,3-tricarboxylic acid [1185-57-5]
Content Ferric Ammonium Citrate contains 14.5–21.0% of iron (Fe = 55.85).
Description Ferric Ammonium Citrate occurs as green, red-brown, deep red, brown, or brownish yellow, transparent flaky crystals, powder, granules, or lumps. It is odorless or has a slight odor of ammonia and a weak iron taste.
Identification
(1) To 5 ml of a solution of Ferric Ammonium Citrate (1 in 10), add 5 ml of sodium hydroxide solution (1 in 25), and heat. An odor of ammonia is evolved, and a red-brown precipitate is formed.
(2) To a solution of Ferric Ammonium Citrate (1 in 100), add ammonia TS. A black color develops, and no precipitate is formed.
(3) To 10 ml of a solution of Ferric Ammonium Citrate (1 in 10), add 4 ml of potassium hydroxide solution (1 in 15), heat, and filter. Measure 4 ml of the filtrate, add diluted acetic acid (1 in 4) to make slightly acidic, and cool. Add 2 ml of calcium chloride solution (3 in 40), and boil. A white crystalline precipitate is formed.
Purity
(1) Sulfate Not more than 0.48% as SO_4.
Proceed as directed in Purity (2) for Ferric Citrate.
(2) Heavy metals Not more than 20 μg/g as Pb.
Proceed as directed in Purity (4) for Ferric Citrate.
(3) Arsenic Not more than 4.0 μg/g as As_2O_3.
Test Solution Weigh 1.0 g of Ferric Ammonium Citrate, add 5 ml of water, 1 ml of sulfuric acid, and 10 ml of sulfurous acid, evaporate to about 2 ml, and add water to make 10 ml. Perform the test, using 5 ml of this solution as the test solution.
Apparatus Use Apparatus B.
(4) Ferric citrate Weigh 0.10 g of Ferric Ammonium Citrate, dissolve it in 10 ml of water, and add 1 drop of freshly prepared potassium ferrocyanide solution (1 in 10). No blue precipitate is formed.
Assay Weigh accurately about 1 g of Ferric Ammonium Citrate into a flask with a ground-glass stopper, dissolve it in 25 ml of water, add 5 ml of hydrochloric acid and 4 g of potassium iodide, immediately stopper tightly, and allow to stand for 15 minutes in a dark place. Add 100 ml of water, and titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: starch TS). Perform a blank test in the same manner, and make any necessary correction.
Each ml of 0.1 mol/L potassium thiosulfate = 5.585 mg of Fe

Ferric Chloride

塩化第二鉄

$FeCl_3 \cdot 6H_2O$ Mol. Wt. 270.29
Iron(III) chloride hexahydrate [10025-77-1]
Content Ferric Chloride contains 98.5–102.0% of ferric chloride ($FeCl_3 \cdot 6H_2O$).
Description Ferric Chloride occurs as deliquescent, yellow-brown crystals or lumps.
Identification Ferric Chloride responds to all tests for Ferric Salt and for Chloride in the Qualitative Tests.
Purity
(1) Clarity of solution Very slightly turbid.
Weigh 1.0 g of Ferric Chloride, add 10 ml of diluted hydrochloric acid (1 in 100), and dissolve by heating.
(2) Free acid Weigh 2.0 g of Ferric Chloride, dissolve it in 5 ml of water, and bring a glass rod wetted with ammonia solution close to it. No white fumes are evolved.
(3) Nitrate Weigh 5.0 g of Ferric Chloride, dissolve it in 25 ml of water, boil, and add 25 ml of ammonia solution. After cooling, add water to make 100 ml, and filter. Use this solution as the sample solution. Measure 5.0 ml of the sample solution, and add 5 ml of water, 0.1 ml of indigo carmine TS, and 10 ml of sulfuric acid. A blue color persists for not less than 5 minutes.
(4) Sulfate Not more than 0.019% as SO_4.
Test Solution Measure 20 ml of the sample solution prepared in Purity (3) above, add 3 ml of anhydrous sodium carbonate solution (1 in 8), evaporate to dryness in a water bath, and heat over a small flame until the white fumes are no longer evolved. After cooling, add 10 ml of water and 3 ml of diluted hydrochloric acid (1 in 4), and evaporate to dryness in a water bath. Dissolve the residue in 0.3 ml of diluted hydrochloric acid (1 in 4), and then add water to make 50 ml.
Control Solution To 0.40 ml of 0.005 mol/L sulfuric acid, add 1 ml of diluted hydrochloric acid (1 in 4) and water to make 50 ml.
(5) Heavy metals Not more than 20 μg/g as Pb.
Test Solution Weigh 1.0 g of Ferric Chloride into a porcelain dish, dissolve it in 3 ml of aqua regia, and evaporate to dryness in a water bath. Dissolve the residue by adding 5 ml of diluted hydrochloric acid (1 in 2), and transfer the resulting solution to a separating funnel. Wash the porcelain dish

twice with 5 ml of diluted hydrochloric acid (1 in 2) each time, adding the washings to the separating funnel. Wash the aqueous layer twice with 40 ml of diethyl ether each time and again once with 20 ml of ether, discarding the washings each time. Dissolve 0.05 g of hydroxylamine hydrochloride in the aqueous layer, heat in a water bath for 10 minutes, add 1 drop of phenolphthalein TS, and add ammonia solution until a pink color develops. After cooling, add diluted hydrochloric acid (1 in 2) dropwise until the solution becomes almost completely colorless, and add 4 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, transfer into a porcelain dish, add 3 ml of aqua regia, and proceed as directed for the test solution .

(6) Zinc Not more than 30 μg/g as Zn.

Test Solution Measure 20 ml of the sample solution prepared in Purity (3) above, transfer into a Nessler tube, neutralize with hydrochloric acid, add water to make 30 ml, and add 3 ml of diluted hydrochloric acid (1 in 4) and 0.2 ml of freshly prepared potassium ferrocyanide solution (1 in 10).

Control Solution Measure 3.0 ml of Zinc Standard Solution, transfer into a Nessler tube, add water to make 30 ml, and then proceed as directed for the test solution.

Procedure Allow the test solution and the control solution to stand for 15 minutes. The test solution is not more turbid than the control solution.

(7) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.50 g of Ferric Chloride, dissolve it in 20 ml of water, and add 0.2 g of L-ascorbic acid to dissolve in the solution.

Standard Color Measure 2.0 ml of Arsenic Standard Solution, add 20 ml of water, and then dissolve 0.2 g of L-ascorbic acid in this solution.

Apparatus Use Apparatus B.

Procedure Perform the test without neutralizing with ammonia solution.

(8) Free chlorine Weigh 2.0 g of Ferric Chloride, dissolve it in 5 ml of water, heat, and bring a filter paper wetted with zinc iodide–starch TS close to it. No blue color develops.

Assay Weigh accurately about 0.6 g of Ferric Chloride into a flask with a ground-glass stopper, dissolve it in about 50 ml of water, add 3 ml of hydrochloric acid and 3 g of potassium iodide, and immediately stopper tightly. Allow to stand for 15 minutes in a dark place, and titrate with 0.1 mol/L sodium thiosulfate (indicator: starch TS). Perform a blank test in the same manner, and make any necessary correction.

Each ml of 0.1 mol/L sodium thiosulfate = 27.03 mg of $FeCl_3 \cdot 6H_2O$

Ferric Citrate

クエン酸鉄

Iron(III) salt of 2-hydroxypropane-1,2,3-tricarboxylic acid

Content Ferric Citrate contains 16.5–18.5% of iron (Fe = 55.85).

Description Ferric Citrate occurs as a brown powder or as transparent, red-brown laminae.

Identification Ferric Citrate responds to all tests for Ferric Salt and to test (2) for Citrate in the Qualitative Tests.

Purity

(1) Clarity of solution Almost clear.

Test Solution Weigh 1.0 g of Ferric Citrate, add 20 ml of water, and dissolve by heating in a water bath.

(2) Sulfate Not more than 0.48% as SO_4.

Proceed as directed under Purity (4) for Sodium Ferrous Citrate.

(3) Ammonium salt Weigh 1.0 g of Ferric Citrate, add 10 ml of water and 5 ml of potassium hydroxide solution (1 in 15), and boil. No odor of ammonia is evolved.

(4) Heavy metals Not more than 20 μg/g as Pb.

Proceed as directed under Purity (3) for Sodium Ferrous Citrate.

(5) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 1.0 g of Ferric Citrate, add 5 ml of water, 1 ml of sulfuric acid, and 10 ml of sulfurous acid, evaporate to about 2 ml, and add water to make 10 ml. Perform the test, using 5 ml of this solution.

Apparatus Use Apparatus B.

Assay Weigh accurately about 1 g of Ferric Citrate, transfer into a flask with a ground-glass stopper, add 5 ml of hydrochloric acid and 30 ml of water, dissolve by heating, and cool. Add 4 g of potassium iodide, immediately stopper tightly, and allow to stand for 15 minutes in a dark place. Add 100 ml of water, and titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: starch TS). Perform a blank test in the same manner, and make any necessary correction.

Each ml of 0.1 mol/L sodium thiosulfate = 5.585 mg of Fe

Ferric Pyrophosphate

ピロリン酸第二鉄

$Fe_4(P_2O_7)_3$ Mol. Wt. 745.21

Iron(III) diphosphate

Content Ferric Pyrophosphate, when ignited, contains not less than 95.0% of ferric pyrophosphate ($Fe_4(P_2O_7)_3$).

Description Ferric Pyrophosphate occurs as a yellow to yellow-brown powder. It is odorless and has a slight, iron taste.

Identification

(1) To 0.2 g of Ferric Pyrophosphate, add 10 ml of sodium hydroxide solution (1 in 25), and filter the red-brown precipitate formed. Dissolve the residue on the filter paper by adding diluted hydrochloric acid (1 in 4). The resulting solution responds to the test for Ferric Salt.

(2) Add diluted nitric acid (1 in 10) to the filtrate obtained in Identification (1) to make slightly acidic, and add silver nitrate solution (1 in 50). A white precipitate is formed.

Purity

(1) Clarity of solution Very slightly turbid.

Weigh 0.10 g of Ferric Pyrophosphate, dissolve it in 5.0 ml of diluted hydrochloric acid (1 in 2), and add water to make 20 ml.

(2) Chloride Not more than 3.55% as Cl.

Sample Solution Weigh 1.00 g of Ferric Pyrophosphate, add 5 ml of diluted nitric acid (1 in 2), and dissolve by heat-

ing in a water bath. Add several drops of phenolphthalein TS and 50 ml of sodium hydroxide solution (1 in 25), shake well, add water to make 100 ml, allow to stand for about 10 minutes, and filter through a dry filter paper. Measure 10 ml of the filtrate, and add water to make 100 ml. Measure 2.0 ml of this solution, and neutralize with diluted nitric acid (1 in 10).

Control Solution Use 0.20 ml of 0.01 mol/L hydrochloric acid.

(3) Sulfate Not more than 0.12% as SO_4.

Sample Solution Measure 40 ml of the filtrate obtained in Purity (2), and neutralize with diluted hydrochloric acid (1 in 4).

Control Solution Use 1.0 ml of 0.005 mol/L sulfuric acid.

(4) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 0.50 g of Ferric Pyrophosphate, transfer into a porcelain dish, dissolve by adding 3 ml of aqua regia, and evaporate to dryness gently in a water bath. Dissolve the residue by adding 5 ml of diluted hydrochloric acid (1 in 2), and transfer the solution to a separating funnel. Wash the porcelain dish three times with 5 ml of diluted hydrochloric acid (1 in 2) each time, adding the washings to the separating funnel. Wash the content five times by shaking with diethyl ether (two 40 ml portions followed by three 20 ml portions). After each washing, allow to stand, and discard the separated diethyl ether layer. Dissolve 0.2 g of hydroxylamine hydrochloride in the aqueous layer, and heat in a water bath for 10 minutes. Cool, add 1 drop of phenolphthalein TS, and add ammonia solution until a pink color develops. Add, dropwise, diluted hydrochloric acid (1 in 2) until the solution is almost colorless, then add 1 ml of diluted hydrochloric acid (1 in 2), 4 ml of diluted acetic acid (1 in 20), 4 ml of sodium acetate solution (2 in 15), and water to make 50 ml.

Control Solution Measure 1.0 ml of Lead Standard Solution, transfer into a porcelain dish, add 3 ml of aqua regia, and proceed in the same manner as for the test solution. In this case, after adding diluted hydrochloric acid (1 in 2) dropwise until the solution is almost colorless, the volume of the diluted hydrochloric acid to be added is 0.5 ml.

(5) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.50 g of Ferric Pyrophosphate, dissolve it in 5 ml of diluted hydrochloric acid (1 in 2), and then add 0.2 g of L-ascorbic acid to dissolve.

Standard Color Add 5 ml of diluted hydrochloric acid (1 in 2) to 1.0 ml of Arsenic Standard Solution, and dissolve 0.2 g of L-ascorbic acid in the solution obtained.

Apparatus Use Apparatus B.

Procedure Proceed as directed in the Arsenic Limit Test. In the procedure, omit the neutralization of the test solution with ammonia solution.

Loss on Ignition Not more than 20.0% (1 hour).

Assay Immediately weigh accurately about 0.3 g Ferric Pyrophosphate, previously ignited, dissolve it in 20 ml of diluted hydrochloric acid (1 in 2), and transfer the solution to a flask with a ground–glass stopper with 20 ml of water. Add 3 g of potassium iodide, immediately stopper tightly, and allow to stand in a dark place for 15 minutes. Add 100 ml of water, and titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: starch TS). Perform a blank test in the same manner.

Each ml of 0.1 mol/L sodium thiosulfate = 18.63 mg of $Fe_4(P_2O_7)_3$

Ferric Pyrophosphate Solution

ピロリン酸第二鉄液

Content Ferric Pyrophosphate Solution contains 2.5–3.5% of ferric pyrophosphate ($Fe_4(P_2O_7)_3$ = 745.22).

Description Ferric Pyrophosphate Solution is a white to light yellow, milky liquid. It is odorless and has a slight, iron taste.

Identification

(1) To Ferric Pyrophosphate Solution, add sodium hydroxide solution (1 in 25) in excess, and filter the red-brown precipitate formed. Dissolve the residue on the filter paper with diluted hydrochloric acid (1 in 3). The solution responds to all tests for Ferric Salt in the Qualitative Tests.

(2) Add diluted nitric acid (1 in 10) to the filtrate obtained in Identification (1) to make slightly acidic, and add silver nitrate solution (1 in 50). A white precipitate is formed.

Purity

(1) Clarity of solution Very slightly turbid.

Weigh 2.0 g of Ferric Pyrophosphate Solution, dissolve it in 5.0 ml of diluted hydrochloric acid (1 in 2), and add water to make 20 ml.

(2) Chloride Not more than 0.35% as Cl.

Sample Solution Weigh 10 g of Ferric Pyrophosphate Solution, add several drops of phenolphthalein TS and 7 ml of sodium hydroxide solution (1 in 25), shake well, add water to make 100 ml, allow to stand for about 10 minutes, and filter through a dry filter paper. Measure 10 ml of the filtrate, add water to make 100 ml, then measure 2.0 ml of the solution obtained, and neutralize with diluted nitric acid (1 in 10).

Control Solution Use 0.20 ml of 0.01 mol/L hydrochloric acid.

(3) Sulfate Not more than 0.002% as SO_4.

Test Solution Measure 40 ml of the filtrate obtained in Purity (2), and neutralize with diluted hydrochloric acid (1 in 4).

Control Solution 0.20 ml of 0.005 mol/L sulfuric acid.

(4) Heavy metals Not more than 4.0 μg/g as Pb.

Test Solution Weigh 5.0 g of Ferric Pyrophosphate Solution into a porcelain dish, dissolve it in 5 ml of aqua regia, and evaporate to dryness in a water bath. Dissolve the residue by adding 5 ml of diluted hydrochloric acid (1 in 2), and transfer into a separating funnel. Wash the porcelain dish twice with 5 ml of dilute hydrochloric acid (1 in 2) each time, and add the washings to the separating funnel. Wash the content three times by shaking with diethyl ether (two 40 ml portions followed by one 20 ml portion). After each washing, allow to stand, and discard the diethyl ether layer. Dissolve 0.05 g of hydroxylamine hydrochloride in the aqueous layer, heat 10 minutes in a water bath, add 1 drop of phenolphthalein TS, then add ammonia solution until a pink color develops. After cooling, add diluted hydrochloric acid (1 in 2) dropwise until the solution is almost colorless, and then add 4 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Place 2.0 ml of Lead Standard Solution into a porcelain dish, add 5 ml of aqua regia, and proceed as directed for the test solution.

(5) Arsenic Not more than 0.2 μg/g as As_2O_3.

Test Solution Dissolve 0.2 g of L-ascorbic acid in 10 g of Ferric Pyrophosphate Solution.

Apparatus Use Apparatus B.

Standard Color Add 4 ml of water to 2.0 ml of Arsenic Standard Solution, dissolve 0.1 g of L-ascorbic acid in the solution obtained, and proceed in the same manner as for the test solution.

Procedure Proceed as directed as the Arsenic Limit Test. In the procedure, omit the neutralization of the test solution with ammonia solution.

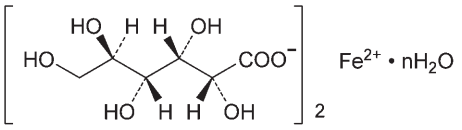
Assay Weigh accurately about 10 g of Ferric Pyrophosphate Solution, transfer into a flask with a ground-glass stopper with about 30 ml of water, and dissolve by adding 10 ml of hydrochloric acid. Add 3 g of potassium iodide, immediately stopper tightly, and allow to stand in a dark place for 15 minutes. Add 100 ml of water, and titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: starch TS). Perform a blank test in the same manner.

Each ml of 0.1 mol/L sodium thiosulfate = 18.63 mg of $Fe_4(P_2O_7)_3$

Ferrous Gluconate

Iron Gluconate

グルコン酸第一鉄



n = 2 or 0

$C_{12}H_{22}FeO_{14}\cdot nH_2O$ (n=2 or 0) Mol. Wt. dihydrate 482.17
anhydrous 446.14

Monoiron(II) bis(D-gluconate) dihydrate

Monoiron(II) bis(D-gluconate) [299-29-6]

Content Ferrous Gluconate, when dried, contains not less than 95.0% of ferrous gluconate ($C_{12}H_{22}FeO_{14}$).

Description Ferrous Gluconate occurs as a yellow-gray to green-yellow powder or granules having a slight, characteristic odor.

Identification

(1) Measure 5 ml of a solution of Ferrous Gluconate in warm water (1 in 10), and proceed as directed in Identification (2) for Glucono-δ-Lactone.

(2) A solution of Ferrous Gluconate (1 in 20) responds to all tests for Ferrous Salt in the Qualitative Tests.

Purity

(1) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of Ferrous Gluconate, transfer into a crucible, moisten with 2 ml of sulfuric acid, heat gradually until it is almost incinerated, and cool. Add 1 ml of sulfuric acid, and heat gradually until the fumes of sulfuric acid are almost no longer evolved. Ignite at 450–550°C until the residue incinerates. After cooling, dissolve the residue by adding 5 ml of diluted hydrochloric acid (1 in 2), and transfer the solution to a separating funnel. Wash the crucible twice with 5 ml of diluted hydrochloric acid (1 in 2) each time, and add the washings to the separating funnel. Wash the content three times by shaking with diethyl ether (two 40-ml portions followed by one 20-ml portion). After each washing, allow to stand, and discard the separated diethyl ether layer. Dissolve 0.05 g of hydroxylamine hydrochloride in the aqueous layer, heat on a water bath for 10 minutes, add 1 drop of phenolphthalein TS, and add ammonia solution until a pink color develops. After cooling, add diluted hydrochloric acid (1 in 2) dropwise until the solution becomes almost colorless, and add 4 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Using 2.0 ml of Lead Standard Solution in place of the sample, proceed as directed for the test solution.

(2) Ferric salt Not more than 2.0% as Fe^{3+}.

Weigh 5.0 g of Ferrous Gluconate, add 100 ml of water and 10 ml of hydrochloric acid to dissolve, then add 3 g of potassium iodide, and shake. Allow to stand in a dark place for 5 minutes. Titrate with 0.1 mol/L sodium thiosulfate (indicator: starch TS). The volume of the sodium thiosulfate consumed is not more than 18 ml.

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(4) Oxalate Weigh 1.0 g of Ferrous Gluconate, add 10 ml of water and 2 ml of hydrochloric acid to dissolve, and transfer into a separating funnel. Perform extraction twice with 50 ml and 20 ml of diethyl ether. Combine the extracts, add 10 ml of water, evaporate the diethyl ether on a water bath, and add 1 drop of acetic acid and 1 ml of calcium acetate solution (1 in 20). No turbidity appears within 5 minutes.

(5) Sucrose or reducing sugars Weigh 0.5 g of Ferrous Gluconate, add 10 ml of water, dissolve by warming, and add 1 ml of ammonia TS. Pass hydrogen sulfide through the solution, allow to stand for 30 minutes, and filter. Wash the residue on the filter paper twice with 5 ml of water each time, combine the filtrate and the washings, neutralize with hydrochloric acid, and add 2 ml of diluted hydrochloric acid (1 in 4). Concentrate the solution to about 10 ml, cool, add 5 ml of anhydrous sodium carbonate solution (1 in 8) and 20 ml of water, and filter. Add water to the filtrate to make 100 ml. To 5 ml of this solution, add 2 ml of Fehling's TS, and boil for 1 minute. A yellow to red precipitate is not formed immediately.

Loss on Drying Not more than 10.0% (105°C, 4 hours).

Assay Weigh accurately about 1.5 g of Ferrous Gluconate, previously dried, add 75 ml of water and 15 ml of diluted sulfuric acid (1 in 20) to dissolve, and then add 0.25 g of zinc dust. Allow to stand for 20 minutes, filter, with suction, through a crucible-shaped glass filter (1G4) on which zinc dust is placed in thin layer, and wash the residue with 10 ml of diluted sulfuric acid (1 in 20) and 10 ml of water. Combine the filtrate and the washings, add 2 drops of *o*-phenanthroline TS, filter with suction if necessary, and titrate immediately with 0.1 mol/L ceric sulfate. Perform a blank test in the same manner, and make any necessary correction.

Each ml of 0.1 mol/L ceric sulfate = 44.61 mg of $C_{12}H_{22}FeO_{14}$

Ferrous Sulfate

硫酸第一鉄

$FeSO_4$

Iron(II) sulfate hydrate [13463-43-9]

Definition Ferrous Sulfate occurs in crystalline form (heptahydrate), called Ferrous Sulfate (crystal), and in dried form (monohydrate to sesquihydrate), called Ferrous Sulfate (dried).

Content Ferrous Sulfate (crystal) contains 98.0–104.0% of ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$ = 278.02), and Ferrous Sulfate (dried) contains not less than 85.0% of ferrous sulfate ($FeSO_4$ = 151.91).

Description Ferrous Sulfate (crystal) occurs as whitish green crystals or crystalline powder. Ferrous Sulfate (dried) occurs as a gray-white powder.

Identification A solution of Ferrous Sulfate (1 in 100) responds to all tests for Ferrous Salt and for Sulfate in the Qualitative Tests.

Purity

(1) pH Acidic, not less than 3.4 (crystal 1.0 g, water 10 ml).

(2) Heavy metals

Crystal: Not more than 40 μg/g as Pb.

Dried: Not more than 60 μg/g as Pb.

Test Solution Weigh 0.50 g of Ferrous Sulfate (crystal) or 0.33 g of Ferrous Sulfate (dried) into a porcelain dish, dissolve it in 3 ml of aqua regia, and evaporate to dryness in a water bath. Dissolve the residue in 5 ml of diluted hydrochloric acid (1 in 2), and transfer the solution to a separating funnel. Wash the porcelain dish twice with 5 ml of diluted hydrochloric acid (1 in 2) each time, and add the washings to the separating funnel. Wash the content in the funnel three times by shaking with diethyl ether (two 40-ml portions followed by one 20-ml portion). After each washing, allow to stand, and discard the separated diethyl ether layer. Dissolve 0.05 g of hydroxylamine hydrochloride in the aqueous layer, heat on a water bath for 10 minutes, add 1 drop of phenolphthalein TS, and add ammonia solution until a pink color develops. After cooling, add diluted hydrochloric acid (1 in 2) dropwise until the solution is almost colorless, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Place 2.0 ml of Lead Standard Solution into a porcelain dish, add 3 ml of aqua regia, and then proceed as directed for the test solution.

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

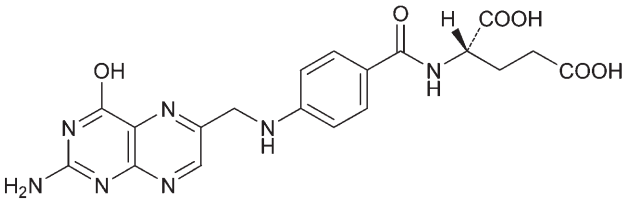
Assay Weigh accurately about 0.5 g of Ferrous Sulfate, dissolve it in a mixture of 25 ml of diluted sulfuric acid (1 in 25) and 25 ml of freshly boiled and cooled water, and titrate with 0.02 mol/L potassium permanganate.

Ferrous Sulfate (crystal): Each ml of 0.02 mol/L potassium permanganate = 27.80 mg of $FeSO_4 \cdot 7H_2O$

Ferrous Sulfate (dried): Each ml of 0.02 mol/L potassium permanganate = 15.19 mg of $FeSO_4$

Folic Acid

葉酸



$C_{19}H_{19}N_7O_6$ Mol. Wt. 441.40

N-{4-[(2-Amino-4-hydroxypteridin-6-ylmethyl)amino]benzoyl}-L-glutamic acid [59-30-3]

Content Folic Acid contains 98.0%–102.0% of folic acid ($C_{19}H_{19}N_7O_6$).

Description Folic Acid occurs as a yellow to orange-yellow crystalline powder. It is odorless.

Identification Dissolve 1.5 mg of Folic Acid in sodium hydroxide solution (1 in 250) to make 100 ml. The solution exhibits absorption maxima at wavelengths of 255–257 nm, 281–285 nm, and 361–369 nm.

Purity Free amine Not more than 1.0%.

Weigh accurately about 0.05 g of *p*-Aminobenzoylglutamic Acid Reference Standard, previously dried for 4 hours under reduced pressure in a desiccator, and dissolve it in 40% (vol) ethanol to make exactly 100 ml. Measure exactly 3 ml of this solution, and add water to make exactly 1,000 ml. Using exactly 4 ml of the resulting solution, prepare a solution as directed for the preparation of solution S_3 in the Assay (preparation of solution S_3 from solution S_2), and measure the absorbance (A_S'). Calculate the amount of free amine by the formula below from A_S' and A_C obtained in the Assay.

$$\text{Amount (\%) of free amine} = \frac{\left(\dfrac{\text{Weight (g) of } p\text{-Aminobenzoylglutamic Acid}}{\text{Reference Standard}}\right)}{\text{Anhydrous basis weight (g) of the sample used in Assay}} \times \frac{A_C}{A_S'}$$

Water Content Not more than 8.5% (0.2 g, Back Titration).

Use 5 ml of pyridine for water determination, besides 20 ml of methanol for water determination. Add a constant amount of excess water determination TS, and stir for 30 minutes before back titration.

Residue on Ignition Not more than 0.50%.

Assay Weigh accurately about 0.05 g each of Folic Acid and Folic Acid Reference Standard (the water content should be measured previously in the same manner as for Folic Acid), add 50 ml of sodium hydroxide solution (1 in 250) to each, and dissolve by shaking well. Add sodium hydroxide solution (1 in 250) to make exactly 100 ml of each. Refer to these solutions as solution T_1 and solution S_1, respectively.

Measure exactly 30 ml each of solution T_1 and solution S_1, add 20 ml of diluted hydrochloric acid (1 in 4), then add water to make exactly 100 ml of each. Measure exactly 60 ml of each, add 0.5 g of zinc dust to each, and allow to stand

for 20 minutes with occasional shaking. Filter each solution through a dry filter paper, discard the initial 10 ml of filtrate, measure exactly the subsequent 10 ml of filtrate, and add water to make exactly 100 ml of each. Refer to these solutions as solution T_2 and solution S_2, respectively.

Measure exactly 4 ml each of solution T_2 and solution S_2, add 1 ml of water, 1 ml of diluted hydrochloric acid (1 in 4), and 1 ml of sodium nitrite solution (1 in 1,000) to each, mix, and allow to stand for 2 minutes. Add 1 ml of ammonium sulfamate solution (1 in 200) to each, shake well, and allow to stand for another 2 minutes. Then add 1 ml of *N*-1-naphthyl-*N'*-diethylethylenediamine oxalate solution (1 in 1,000), shake, allow to stand for 10 minutes, and add water to make exactly 20 ml of each. Refer to these solutions as solution T_3 and solution S_3, respectively.

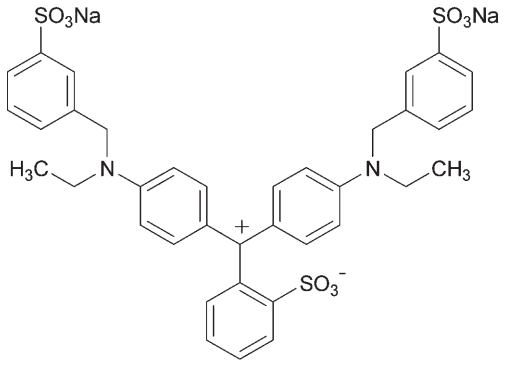
Measure exactly 30 ml of solution T_1, and add 20 ml of diluted hydrochloric acid (1 in 4) and water to make exactly 100 ml. With exactly 4 ml of this solution, prepare solution C, according to the direction in which solution T_3 was prepared using solution T_2. Measure the absorbances (A_T, A_S, and A_C) of solutions T_3, S_3, and C at a wavelength of 550 nm against a reference prepared with 4 ml of water, according to the direction in which solution T_3 was prepared using solution T_2. Calculate the content by the formula:

$$\text{Content (\%) of folic acid } (C_{19}H_{19}N_7O_6) = \frac{\left(\begin{array}{c}\text{Dry basis weight (g) of}\\ \text{Folic Acid Reference Standard}\end{array}\right)}{\text{Anhydrous basis weight (g) of the sample}} \times \frac{A_T - 0.1 \times A_C}{A_S} \times 100$$

Food Blue No. 1

Brilliant Blue FCF
FD&C Blue No. 1

食用青色1号



$C_{37}H_{34}N_2Na_2O_9S_3$ Mol. Wt. 792.85
Disodium 2-(bis{4-[*N*-ethyl-*N*-(3-sulfonatophenylmethyl)amino]phenyl}methyliumyl)benzenesulfonate
[3844-45-9]

Definition Food Blue No. 1 consists mainly of disodium 2-(bis{4-[*N*-ethyl-*N*-(3-sulfonatophenylmethyl)amino]phenyl}-methyliumyl)benzenesulfonate.

Content Food Blue No. 1 contains the equivalent of not less than 85.0% of disodium 2-(bis{4-[*N*-ethyl-*N*-(3-sulfonatophenylmethyl)amino]phenyl}methyliumyl)-benzenesulfonate ($C_{37}H_{34}N_2Na_2O_9S_3$).

Description Food Blue No. 1 occurs as a reddish purple powder or granules. It is odorless and has a metallic luster.

Identification

(1) A solution of Food Blue No. 1 (1 in 2,000) is blue.

(2) To 5 ml of a solution of Food Blue No. 1 (1 in 1,000), add 1 ml of hydrochloric acid. The color of the solution changes to dark yellow-green.

(3) A solution of Food Blue No. 1 in sulfuric acid (1 in 100) is dark orange. Add 2 to 3 drops of this solution to 5 ml of water. The color changes to green.

(4) To 5 ml of a solution of Food Blue No. 1 (1 in 1,000), add 5 ml of sodium hydroxide solution (1 in 5), and heat in a water bath. The color of solution changes to purple-red.

(5) Dissolve 0.1 g of Food Blue No. 1 in 200 ml of ammonium acetate solution (3 in 2,000). To 1 ml of this solution, add ammonium acetate solution (3 in 2,000) to make 100 ml. The solution exhibits absorption maximum at a wavelength of 628–632 nm.

Purity

(1) Water-insoluble substances Not more than 0.20% (Coloring Matter Tests).

(2) Chloride and sulfate Not more than 4.0% as total amount (Coloring Matter Tests).

(3) Heavy metals

Not more than 50 μg/g as Cr (Coloring Matter Tests, Heavy Metals (2)).

Not more than 50 μg/g as Mn (Coloring Matter Tests, Heavy Metals (4)).

Not more than 20 μg/g as Pb (Coloring Matter Tests, Heavy Metals (5)).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (Coloring Matter Tests).

(5) Other coloring matters (Coloring Matter Tests, Other Coloring Matters (4)).

Loss on Drying Not more than 10.0% (135°C, 6 hours).

Assay Weigh accurately about 4.8 g of Food Blue No. 1, and dissolve it in water to make exactly 250 ml. Use exactly 50 ml of this solution as the test solution. Proceed as directed in Titanium Trichloride Method (ii) in the Assay in the Coloring Matter Tests.

Each ml of 0.1 mol/L titanium trichloride = 39.64 mg of $C_{37}H_{34}N_2Na_2O_9S_3$

Food Blue No. 1 Aluminum Lake

Brilliant Blue FCF Aluminum Lake

食用青色1号アルミニウムレーキ

Definition Food Blue No. 1 Aluminum Lake is prepared by adsorbing Food Blue No. 1 to a solution of aluminum salt that was reacted with alkali. Following lake formation, the product is filtered, dried, and crushed.

Content Food Blue No. 1 Aluminum Lake contains the

equivalent of not less than 10.0% of disodium 2-(bis {4-[*N*-ethyl-*N*-(3-sulfonatophenylmethyl)amino]phenyl}-methyliumyl)benzenesulfonate ($C_{37}H_{34}N_2Na_2O_9S_3$ = 792.85).

Description Food Blue No. 1 Aluminum Lake occurs as a fine blue powder. It is odorless.

Identification

(1) To 0.1 g of Food Blue No. 1 Aluminum Lake, add 5 ml of diluted hydrochloric acid (1 in 4), and heat in a water bath for about 5 minutes with occasional shaking. It dissolves almost clearly, and the solution is green to dark green. Cool, and neutralize with ammonia TS. A blue color develops, and a gelatinous precipitate of the same color is formed.

(2) To 0.1 g of Food Blue No. 1 Aluminum Lake, add 5 ml of sulfuric acid, and heat in a water bath for about 5 minutes with occasional shaking. A dark yellow to dark gray-brown color develops. Cool, and add 2 to 3 drops of the supernatant to 5 ml of water. A blue to blue-green color develops.

(3) To 0.1 g of Food Blue No. 1 Aluminum Lake, add 5 ml of sodium hydroxide solution (1 in 10), and heat in a water bath for about 5 minutes with occasional shaking. It dissolves almost clearly, and the solution is purple-red to red-purple. After cooling, neutralize with diluted hydrochloric acid (1 in 4). A blue to red-purple color develops, and a gelatinous precipitate of the same color is formed.

(4) To 0.1 g of Food Blue No. 1 Aluminum Lake, add 5 ml of diluted sulfuric acid (1 in 20), stir well, and add ammonium acetate solution (3 in 2,000) to make 200 ml. If the solution is not clear, centrifuge. Measure 1 to 10 ml of this solution so that the absorbance is between 0.2 and 0.7, and add ammonium acetate solution (3 in 2,000) to make 100 ml. The solution exhibits absorption maximum at a wavelength of 628–632 nm.

(5) To 0.1 g of Food Blue No. 1 Aluminum Lake, add 10 ml of diluted hydrochloric acid (1 in 4), heat in a water bath to dissolve most of it, add 0.5 g of active carbon, shake well, and filter. Neutralize the colorless filtrate with sodium hydroxide solution (1 in 10). The solution responds to all tests for Aluminum Salt in the Qualitative Tests.

Purity

(1) Hydrochloric acid- and ammonia-insoluble substances
Not more than 0.5% (Coloring Matter Aluminum Lake Tests).

(2) Heavy metals Not more than 20 μg/g as Pb (Coloring Matter Aluminum Lake Tests, Heavy Metals (3)).

(3) Barium Not more than 500 μg/g as Ba (Coloring Matter Aluminum Lake Tests).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (Coloring Matter Aluminum Lake Test).

(5) Other coloring matter lakes (Coloring Matter Aluminum Lake Tests, Other Coloring Matter Lakes (3)).

Loss on Drying Not more than 30.0% (135°C, 6 hours).

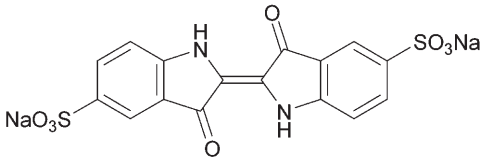
Assay Weigh accurately an amount of Food Blue No. 1 Aluminum Lake so that the volume of 0.1 mol/L titanium trichloride consumed is about 20 ml, and proceed as directed in Assay (2) in the Coloring Matter Aluminum Lake Tests.

Each ml of 0.1 mol/L titanium trichloride = 39.64 mg of $C_{37}H_{34}N_2Na_2O_9S_3$

Food Blue No. 2

Indigo Carmine
Indigotine
FD&C Blue No.2

食用青色２号



$C_{16}H_8N_2Na_2O_8S_2$ Mol. Wt. 466.35

Disodium 2,2′-bi(3-oxo-1*H*-indolin-2-ylidene)-5,5′-disulfonate [860-22-0]

Definition Food Blue No. 2 consists principally of disodium 2,2′-bi(3-oxo-1*H*-indolin-2-ylidene)-5,5′-disulfonate.

Content Food Blue No. 2 contains the equivalent of not less than 85.0% of disodium 2,2′-bi(3-oxo-1*H*-indolin-2-ylidene)-5,5′-disulfonate ($C_{16}H_8N_2Na_2O_8S_2$).

Description Food Blue No. 2 occurs as a dark purple-blue to dark purple-brown powder or granules. It is odorless.

Identification

(1) A solution of Food Blue No. 2 (1 in 2,000) is purple-blue.

(2) A solution of Food Blue No. 2 in sulfuric acid (1 in 100) is deep purple. Add 2 to 3 drops of this solution to 5 ml of water. A purple-blue color develops.

(3) To 5 ml of a solution of Food Blue No. 2 (1 in 1,000), add 1 ml of sodium hydroxide solution (1 in 10). The color of the solution changes to yellow-green.

(4) Dissolve 0.1 g of Food Blue No. 2 in 100 ml of ammonium acetate solution (3 in 2,000). To 1 ml of this solution, add ammonium acetate solution (3 in 2,000) to make 100 ml. The solution exhibits absorption maximum at a wavelength of 610–614 nm.

Purity

(1) Water-insoluble substances Not more than 0.20% (Coloring Matter Tests).

(2) Chloride and sulfate Not more than 7.0% as total amount (Coloring Matter Tests).

(3) Heavy metals
Not more than 500 μg/g as Fe (Coloring Matter Tests, Heavy Metals (3)).
Not more than 20 μg/g as Pb (Coloring Matter Tests, Heavy Metals (5)).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (Coloring Matter Tests).

(5) Other coloring matters (Coloring Matter Tests, Other Coloring Matters(1)).

Loss on Drying Not more than 10.0% (135°C, 6 hours).

Assay Weigh accurately about 2.7 g of Food Blue No. 2, and dissolve it in water to make exactly 500 ml. Use exactly 100 ml of this solution as the test solution. Proceed as directed in Titanium Trichloride Method (ii) in the Assay in the Coloring Matter Tests.

Each ml of 0.1 mol/L titanium trichloride = 23.32 mg of $C_{16}H_8N_2Na_2O_8S_2$

Food Blue No. 2 Aluminum Lake

Indigo Carmine Aluminum Lake

食用青色2号アルミニウムレーキ

Definition Food Blue No. 2 Aluminum Lake is prepared by adsorbing Food Blue No. 2 to a solution of aluminum salt that was reacted with alkali. Following lake formation, the product is filtered, dried, and crushed.

Content Food Blue No. 2 Aluminum Lake contains the equivalent of not less than 10.0% of disodium 2,2′-bi(3-oxo-1*H*-indolin-2-ylidene)-5,5′-disulfonate ($C_{16}H_8N_2Na_2O_8S_2$ = 466.35).

Description Food Blue No. 2 Aluminum Lake occurs as a fine purplish-blue powder. It is odorless.

Identification

(1) To 0.1 g of Food Blue No. 2 Aluminum Lake, add 5 ml of sulfuric acid, and heat in a water bath for about 5 minutes with occasional shaking. A deep purple-blue color develops. Cool, and add 2 to 3 drops of the supernatant to 5 ml of water. A purple-blue color develops.

(2) To 0.1 g of Food Blue No. 2 Aluminum Lake, add 5 ml of sodium hydroxide solution (1 in 10), and heat in a water bath for about 5 minutes with occasional shaking. It dissolves almost clearly, and the solution is yellow-brown. Cool, and neutralize with diluted hydrochloric acid (1 in 4). A purple-blue to light green color develops, and a gelatinous precipitate of the same color is formed.

(3) To 0.1 g of Food Blue No. 2 Aluminum Lake, add 5 ml of diluted sulfuric acid (1 in 20), stir well, and add ammonium acetate solution (3 in 2,000) to make 100 ml. If the solution is not clear, centrifuge. Measure 1 to 10 ml of this solution so that the absorbance is between 0.2 to 0.7, and add ammonium acetate solution (3 in 2,000) to make 100 ml. The solution exhibits absorption maximum at a wavelength of 610–614 nm.

(4) To 0.1 g of Food Blue No. 2 Aluminum Lake, add 10 ml of diluted hydrochloric acid (1 in 4), heat in a water bath to dissolve most of it, add 0.5 g of active carbon, shake well, and filter. Neutralize the colorless filtrate with sodium hydroxide solution (1 in 10). The solution responds to all tests for Aluminum Salt in the Qualitative Tests.

Purity

(1) Hydrochloric acid- and ammonia-insoluble substances
Not more than 0.5% (Coloring Matter Aluminum Lake Tests).

(2) Heavy metals
Not more than 250 μg/g as Fe (Coloring Matter Aluminum Lake Tests, Heavy Metals (2)).
Not more than 20 μg/g as Pb (Coloring Matter Aluminum Lake Tests, Heavy Metals (3)).
In the test for Iron, use 4.0 ml each of the sample solution and the blank solution.

(3) Barium Not more than 500 μg/g as Ba (Coloring Matter Aluminum Lake Tests).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (Coloring Matter Aluminum Lake Tests).

(5) Other coloring matter lakes (Coloring Matter Aluminum Lake Tests, Other Coloring Matter Lakes (4)).

Loss on Drying Not more than 30.0% (135°C, 6 hours).

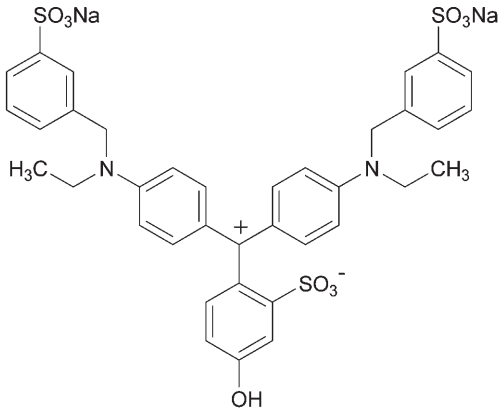
Assay Weigh accurately an amount of Food Blue No. 2 Aluminum Lake so that the volume of 0.1 mol/L titanium trichloride consumed is about 20 ml, and proceed as directed in Assay (2) in the Coloring Matter Aluminum Lake Tests.

Each ml of 0.1 mol/L titanium trichloride solution = 23.32 mg of $C_{16}H_8N_2Na_2O_8S_2$

Food Green No. 3

Fast Green FCF
FD&C Green No.3

食用緑色3号



$C_{37}H_{34}N_2Na_2O_{10}S_3$ Mol. Wt. 808.85

Disodium 2-(bis{4-[*N*-ethyl-*N*-(3-sulfonatophenylmethyl)amino]phenyl}methyliumyl)-5-hydroxybenzenesulfonate [2353-45-9]

Definition Food Green No. 3 consists principally of disodium 2-(bis{4-[*N*-ethyl-*N*-(3-sulfonatophenylmethyl)-amino]phenyl}methyliumyl)-5-hydroxybenzenesulfonate.

Content Food Green No. 3 contains the equivalent of not less than 85.0% of disodium 2-(bis{4-[*N*-ethyl-*N*-(3-sulfonatophenylmethyl)amino]phenyl}methyliumyl)-5-hydroxybenzenesulfonate ($C_{37}H_{34}N_2Na_2O_{10}S_3$).

Description Food Green No. 3 occurs as a dark green powder or granules. It is odorless and has a metallic luster.

Identification

(1) A solution of Food Green No. 3 (1 in 2,000) is blue-green.

(2) To 5 ml of a solution of Food Green No. 3 (1 in 1,000), add 1 ml of hydrochloric acid. The color of the solution changes to brown.

(3) To 5 ml of a solution of Food Green No. 3 (1 in 1,000), add 1 ml of sodium hydroxide solution (1 in 10). The color of the solution changes to blue-purple.

(4) A solution of Food Green No. 3 in sulfuric acid (1 in 100) is orange. When 2 to 3 drops of this solution is added to 5 ml of water. A green color develops.

(5) Dissolve 0.1 g of Food Green No. 3 in 200 ml of ammonium acetate solution (3 in 2,000). To 1 ml of this solution, add ammonium acetate solution (3 in 2,000) to make 100 ml. The solution exhibits absorption maximum at a wavelength of 622–626 nm.

Purity

(1) Water-insoluble substances Not more than 0.20% (Coloring Matter Tests).

(2) Chloride and sulfate Not more than 5.0% as total amount (Coloring Matter Tests).

(3) Heavy metals

Not more than 50 μg/g as Cr (Coloring Matter Tests, Heavy Metals (2)).

Not more than 50 μg/g as Mn (Coloring Matter Tests, Heavy Metals (4)).

Not more than 20 μg/g as Pb (Coloring Matter Tests, Heavy Metals (5)).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (Coloring Matter Tests).

(5) Other coloring matters (Coloring Matter Tests, Other Coloring Matters (4)).

Loss on Drying Not more than 10.0% (135°C, 6 hours).

Assay Weigh accurately about 4.7 g of Food Green No. 3, and dissolve it in water to make exactly 250 ml. Use exactly 50 ml of this solution as the test solution, and proceed as directed in Titanium Trichloride Method (ii) in the Assay in the Coloring Matter Tests.

Each ml of 0.1 mol/L titanium trichloride = 40.44 mg of $C_{37}H_{34}N_2Na_2O_{10}S_3$

Food Green No. 3 Aluminum Lake

Fast Green FCF Aluminum Lake

食用緑色3号アルミニウムレーキ

Definition Food Green No. 3 Aluminum Lake is prepared by adsorbing Food Green No. 3 to a solution of aluminum salt that was reacted with alkali. Following lake formation, the product is filtered, dried, and crushed.

Content Food Green No. 3 Aluminum Lake contains the equivalent of not less than 10.0% of disodium 2-(bis{4-[*N*-ethyl-*N*-(3-sulfonatophenylmethyl)amino]phenyl}-methyliumyl)-5-hydroxybenzenesulfonate ($C_{37}H_{34}N_2Na_2O_{10}S_3$ = 808.85).

Description Food Green No. 3 Aluminum Lake occurs as a fine, dark green-blue powder. It is odorless.

Identification

(1) To 0.1 g of Food Green No. 3 Aluminum Lake, add 5 ml of diluted hydrochloric acid (1 in 4), and heat in a water bath for about 5 minutes with occasional shaking. It dissolves almost clearly, and the solution is dark green. Cool, and neutralize with ammonia TS. A blue-green color develops, and a gelatinous precipitate of the same color is formed.

(2) To 0.1 g of Food Green No. 3 Aluminum Lake, add 5 ml of sulfuric acid, and heat in a water bath for about 5 minutes with occasional shaking. A dark orange color develops. Cool, and add 2 to 3 drops of the supernatant to 5 ml of water. A green color develops.

(3) To 0.1 g of Food Green No. 3 Aluminum Lake, add 5 ml of sodium hydroxide solution (1 in 10), and heat in a water bath for about 5 minutes with occasional shaking. It dissolves almost clearly, and the solution is purple-red. After cooling, neutralize with diluted hydrochloric acid (1 in 4). A blue-green color develops, and a gelatinous precipitate of the same color is formed.

(4) To 0.1 g of Food Green No. 3 Aluminum Lake, add 5 ml of diluted sulfuric acid (1 in 20), stir well, and add ammonium acetate solution (3 in 2,000) to make 200 ml. If the solution is not clear, centrifuge. Measure 1 to 10 ml of the solution so that the absorbance is between 0.2 and 0.7, and add ammonium acetate solution (3 in 2,000) to make 100 ml. The solution exhibits absorption maximum at a wavelength of 622–626 nm.

(5) To 0.1 g of Food Green No. 3 Aluminum Lake, add 10 ml of diluted hydrochloric acid (1 in 4), and heat in a water bath to dissolve most of the sample. Add 0.5 g of active carbon, shake well, and filter. Neutralize the colorless filtrate with sodium hydroxide solution (1 in 10). The solution responds to all tests for Aluminum Salt in the Qualitative Tests.

Purity

(1) Hydrochloric acid- and ammonia-insoluble substances Not more than 0.5% (Coloring Matter Aluminum Lake Tests).

(2) Heavy metals Not more than 20 μg/g as Pb (Coloring Matter Aluminum Lake Tests, Heavy Metals (3)).

(3) Barium Not more than 500 μg/g as Ba (Coloring Matter Aluminum Lake Tests).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (Coloring Matter Aluminum Lake Tests).

(5) Other coloring matter lakes (Coloring Matter Aluminum Lake Tests, Other Coloring Matter Lakes (3)).

Loss on Drying Not more than 30.0% (135°C, 6 hours).

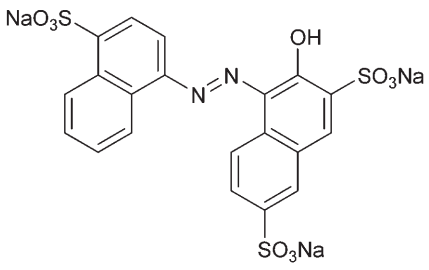
Assay Weigh accurately Food Green No. 3 Aluminum Lake so that the volume of 0.1 mol/L titanium trichloride consumed is about 20 ml, and proceed as directed in Assay (2) in the Coloring Matter Aluminum Lake Tests.

Each ml of 0.1 mol/L titanium trichloride solution = 40.44 g of $C_{37}H_{34}N_2Na_2O_{10}S_3$

Food Red No. 2

Amaranth

食用赤色2号



$C_{20}H_{11}N_2Na_3O_{10}S_3$ Mol. Wt. 604.48

Trisodium 3-hydroxy-4-[(4-sulfonatonaphthalen-1-yl)diazenyl]naphthalene-2,7-disulfonate [915-67-3]

Definition Food Red No. 2 is obtained by diazotizing 4-amino-1-naphthalenesulfonic acid, coupling the obtained

diazo compound with 3-hydroxy-2,7-naphthalenedisulfonic acid, and then salting out and refining the resulting dye. It consists principally of trisodium 3-hydroxy-4-[(4-sulfonatonaphthalen-1-yl)diazenyl]naphthalene-2,7-disulfonate.

Content Food Red No. 2 contains the equivalent of not less than 85.0% of trisodium 3-hydroxy-4-[(4-sulfonatonaphthalen-1-yl)diazenyl]naphthalene-2,7-disulfonate ($C_{20}H_{11}N_2Na_3O_{10}S_3$).

Description Food Red No. 2 occurs as a red-brown to dark red-brown powder or granules. It is odorless.

Identification

(1) A solution of Food Red No. 2 (1 in 1,000) is purplish red.

(2) A solution of Food Red No. 2 in sulfuric acid (1 in 100) is purple. Add 2 to 3 drops of this solution to 5 ml of water. A purplish red color develops.

(3) Dissolve 0.1 g of Food Red No. 2 in 100 ml of ammonium acetate solution (3 in 2,000). To 1 ml of this solution, add ammonium acetate solution (3 in 2,000) to make 100 ml. The solution exhibits absorption maximum at a wavelength of 518–522 nm.

Purity

(1) Water-insoluble substances Not more than 0.20% (Coloring Matter Tests).

(2) Chloride and sulfate Not more than 5.0% as total amount (Coloring Matter Tests).

(3) Heavy metals Not more than 20 μg/g as Pb (Coloring Matter Tests, Heavy Metals (5)).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (Coloring Matter Tests).

(5) Other coloring matters (Coloring Matter Tests, Other Coloring Matters (1)).

(6) Unreacted raw materials and products of side reactions
Not more than 0.5% as the total of:
monosodium 4-amino-1-naphthalenesulfonate,
disodium 7-hydroxy-1,3-naphthalenedisulfonate,
disodium 3-hydroxy-2,7-naphthalenedisulfonate,
monosodium 6-hydroxy-2-naphthalenesulfonate, and
trisodium 7-hydroxy-1,3,6-naphthalenetrisulfonate.

Test Solution Weigh accurately about 0.1 g of Food Red No. 2, dissolve it in ammonium acetate solution (1.54 in 1,000) to make exactly 100 ml.

Standard Solutions Weigh 0.0100 g each of monosodium 4-amino-1-naphthalenesulfonate, disodium 7-hydroxy-1,3-naphthalenedisulfonate, disodium 3-hydroxy-2,7-naphthalenedisulfonate, monosodium 6-hydroxy-2-naphthalenesulfonate, and trisodium 7-hydroxy-1,3,6-naphthalenetrisulfonate, dried previously in a vacuum desiccator for 24 hours. Dissolve separately in ammonium acetate solution (1.54 in 1,000) to make standard stock solutions of exactly 100 ml each. Proceed as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions).

Procedure Determine the amount of each salt in the test solution as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions), and calculate the total amount.

Operating Conditions

Determination wavelength: 238 nm.

Mobile phase

A: Ammonium acetate solution (1.54 in 1,000).

B: Acetonitrile.

Concentration gradient (A/B): Maintain 100% A for 5 minutes, and run a linear gradient from 100% A to 70% A over 50 minutes.

(7) Unsulfonated primary aromatic amines
Not more than 0.01% calculated as aniline,
Not more than 1.0 μg/g as α-naphthylamine (Coloring Matter Tests).

Loss on Drying Not more than 10.0% (135°C, 6 hours).

Assay Weigh accurately about 1.7 g of Food Red No. 2, and dissolve it in water to make exactly 250 ml. Use exactly 50 ml of this solution as the test solution, and proceed as directed under Titanium Trichloride Method (i) in the Assay in the Coloring Matter Tests.

Each ml of 0.1 mol/L titanium trichloride = 15.11 mg of $C_{20}H_{11}N_2Na_3O_{10}S_3$

Food Red No. 2 Aluminum Lake

Amaranth Aluminum Lake

食用赤色2号アルミニウムレーキ

Definition Food Red No. 2 Aluminum Lake is prepared by adsorbing Food Red No. 2 to a solution of aluminum salt that was reacted with alkali. Following lake formation, the product is filtered, dried, and crushed.

Content Food Red No. 2 Aluminum Lake contains the equivalent of not less than 10.0% of trisodium 3-hydroxy-4-[(4-sulfonatonaphthalen-1-yl)diazenyl]naphthalene-2,7-disulfonate ($C_{20}H_{11}N_2Na_3O_{10}S_3$ = 604.48).

Description Food Red No. 2 Aluminum Lake occurs as a fine, purplish red powder. It is odorless.

Identification

(1) To 0.1 g of Food Red No. 2 Aluminum Lake, add 5 ml of sulfuric acid, and heat in a water bath for about 5 minutes with occasional shaking. A purple color develops. Cool, and add 2 to 3 drops of the supernatant to 5 ml of water. A purplish red color develops.

(2) To 0.1 g of Food Red No. 2 Aluminum Lake, add 5 ml of diluted sulfuric acid (1 in 20), stir well, and add ammonium acetate solution (3 in 2,000) to make 100 ml. If the solution is not clear, centrifuge. Measure 1 to 10 ml of this solution so that the absorbance is between 0.2 and 0.7, and add ammonium acetate solution (3 in 2,000) to make 100 ml. The solution exhibits absorption maximum at a wavelength of 518–522 nm.

(3) To 0.1 g of Food Red No. 2 Aluminum Lake, add 10 ml of diluted hydrochloric acid (1 in 4), heat in a water bath until most of it dissolves, add 0.5 g of active carbon, shake well, and filter. Neutralize the colorless filtrate with sodium hydroxide solution (1 in 10). The solution responds to all tests for Aluminum Salt in the Qualitative Tests.

Purity

(1) Hydrochloric acid- and ammonia-insoluble substances
Not more than 0.5% (Coloring Matter Aluminum Lake Tests).

(2) Heavy metals Not more than 20 μg/g as Pb (Coloring Matter Aluminum Lake Tests, Heavy Metals (3)).

(3) Barium Not more than 500 μg/g as Ba (Coloring Matter Aluminum Lake Tests).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (Coloring

Matter Aluminum Lake Tests).

(5) Other coloring matter lakes (Coloring Matter Aluminum Lake Tests, Other Coloring Matter Lakes (1)).

Loss on Drying Not more than 30.0% (135°C, 6 hours).

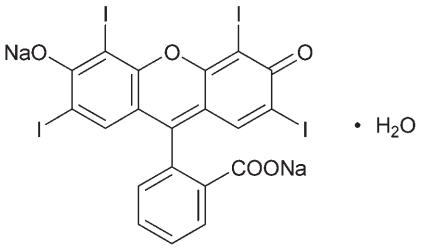
Assay Weigh accurately an amount of Food Red No. 2 Aluminum Lake so that the volume of 0.1 mol/L titanium trichloride consumed is about 20 ml, and proceed as directed in Assay (1) in the Coloring Matter Aluminum Lake Tests.

Each ml of 0.1 mol/L titanium trichloride = 15.11 mg of $C_{20}H_{11}N_2Na_3O_{10}S_3$

Food Red No. 3

Erythrosine
FD&C Red No. 3

食用赤色3号



$C_{20}H_6I_4Na_2O_5 \cdot H_2O$ Mol. Wt. 897.87

Disodium 2-(2,4,5,7-tetraiodo-6-oxido-3-oxo-3*H*-xanthen-9-yl)benzoate monohydrate
[anhydrous 16423-68-0]

Definition Food Red No. 3 consists principally of disodium 2-(2,4,5,7-tetraiodo-6-oxido-3-oxo-3*H*-xanthen-9-yl)benzoate monohydrate.

Content Food Red No. 3 contains the equivalent of not less than 85.0% of disodium 2-(2,4,5,7-tetraiodo-6-oxido-3-oxo-3*H*-xanthen-9-yl)benzoate monohydrate ($C_{20}H_6I_4Na_2O_5 \cdot H_2O$).

Description Food Red No. 3 occurs as a red to brown powder or granules. It is odorless.

Identification

(1) A solution (1 in 1,000) of Food Red No. 3 is bluish red.

(2) To 5 ml of a solution (1 in 1,000) of Food Red No. 3, add 1 ml of hydrochloric acid. A red precipitate is formed.

(3) A solution (1 in 100) of Food Red No. 3 in sulfuric acid is brown-yellow. Add 2 to 3 drops of this solution to 5 ml of water. An orange-red precipitate is formed.

(4) Dissolve 0.1 g of Food Red No. 3 in 500 ml of ammonium acetate solution (3 in 2,000). To 3 ml of this solution, add ammonium acetate solution (3 in 2,000) to make 200 ml. The solution exhibits absorption maximum at a wavelength of 524–528 nm.

Purity

(1) Water-insoluble substances Not more than 0.20% (Coloring Matter Tests).

(2) pH 6.5–10.0 (1.0 g, water 100 ml).

(3) Chloride and sulfate Not more than 2.0% as total amount (Coloring Matter Tests).

(4) Iodide Not more than 0.4% (Coloring Matter Tests).

(5) Heavy metals

Not more than 200 μg/g as Zn (Coloring Matter Tests, Heavy Metals (1)),

Not more than 20 μg/g as Pb (Coloring Matter Tests, Heavy Metals (5)).

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (Coloring Matter Tests).

(7) Other coloring matters (Coloring Matter Tests, Other Coloring Matters (2)).

Loss on Drying Not more than 12.0% (135°C, 6 hours).

Assay Weigh accurately about 1 g of Food Red No. 3, and dissolve it in water to make exactly 100 ml. Use exactly 50 ml of this solution as the test solution, and proceed as directed in the Mass Method in the Assay in the Coloring Matter Tests.

$$\text{Content (\%) Food Red No.3 } (C_{20}H_6I_4Na_2O_5 \cdot H_2O) = \frac{\text{Mass (g) of the precipitate} \times 2.148}{\text{Weight (g) of the sample}} \times 100$$

Food Red No. 3 Aluminum Lake

Erythrosine Aluminum Lake

食用赤色 3 号アルミニウムレーキ

Definition Food Red No. 3 Aluminum Lake is prepared by adsorbing Food Red No. 3 to a solution of aluminum salt that was reacted with alkali. Following lake formation, the product is filtered, dried, and crushed.

Content Food Red No. 3 Aluminum Lake contains the equivalent of not less than 10.0% of disodium 2-(2,4,5,7-tetraiodo-6-oxido-3-oxo-3*H*-xanthen-9-yl)benzoate monohydrate ($C_{20}H_6I_4Na_2O_5 \cdot H_2O$ = 897.87).

Description Food Red No. 3 Aluminum Lake occurs as a fine, red powder. It is odorless.

Identification

(1) To 0.1 g of Food Red No. 3 Aluminum Lake, add 5 ml of sulfuric acid, and heat in a water bath for about 5 minutes with occasional shaking. A light brown-orange color develops. Cool, and add 2 to 3 drops of the supernatant to 5 ml of water. An orange-red precipitate is formed.

(2) To 0.1 g of Food Red No. 3 Aluminum Lake, add 5 ml of sodium hydroxide solution (1 in 10), dissolve by heating on a water bath, and add ammonium acetate solution (3 in 2,000) to make 100 ml. If the solution is not clear, centrifuge. Measure 0.5 to 5 ml of this solution so that the absorbance is between 0.2–0.7, and add ammonium acetate solution (3 in 2,000) to make 100 ml. The solution exhibits absorption maximum at a wavelength of 524–528 nm.

(3) To 0.1 g of Food Red No. 3 Aluminum Lake, add 10 ml of diluted hydrochloric acid (1 in 4), heat in a water bath until most of it dissolves, add 0.5 g of active carbon, shake well, and filter. Neutralize the colorless filtrate with sodium hydroxide solution (1 in 10). The solution responds to all tests for Aluminum Salt in the Qualitative Tests.

Purity

(1) Hydrochloric acid- and ammonia-insoluble substances

Not more than 0.5% (Coloring Matter Aluminum Lake Tests).

(2) Iodide Not more than 0.2% (Coloring Matter Aluminum Lake Tests).

(3) Heavy metals

Not more than 50 μg/g as Zn (Coloring Matter Aluminum Lake Tests, Heavy Metals (1)).

Not more than 20 μg/g as Pb (Coloring Matter Aluminum Lake Tests, Heavy Metals (3)).

(4) Barium Not more than 500 μg/g as Ba (Coloring Matter Aluminum Lake Tests).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (Coloring Matter Aluminum Lake Tests).

(6) Other coloring matter lakes (Coloring Matter Aluminum Lake Tests, Other Coloring Matter Lakes (2)).

Loss on Drying Not more than 30.0% (135°C, 6 hours).

Assay

Test Solution Weigh accurately about 0.1 g of Food Red No. 3 Aluminum Lake, transfer into a 100-ml beaker, add 50 ml of sodium hydroxide solution (1 in 250) to dissolve, and transfer into a 500-ml volumetric flask. Wash the beaker with ammonium acetate solution (3 in 2,000), add the washings to the volumetric flask, and add ammonium acetate solution (3 in 2,000) to make exactly 500 ml. Use this solution as the sample solution. Measure exactly a certain volume, between 10 and 20 ml, of the sample solution so that the absorbance is between 0.2 and 0.7, and add ammonium acetate solution (3 in 2,000) to make exactly 200 ml.

Procedure Measure the absorbance (A) of the test solution at a wavelength of 526 nm, and calculate the content by the formula:

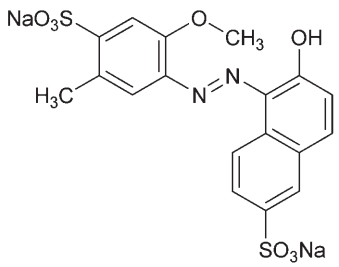
$$\text{Content (\%) of Food Red No.3 } (C_{20}H_6I_4Na_2O_5\cdot H_2O) = \frac{A \times 0.1}{0.111 \times S \times \text{Weight (g) of the sample}} \times 100$$

S = volume (ml) of the sample solution used for the preparation of the test solution.

Food Red No. 40

Allura Red AC
FD&C Red No. 40

食用赤色40号



$C_{18}H_{14}N_2Na_2O_8S_2$ Mol. Wt. 496.42

Disodium 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfonatophenyl)diazenyl]naphthalene-2-sulfonate

[25956-17-6]

Definition Food Red No. 40 is obtained by diazotizing 4-amino-5-methoxy-2-methylbenzenesulfonic acid, coupling the obtained diazo compound with 6-hydroxy-2-naphthalenesulfonic acid, and then salting out and refining the resulting dye. It consists principally of disodium 6-hydroxy-5-[(2-methoxy-5-methyl4-sulfonatophenyl)diazenyl]naphthalene-2-sulfonate.

Content Food Red No. 40 contains the equivalent of not less than 85.0% of disodium 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfonatophenyl)diazenyl]naphthalene-2-sulfonate ($C_{18}H_{14}N_2Na_2O_8S_2$).

Description Food Red No. 40 occurs as a dark red powder or granules. It is odorless.

Identification

(1) A solution of Food Red No. 40 (1 in 1,000) is red.

(2) A solution of Food Red No. 40 in sulfuric acid (1 in 100) is dark red-purple. Add 2 to 3 drops of this solution to 5 ml of water. A red color develops.

(3) Dissolve 0.1 g of Food Red No. 40 in 100 ml of ammonium acetate solution (3 in 2,000). To 1 ml of this solution, add ammonium acetate solution (3 in 2,000) to make 100 ml. This solution exhibits absorption maximum at a wavelength of 497–501 nm.

Purity

(1) Water-insoluble substances Not more than 0.20% (Coloring Matter Tests).

(2) Chloride and sulfate Not more than 5.0% as total amount (Coloring Matter Tests).

(3) Heavy metals Not more than 20 μg/g as Pb (Coloring Matter Tests, Heavy Metals (5)).

(4) Lead Not more than 10 μg/g as Pb.

Test Solution Use 10 ml of the sample solution prepared in Purity (3).

Control Solution To 1.0 ml of Lead Standard Solution, add diluted hydrochloric acid (1 in 4) to make 20 ml.

Procedure Proceed as directed in Method 1 in the Lead Limit Test.

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (Coloring Matter Tests).

(6) Lower sulfonated subsidiary colors Not more than 1.0%.

Test Solution Weigh accurately about 0.1 g of Food Red No. 40, dissolve it in ammonium acetate solution (7.7 in1,000) to make exactly 100 ml.

Standard Solutions Weigh 0.0100 g each of cresidine sulfonic acid azo β-naphthol and cresidine azo Schaeffer's salt, dried previously in a vacuum desiccator for 24 hours. dissolve separately in ammonium acetate solution (7.7 in 1,000) to prepare standard stock solutions of exactly 100 ml each. Proceed as directed in the Coloring Matter Tests (Subsidiary Colors).

Procedure Determine the amounts of cresidine sulfonic acid azo β-naphthol and cresidine azo Schaeffer's salt in the test solution as directed in the Coloring Matter Tests (Subsidiary Colors), and calculate the total amount.

Operating Conditions

Determination wavelength: 515 nm.

Mobile phase

A: Ammonium acetate solution (7.7 in 1,000).

B: Methanol.

Concentration gradient (A/B): Run a linear gradient from 100% A to 0% A over 50 minutes.

(7) Higher sulfonated subsidiary colors Not more than 1.0%.

Test Solution Use 20 μl of the test solution prepared in Purity (6).

Standard Solutions Weigh 0.0100 g of each of cresidine sulfonic acid azo G salt and cresidine sulfonic acid azo R salt, dried previously in a vacuum desiccator for 24 hours, dissolve separately in ammonium acetate solution (7.7 in 1,000) to prepare standard stock solutions of exactly 100 ml each. Proceed as directed in the Coloring Matter Tests (Subsidiary Colors).

Procedure Analyze equal portions of the test solution and the standard solutions by liquid chromatography using the operating conditions specified in Purity (6), as directed in the Coloring Matter Tests (Subsidiary Colors). Determine the amounts of cresidine sulfonic acid azo G salt and cresidine sulfonic acid azo R salt in the test solution, and calculate the total amount.

(8) Monosodium 6-hydroxy-2-naphthalenesulfonate Not more than 0.3%.

Test Solution Use 20 μl of the test solution prepared in Purity (6).

Standard Solutions Weigh 0.0100 g of monosodium 6-hydroxy-2-naphthalenesulfonate, dried in a vacuum desiccator for 24 hours, dissolve it in ammonium acetate solution (7.7 in 1,000) to prepare a standard stock solution of exactly 100 ml. Proceed as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions).

Procedure Determine the amount of monosodium 6-hydroxy-2-naphthalenesulfonate in the test solution, as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions).

Operating Conditions

Determination wavelength: 290 nm.

Mobile phase

A: Ammonium acetate solution (7.7 in 1,000).

B: Methanol.

Concentration gradient (A/B): Run a linear gradient from 100% A to 0% A over 50 minutes.

(9) 4-Amino-5-methoxy-2-methylbenzenesulfonic acid Not more than 0.2%.

Test Solution Use 20 μl of the test solution prepared in Purity (6).

Standard Solutions Weigh 0.0100 g of 4-amino-5-methoxy-2-methylbenzenesulfonic acid, dried in a vacuum desiccator for 24 hours, and dissolve in ammonium acetate solution (7.7 in 1,000) to prepare a standard stock solution of exactly 100 ml. Proceed as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions).

Procedure Analyze equal portions of the test solution and the standard solutions by liquid chromatography using the operating conditions specified in Purity (8), as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions), and determine the amount of 4-amino-5-methoxy-2-methylbenzenesulfonic acid in the test solution.

(10) Disodium 6,6′-oxybis(2-naphthalenesulfonate) Not more than 1.0%.

Test Solution Use 20 μl of the test solution prepared in Purity (6).

Standard Solutions Weigh 0.0100 g of disodium 6,6′-oxybis(2-naphthalenesulfonate), dried in a vacuum desiccator for 24 hours, and dissolve in ammonium acetate solution (7.7 in 1,000) to prepare a standard stock solution of exactly 100 ml. Proceed as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions).

Procedure Analyze equal portions of the test solution and the standard solutions by liquid chromatography using the operating conditions specified under Purity (8), as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions), and determine the amount of disodium 6,6′-oxybis(2-naphthalenesulfonate) in the test solution.

(11) Unsulfonated primary aromatic amines

Not more than 0.01% as aniline (Coloring Matter Tests).

Not more than 10 μg/g as *p*-cresidine (Coloring Matter Tests).

Loss on Drying Not more than 10.0% (135°C, 6 hours).

Assay Weigh accurately about 1.5 g of Food Red No. 40, and dissolve it in water to make exactly 250 ml. Use exactly 50 ml of this solution as the test solution, and proceed as directed in Titanium Trichloride Method (i) in the Assay in the Coloring Matter Tests.

Each ml of 0.1 mol/L titanium trichloride = 12.41 mg of $C_{18}H_{14}N_2Na_2O_8S_2$

Food Red No. 40 Aluminum Lake

Allura Red AC Aluminum Lake

食用赤色40号アルミニウムレーキ

Definition Food Red No. 40 Aluminum Lake is prepared by adsorbing Food Red No. 40 to a solution of aluminum salt that was reacted with alkali. Following lake formation, the product is filtered, dried, and crushed.

Content Food Red No. 40 Aluminum Lake contains the equivalent of not less than 10.0% of disodium 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfonatophenyl)diazenyl]-naphthalene-2-sulfonate ($C_{18}H_{14}N_2Na_2O_8S_2$ = 496.42).

Description Food Red No. 40 Aluminum Lake occurs as an

orange-red fine powder. It is odorless.

Identification

(1) To 0.1 g of Food Red No. 40 Aluminum Lake, add 5 ml of sulfuric acid, and heat in a water bath for about 5 minutes with occasional shaking. A dark purple-red color develops. Cool, add 2 to 3 drops of the supernatant to 5 ml of water. A red color develops.

(2) To 0.1 g of Food Red No. 40 Aluminum Lake, add 60 ml of diluted ammonia solution (4 in 100), heat to boil, and concentrate to about 40 ml. Cool and centrifuge the liquid, and take the supernatant. To the residue, add 10 ml of water, mix, centrifuge again, and take the supernatant. Combine both supernatants, and add ammonium acetate solution (7.7 in 1,000) to make 100 ml. Measure a certain volume, between 1 to 10 ml, of this solution so that the absorbance is between 0.2 and 0.7, and add ammonium acetate solution (7.7 in 1,000) to make 100 ml. The solution exhibits absorption maximum at a wavelength of 497–501 nm.

(3) To 0.1 g of Food Red No. 40 Aluminum Lake, add 10 ml of diluted hydrochloric acid (1 in 4), heat in a water bath until most of it dissolves, add 0.5 g of active carbon, shake well, and filter. Neutralize the colorless filtrate with sodium hydroxide solution (1 in 10). The solution responds to all tests for Aluminum Salt in the Qualitative Tests.

Purity

(1) Hydrochloric acid- and ammonia-insoluble substances
Not more than 0.5% (Coloring Matter Aluminum Lake Tests).

(2) Heavy metals Not more than 20 µg/g as Pb (Coloring Matter Aluminum Lake Tests, Heavy Metals (3)).

(3) Lead Not more than 10 µg/g as Pb.

Test Solution Use 10 ml of the test solution prepared in Purity (2).

Control Solution To 1.0 ml of Lead Standard Solution, add diluted hydrochloric acid (1 in 4) to make 20 ml.

Procedure Proceed as directed in Method 1 in the Lead Limit Test.

(4) Barium Not more than 500 µg/g as Ba (Coloring Matter Aluminum Lake Tests).

(5) Arsenic Not more than 4.0 µg/g as As_2O_3 (Coloring Matter Aluminum Lake Tests).

(6) Lower sulfonated subsidiary colors Not more than 1.0% (when the content of Food Red No. 40 used for production is 85.0%).

Test Solution Weigh 0.10 g of Food Red No. 40 Aluminum Lake, add 60 ml of diluted ammonia solution (4 in 100), heat to boil, and concentrate to about 40 ml. Cool and centrifuge the liquid, and take the supernatant. To the residue, add 10 ml of methanol, mix, centrifuge again, and take the supernatant. Combine both supernatants, and add ammonium acetate solution (7.7 in 1,000) to make 100 ml.

Procedure Proceed as directed in Purity (6) for Food Red No. 40.

(7) Higher sulfonated subsidiary colors Not more than 1.0% (when the content of Food Red No. 40 used for production is 85.0%).

Test Solution Use 20 µl of the test solution prepared under Purity (6).

Procedure Proceed as directed in Purity (7) for Food Red No. 40.

(8) Monosodium 6-hydroxy-2-naphthalenesulfonate Not more than 0.3% (when the content of Food Red No. 40 used for production is 85.0%).

Test Solution Use 20 µl of the test solution prepared in Purity (6).

Procedure Proceed as directed in Purity (8) for Food Red No. 40.

(9) 4-Amino-5-methoxy-2-methylbenzenesulfonic acid
Not more than 0.2% (when the content of Food Red No. 40 used for production is 85.0%).

Test Solution Use 20 µl of the test solution prepared in Purity (6).

Procedure Proceed as directed in Purity (9) for Food Red No. 40.

(10) Disodium 6,6′-oxybis(2-naphthalenesulfonate) Not more than 1.0% (when the content of Food Red No. 40 used for production is 85.0%).

Test Solution Use 20 µl of the test solution prepared in Purity (6).

Procedure Proceed as directed in Purity (10) for Food Red No. 40.

(11) Unsulfonated primary aromatic amines Not more than 0.01% as aniline (when the content of Food Red No. 40 used for production is 85.0%).

Weigh an amount of the sample equivalent to 0.85 g of the tar color, add 70 ml of ethyl acetate, allow to standard for 1 hour with occasional shaking, and filter through a dry filter paper (5C) for quantitative analysis. Wash the residue on the filter paper three times with 10 ml of ethyl acetate each time, and combine the washings with the filtrate. Extract three time from this solution with 10 ml of diluted hydrochloric acid (3 in 10) each time, combine the hydrochloric acid extracts, add water to make exactly 50 ml. Use this solution as the sample solution. Proceed as directed in Purity (11) for Food Red No. 40.

Loss on Drying Not more than 30.0% (135°C, 6 hours).

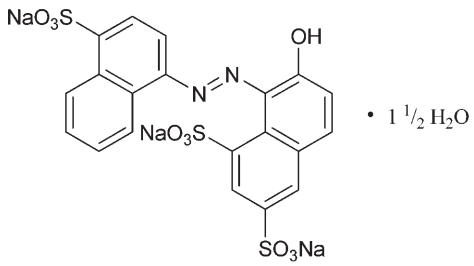
Assay Weigh accurately an amount of Food Red No. 40 Aluminum Lake so that the volume of 0.1 mol/L titanium trichloride consumed is about 20 ml, and proceed as directed in Assay (1) in the Coloring Matter Aluminum Lake Tests.

Each ml of 0.1 mol/L titanium trichloride = 12.41 mg of $C_{18}H_{14}N_2Na_2O_8S_2$

Food Red No. 102

New Coccine
Ponceau 4R

食用赤色 102 号



$C_{20}H_{11}N_2Na_3O_{10}S_3 \cdot 1½H_2O$ Mol. Wt. 631.50
Trisodium 7-hydroxy-8-[(4-sulfonatonaphthalen-1-yl)-diazenyl]naphthalene-1,3-disulfonate sesquihydrate [anhydrous 2611-82-7]

Definition Food Red No. 102 is obtained by diazotizing 4-amino-1-naphthalenesulfonic acid, coupling the obtained diazo compound with 7-hydroxy-1,3-naphthalenedisulfonic acid, and then salting out and refining the resulting dye. It consists principally of trisodium 7-hydroxy-8-[(4-sulfonatonaphthalen-1-yl)diazenyl]naphthalene-1,3-disulfonate sesquihydrate.

Content Food Red No. 102 contains the equivalent of not less than 85.0% of trisodium 7-hydroxy-8-[(4-sulfonatonaphthalen-1-yl)diazenyl]naphthalene-1,3-disulfonate sesquihydrate. ($C_{20}H_{11}N_2Na_3O_{10}S_3 \cdot 1½\ H_2O$).

Description Food Red No. 102 occurs as a red to dark red powder or granules. It is odorless.

Identification

(1) A solution of Food Red No. 102 (1 in 1,000) is red.

(2) A solution of Food Red No. 102 in sulfuric acid (1 in 100) is purple-red. Add 2 to 3 drops of this solution to 5 ml of water. A yellow-red color develops.

(3) Dissolve 0.1 g of Food Red No. 102 in 100 ml of ammonium acetate solution (3 in 2,000). To 1 ml of this solution, add ammonium acetate solution (3 in 2,000) to make 100 ml. The solution exhibits absorption maximum at a wavelength of 506–510 nm.

Purity

(1) Water-insoluble substances Not more than 0.20% (Coloring Matter Tests).

(2) Chloride and sulfate Not more than 8.0% as total amount (Coloring Matter Tests).

(3) Heavy metals Not more than 20 μg/g as Pb (Coloring Matter Tests, Heavy Metals (5)).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (Coloring Matter Tests).

(5) Other coloring matters (Coloring Matter Tests, Other Coloring Matters (1)).

(6) Unreacted raw materials and products of side reactions
Not more than 0.5% as the total of:
monosodium 4-amino-1-naphthalenesulfonate,
disodium 7-hydroxy-1,3-naphthalenedisulfonate,
disodium 3-hydroxy-2,7-naphthalenedisulfonate,
monosodium 6-hydroxy-2-naphthalenesulfonate, and
trisodium 7-hydroxy-1,3,6-naphthalenetrisulfonate.

Test Solution Weigh accurately about 0.1 g of Food Red No. 102, and dissolve it in ammonium acetate solution (1.54 in 1,000) to make exactly 100 ml.

Standard Solutions Weigh 0.0100 g each of monosodium 4-amino-1-naphthalenesulfonate, disodium 7-hydroxy-1,3-naphthalenedisulfonate, disodium 3-hydroxy-2,7-naphthalenedisulfonate, monosodium 6-hydroxy-2-naphthalenesulfonate, and trisodium 7-hydroxy-1,3,6-naphthalenetrisulfonate, dried previously in a vacuum desiccator for 24 hours. Dissolve separately in ammonium acetate solution (1.54 in 1,000) to prepare standard stock solutions of exactly 100 ml each. Proceed as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions).

Procedure Determine the amounts of these salts as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions), and calculate the total amount.

Operating Conditions
Determination wavelength: 238 nm.
Mobile phase
A: Ammonium acetate solution (1.54 in 1,000).
B: Acetonitrile.
Concentration gradient (A/B): Maintain 100% A for 5 minutes, and run a linear gradient from 100% A to 70% A over 50 minutes.

(7) Unsulfonated primary aromatic amines
Not more than 0.01% calculated as aniline.
Not more than 1.0 μg/g as α-naphthylamine.

Loss on Drying Not more than 10.0% (135°C, 6 hours).

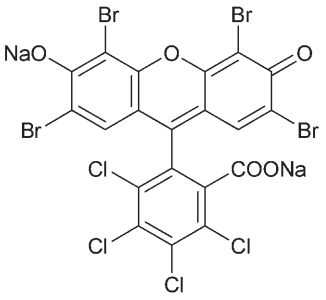
Assay Weigh accurately about 1.7 g of Food Red No. 102, and dissolve it in water to make 250 ml. Use exactly 50 ml of this solution as the test solution, and proceed as directed in Titanium Trichloride Method (i) in the Assay in the Coloring Matter Tests.

Each ml of 0.1 mol/L titanium trichloride = 15.79 mg of $C_{20}H_{11}N_2Na_3O_{10}S_3 \cdot 1½H_2O$

Food Red No. 104

Phloxine

食用赤色 104 号



$C_{20}H_2Br_4Cl_4Na_2O_5$ Mol. Wt. 829.63

Disodium 3,4,5,6-tetrachloro-2-(2,4,5,7-tetrabromo-6-oxido-3-oxo-3*H*-xanthen-9-yl)benzoate [18472-87-2]

Definition Food Red No. 104 consists principally of disodium 3,4,5,6-tetrachloro-2-(2,4,5,7-tetrabromo-6-oxido-3-oxo-3*H*-xanthen-9-yl)benzoate.

Content Food Red No. 104 contains the equivalent of not less than 85.0% of disodium 3,4,5,6-tetrachloro-2-(2,4,5,7-tetrabromo-6-oxido-3-oxo-3*H*-xanthen-9-yl)benzoate ($C_{20}H_2Br_4Cl_4Na_2O_5$).

Description Food Red No. 104 occurs as a red to dark red-brown powder or granules. It is odorless.

Identification

(1) A solution of Food Red No. 104 (1 in 1,000) is orange-red and emits a green-yellow fluorescence.

(2) To 5 ml of a solution of Food Red No. 104 (1 in 1,000), add 1 ml of hydrochloric acid. A light red precipitate is formed, and the fluorescence disappears.

(3) A solution of Food Red No. 104 in sulfuric acid (1 in 100) is brownish yellow and emits no fluorescence. Add 2 to 3 drops of this solution to 5 ml of water. A light red precipitate is formed, and no fluorescence appears.

(4) Dissolve 0.1 g of Food Red No. 104 in 200 ml of ammonium acetate solution (3 in 2,000). To 1 ml of this solution, add ammonium acetate solution (3 in 2,000) to make 100 ml. The solution exhibits absorption maximum at a wavelength of 536–540 nm.

Purity

(1) Water-insoluble substances Not more than 0.20% (Coloring Matter Tests).

(2) pH 6.5–10.0 (1.0 g, water 100 ml).

(3) Chloride and sulfate Not more than 5.0% as total amount (Coloring Matter Tests).

(4) Bromide Not more than 1.0% (Coloring Matter Tests).

(5) Heavy metals

Not more than 200 μg/g as Zn (Coloring Matter Tests, Heavy Metals(1)).

Not more than 20 μg/g as Pb (Coloring Matter Tests, Heavy Metals (5)).

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (Coloring Matter Tests).

(7) Other coloring matters (Coloring Matter Tests, Other Coloring Matters (2)).

(8) Hexachlorobenzene Not more than 5.0 μg/g.

Test Solution Weigh accurately about 0.02 g of Food Red No. 104 into a 50-ml centrifuge tube, and dissolve it in 30 ml of water. Add exactly 10 m of hexane, and shake for 5 minutes. Transfer the hexane layer into a test tube with stopper, add 0.5 g of anhydrous sodium sulfate to the hexane layer, and shake. Use the hexane layer as the test solution.

Standard Solutions Weigh accurately about 0.01 g of hexachlorobenzene, and dissolve it in hexane to make exactly 100 ml. Measure exactly 5 ml of this solution, and add hexane to make exactly 100 ml. Measure exactly 1 ml of the second solution, and add hexane to make exactly 100 ml. Next, transfer exactly 1 ml, 1 ml, 2 ml, 3 ml, and 6 ml of the last solution into separate volumetric flasks. To each, add hexane to make exactly 50 ml, 10 ml, 10 ml, 10 ml, and 10 ml.

Procedure Analyze 1 μl portions of the test solution and the standard solutions by gas chromatography using the conditions given below. Measure the peak areas of hexachlorobenzene for the standard solutions to prepare a calibration curve. Obtain the content of hexachlorobenzene in the test solution from the calibration curve and the peak area of hexachlorobenzene for the test solution.

Operating Conditions

Detector: Electron-capture detector.

Column: A silicate glass capillary (0.25 mm internal diameter and 30 m length) coated with a 0.25-μm thick layer of 5% diphenyl/95% dimethyl polysiloxane for gas chromatography.

Column temperature: Maintain the temperature at 60ºC for 1 minute, thereafter raise to 280ºC, and maintain at 280ºC for 5 minutes. The temperature should be adjusted so that the peak of hexachlorobenzene is separated from the peaks of other components and appears 10–15 minutes after injection.

Injection port temperature: 260ºC.

Detector temperature: 300ºC.

Injection: Splitless.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the peak of hexachlorobenzene appears 10–15 minutes after injection.

Loss on Drying Not more than 10.0% (135°C, 6 hours).

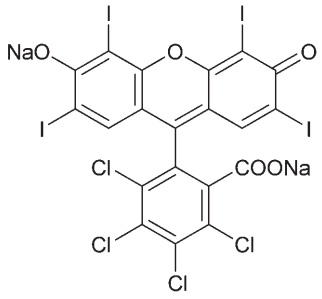
Assay Weigh accurately about 1 g of Food Red No. 104, and dissolve it in water to make exactly 100 ml. Use exactly 50 ml of this solution as the test solution, and proceed as directed in the Mass Method in the Assay in the Coloring Matter Tests.

$$\text{Content (\%) of Food Red No.104 } (C_{20}H_2Br_4C_{l4}Na_2O_5) = \frac{\text{Mass (g) of the precipitate} \times 2.112}{\text{Weight (g) of the sample}} \times 100$$

Food Red No. 105

Rose Bengal

食用赤色 105 号



$C_{20}H_2Cl_4I_4Na_2O_5$ Mol. Wt. 1017.64
Disodium 3,4,5,6-tetrachloro-2-(2,4,5,7-tetraiodo-6-oxido-3-oxo-3*H*-xanthen-9-yl)benzoate [632-69-9]

Definition Food Red No. 105 consists principally of disodium 3,4,5,6-tetrachloro-2-(2,4,5,7-tetraiodo-6-oxido-3-oxo-3*H*-xanthen-9-yl)benzoate.

Content Food Red No. 105 contains the equivalent of not less than 85.0% of disodium 3,4,5,6-tetrachloro-2-(2,4,5,7-tetraiodo-6-oxido-3-oxo-3*H*-xanthen-9-yl)benzoate ($C_{20}H_2Cl_4I_4Na_2O_5$).

Description Food Red No. 105 occurs as a purplish red to red-brown powder or granules. It is odorless.

Identification

(1) A solution of Food Red No. 105 (1 in 1,000) is bluish red.

(2) To 5 ml of a solution of Food Red No. 105 (1 in 1,000), add 1 ml of hydrochloric acid. A bluish red precipitate is formed.

(3) A solution of Food Red No. 105 in sulfuric acid (1 in 100) is brown-yellow. Add 2 to 3 drops of this solution to 5 ml of water. A bluish red precipitate is formed.

(4) Dissolve 0.1 g of Food Red No. 105 in 200 ml of ammonium acetate solution (3 in 2,000). To 1 ml of this solution, add ammonium acetate solution (3 in 2,000) to make 100 ml. The solution exhibits absorption maximum at a wavelength of 546–550 nm.

Purity

(1) Water-insoluble substances Not more than 0.20% (Coloring Matter Tests).

(2) pH 6.5–10.0 (1.0 g, water 100 ml).

(3) Chloride and sulfate Not more than 5.0% as total amount (Coloring Matter Tests).

(4) Iodide Not more than 0.4% (Coloring Matter Tests).

(5) Heavy metals

Not more than 200 μg/g as Zn (Coloring Matter Tests, Heavy Metals (1)).

Not more than 20 μg/g as Pb (Coloring Matter Tests, Heavy Metals (5)).

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (Coloring Matter Tests).

(7) Other coloring matters (Coloring Matter Tests, Other Coloring Matters (2)).

(8) Hexachlorobenzene Not more than 6.5 μg/g.

Proceed as directed in Purity (8) for Food Red No. 104.

Loss on Drying Not more than 10.0% (135°C, 6 hours).

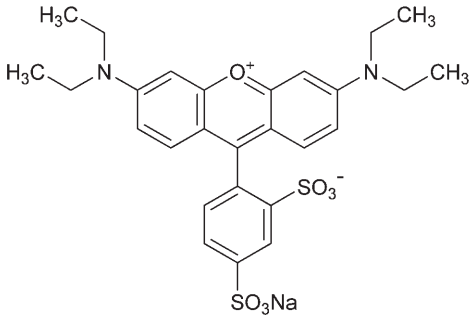
Assay Weigh accurately about 1 g of Food Red No. 105, and dissolve it in water to make exactly 100 ml. Use exactly 50 ml of this solution as the test solution, and proceed as directed in the Mass Method in the Assay in the Coloring Matter Tests.

$$\text{Content (\%) of Food Red No.105 } (C_{20}H_2Cl_4I_4Na_2O_5) = \frac{\text{Mass (g) of the precipitate} \times 2.090}{\text{Weight (g) of the sample}} \times 100$$

Food Red No. 106

Acid Red

食用赤色 106 号



$C_{27}H_{29}N_2NaO_7S_2$ Mol. Wt. 580.65
Monosodium 6-[3,6-bis(diethylamino)xanthenium-9-yl]benzene-1,3-disulfonate [3520-42-1]

Definition Food Red No. 106 consists principally of monosodium 6-[3,6-bis(diethylamino)xanthenium-9-yl]-benzene-1,3-disulfonate.

Content Food Red No. 106 contains the equivalent of not less than 85.0% of monosodium 6-[3,6-bis(diethylamino)xanthenium-9-yl]benzene-1,3-disulfonate ($C_{27}H_{29}N_2NaO_7S_2$).

Description Food Red No. 106 occurs as a purple-brown powder or granules. It is odorless.

Identification

(1) A solution of Food Red No. 106 (1 in 1,000) is bluish red and emits a light yellow fluorescence.

(2) To 5 ml of a solution of Food Red No. 106 (1 in 1,000), add 1 ml of hydrochloric acid. The color of the solution changes to red while the color of fluorescence does not change.

(3) A solution of Food Red No. 106 in sulfuric acid (1 in 100) is orange-yellow and emits a green-yellow fluorescence. Add 2 to 3 drops of this solution to 5 ml of water. A bluish red color develops, and a slight green-yellow fluorescence appears.

(4) Dissolve 0.1 g of Food Red No. 106 in 500 ml of ammonium acetate solution (3 in 2,000). To 3 ml of this solution, add ammonium acetate solution (3 in 2,000) to make 200 ml. The solution exhibits absorption maximum at a

wavelength of 564–568 nm.

Purity

(1) Water-insoluble substances Not more than 0.20% (Coloring Matter Tests).

(2) pH 6.5–10.0 (1.0 g, water 100 ml).

(3) Chloride and sulfate Not more than 5.0% as total amount (Coloring Matter Tests).

(4) Heavy metals

Not more than 25 μg/g as Cr (Coloring Matter Tests, Heavy Metals (2)).

Not more than 50 μg/g as Mn (Coloring Matter Tests, Heavy Metals (4)).

Not more than 20 μg/g as Pb (Coloring Matter Tests, Heavy Metals (5)).

For the Chromium test, use 10.0 ml each of the sample solution and the blank solution.

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (Coloring Matter Tests).

(6) Other coloring matters (Coloring Matter Tests, Other Coloring Matters (3)).

Loss on Drying Not more than 10.0% (135°C, 6 hours).

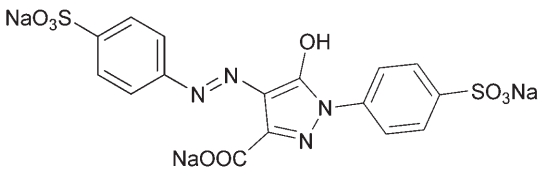
Assay Weigh accurately about 3 g of Food Red No. 106, and dissolve it in water to make exactly 250 ml. Use exactly 50 ml of this solution as the test solution, and proceed as directed in Titanium Trichloride Method (iv) in the Assay in the Coloring Matter Tests.

Each ml of 0.1 mol/L titanium trichloride = 29.03 mg of $C_{27}H_{29}N_2NaO_7S_2$

Food Yellow No. 4

Tartrazine
FD&C Yellow No. 5

食用黄色4号



$C_{16}H_9N_4Na_3O_9S_2$ Mol. Wt. 534.37

Trisodium 5-hydroxy-1-(4-sulfonatophenyl)-4-[(4-sulfonatophenyl)diazenyl]-1*H*-pyrazole-3-carboxylate [1934-21-0]

Definition Food Yellow No. 4 is obtained by diazotizing 4-aminobenzenesulfonic acid, coupling the obtained diazo compound with 5-hydroxy-1-(4-sulfophenyl)-3-pyrazolecarboxylic acid, and then salting out and refining the resulting dye. It consists essentially of trisodium 5-hydroxy-1-(4-sulfonatophenyl)-4-[(4-sulfonatophenyl)diazenyl]-1*H*-pyrazole-3-carboxylate.

Content Food Yellow No. 4 contains the equivalent of not less than 85.0% of trisodium 5-hydroxy-1-(4-sulfonatophenyl)-4-[(4-sulfonatophenyl)diazenyl]-1*H*-pyrazole-3-carboxylate ($C_{16}H_9N_4Na_3O_9S_2$).

Description Food Yellow No. 4 occurs as an orange-yellow to orange powder or granules. It is odorless.

Identification

(1) A solution of Food Yellow No. 4 (1 in 1,000) is yellow.

(2) A solution of Food Yellow No. 4 in sulfuric acid (1 in 100) is yellow. Add 2 to 3 drops of this solution to 5 ml of water. A yellow color develops.

(3) Dissolve 0.1 g of Food Yellow No. 4 in 100 ml of ammonium acetate solution (3 in 2,000). To 1 ml of this solution, add ammonium acetate solution (3 in 2,000) to make 100 ml. The solution exhibits absorption maximum at a wavelength of 426–430 nm.

Purity

(1) Water-insoluble substances Not more than 0.20% (Coloring Matter Tests).

(2) Chloride and sulfate Not more than 6.0% as total amount (Coloring Matter Tests).

(3) Heavy metals Not more than 20 μg/g as Pb (Coloring Matter Tests, Heavy Metals (5)).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (Coloring Matter Tests).

(5) Other coloring matters (Coloring Matter Tests, Other Coloring Matters (1)).

(6) Unreacted raw materials and products of side reactions
Not more than 0.5% as the total of:
4-aminobenzenesulfonic acid,
5-hydroxy-1-(4-sulfophenyl)-3-pyrazolecarboxylic acid,
4-hydrazinobenzenesulfonic acid, and
disodium 4,4′-(diazoamino)dibenzenesulfonate.

Test Solution Weigh accurately about 0.1 g of Food Yellow No. 2, and dissolve it in ammonium acetate solution (1.54 in 1,000) to make exactly 100 ml.

Standard Solutions Weigh 0.0100 g each of 4-aminobenzenesulfonic acid, 5-hydroxy-1-(4-sulfophenyl)-3-pyrazolecarboxylic acid, 4-hydrazinobenzenesulfonic acid, and disodium 4,4′-(diazoamino)dibenzenesulfonate, dried previously in a vacuum desiccator for 24 hours. Dissolve disodium 4,4′-(diazoamino)dibenzenesulfonate in sodium hydroxide solution (4 in 1,000) and each of the remaining salts in ammonium acetate solution (1.54 in 1,000), and prepare standard stock solutions of exactly 100 ml each. For 4-hydrazinobenzenesulfonic acid standard stock solution, prepare fresh before use. Proceed as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions).

Procedure Determine the amounts of these substances in the test solution as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions), and calculate the total amount.

Operating Conditions

Determination wavelengths
4-aminobenzenesulfonic acid: 254 nm,
5-hydroxy-1-(4-sulfophenyl)-3-pyrazolecarboxylic acid: 254 nm,
4-hydrazinobenzenesulfonic acid: 254 nm.
disodium 4,4′-(diazoamino)-dibenzenesulfonate: 358 nm.

Mobile phase
A: Ammonium acetate solution (1.54 in 1,000).
B: Acetonitrile.

Concentration gradient (A/B): Maintain 100% A for 5 minutes, and run a linear gradient from 100% A to 70% A over 50 minutes.

(7) Unsulfonated primary aromatic amines Not more than 0.01% calculated as aniline (Coloring Matter Tests).

Loss on Drying Not more than 10.0% (135°C, 6 hours).
Assay Weigh accurately about 1.5 g of Food Yellow No. 4, and dissolve it in water to make exactly 250 ml. Use exactly 50 ml of this solution as the test solution, and proceed as directed in Titanium Trichloride Method (iii) in the Assay in the Coloring Matter Tests.

Each ml of 0.1 mol/L titanium trichloride = 13.36 mg of $C_{16}H_9N_4Na_3O_9S_2$

Food Yellow No. 4 Aluminum Lake

Tartrazine Aluminum Lake

食用黄色 4 号アルミニウムレーキ

Definition Food Yellow No. 4 Aluminum Lake is prepared by adsorbing Food Yellow No. 4 to a solution of aluminum salt that was reacted with alkali. Following lake formation, the product is filtered, dried, and crushed.
Content Food Yellow No. 4 Aluminum Lake contains the equivalent of not less than 10.0% of trisodium 5-hydroxy-1-(4-sulfonatophenyl)-4-[(4-sulfonatophenyl)diazenyl]-1*H*-pyrazole-3-carboxylate ($C_{16}H_9N_4Na_3O_9S_2$ = 534.37).
Description Food Yellow No. 4 Aluminum Lake occurs as a fine, yellow powder. It is odorless.
Identification

(1) To 0.1 g of Food Yellow No. 4 Aluminum Lake, add 5 ml of sulfuric acid, and heat in a water bath for about 5 minutes with occasional shaking. A yellow color develops. Cool, and add 2 to 3 drops of the supernatant to 5 ml of water. A yellow color develops.

(2) To 0.1 g of Food Yellow No. 4 Aluminum Lake, add 5 ml of diluted sulfuric acid (1 in 20), stir well, and add ammonium acetate solution (3 in 2,000) to make 100 ml. If the solution is not clear, centrifuge. Measure 1 to 10 ml of this solution so that the absorbance is between 0.2 and 0.7, and add ammonium acetate solution (3 in 2,000) to make 100 ml. The solution exhibits absorption maximum at a wavelength of 426–430 nm.

(3) To 0.1 g of Food Yellow No. 4 Aluminum Lake, add 10 ml of diluted hydrochloric acid (1 in 4), heat in a water bath until most of it dissolves, add 0.5 g of active carbon, shake well, and filter. Neutralize the colorless filtrate with sodium hydroxide solution (1 in 10). The solution responds to all tests for Aluminum Salt in the Qualitative Tests.

Purity

(1) Hydrochloric acid- and ammonia-insoluble substances Not more than 0.5% (Coloring Matter Aluminum Lake Tests).

(2) Heavy metals Not more than 20 μg/g as Pb (Coloring Matter Aluminum Lake Tests, Heavy Metals (3)).

(3) Barium Not more than 500 μg/g as Ba (Coloring Matter Aluminum Lake Tests).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (Coloring Matter Aluminum Lake Tests).

(5) Other coloring matter lakes (Coloring Matter Aluminum Lake Tests, Other Coloring Matter Lakes (1)).

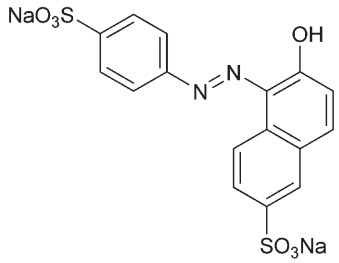
Loss on Drying Not more than 30.0% (135°C, 6 hours).
Assay Weigh accurately an amount of Food Yellow No. 4 Aluminum Lake so that the volume of 0.1 mol/L titanium trichloride consumed is about 20 ml, and proceed as directed in Assay (3) in the Coloring Matter Aluminum Lake Tests.

Each ml of 0.1 mol/L titanium trichloride = 13.36 mg of $C_{16}H_9N_4Na_3O_9S_2$

Food Yellow No. 5

Sunset Yellow FCF
FD&C Yellow No. 6

食用黄色 5 号



$C_{16}H_{10}N_2Na_2O_7S_2$ Mol. Wt. 452.37
Disodium 6-hydroxy-5-[(4-sulfonatophenyl)diazenyl]naphthalene-2-sulfonate
[2783-94-0]
Definition Food Yellow No. 5 is obtained by diazotizing 4-aminobenzenesulfonic acid, coupling the obtained diazo compound with 6-hydroxy-2-naphthalenesulfonic acid, and then salting out and refining the resulting dye. It consists principally of disodium 6-hydroxy-5-[(4-sulfonatophenyl)diazenyl]naphthalene-2-sulfonate.
Content Food Yellow No. 5 contains the equivalent of not less than 85.0% of disodium 6-hydroxy-5-[(4-sulfonatophenyl)diazenyl]naphthalene-2-sulfonate ($C_{16}H_{10}N_2Na_2O_7S_2$).
Description Food Yellow No. 5 occurs as an orange-red powder or granules. It is odorless.
Identification

(1) A solution of Food Yellow No. 5 (1 in 1,000) is orange.

(2) A solution of Food Yellow No. 5 in sulfuric acid (1 in 100) is orange-red. Add 2 to 3 drops of this solution to 5 ml of water. An orange-yellow color develops.

(3) Dissolve 0.1 g of Food Yellow No. 5 in 100 ml of ammonium acetate solution (3 in 2,000). To 1 ml of this solution, add ammonium acetate solution (3 in 2,000) to make 100 ml. The solution exhibits absorption maximum at a wavelength of 480–484 nm.

Purity

(1) Water-insoluble substances Not more than 0.20% (Coloring Matter Tests).

(2) Chloride and sulfate Not more than 5.0% as total amount (Coloring Matter Tests).

(3) Heavy metals Not more than 20 μg/g as Pb (Coloring Matter Tests, Heavy Metals (5)).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (Coloring Matter Tests).

(5) Subsidiary colors
Not more than 5% as the total of:
sulfanilic acid azo G salt color,
sulfanilic acid azo R salt color,
sulfanilic acid azo β-naphthol color, and
aniline azo Schaeffer's salt color; and
not more than 2% of colors other than sulfanilic acid azo R salt color.

Test Solution Weigh accurately about 0.1 g of Food Yellow No. 5, dissolve it in ammonium acetate solution (1.54 in 1,000, pH 8.0) to make exactly 100 ml.

Standard Solutions Weigh 0.0100 g of each of sulfanilic acid azo G salt color, sulfanilic acid azo R salt color, sulfanilic acid azo β-naphthol color, and aniline azo Schaeffer's salt color, dried previously in a vacuumed desiccator for 24 hours. Dissolve separately in ammonium acetate solution (1.54 in 1,000, pH 8.0) to prepare standard stock solutions of exactly 100 ml each. Proceed as directed in the Coloring Matter Tests (Subsidiary Colors).

Procedure Determine the amounts of these colors in the test solution as directed in the Coloring Matter Tests (Subsidiary Colors), and calculate the total amount.

Operating Conditions
Determination wavelength: 482 nm.
Mobile phase
A: Ammonium acetate solution (1.54 in 1,000).
B: Acetonitrile.
Concentration gradient (A/B): Run a linear gradient from 100% A to 60% A over 50 minutes.

(6) Unreacted raw materials and products of side reactions
Not more than 0.5% as the total of:
4-aminobenzenesulfonic acid,
disodium 7-hydroxy-1,3-naphthalenedisulfonate,
disodium 3-hydroxy-2,7-naphthalenedisulfonate,
monosodium 6-hydroxy-2-naphthalenesulfonate,
disodium 6,6′-oxybis(2-naphthalenesulfonate), and
disodium 4,4′-(diazoamino)dibenzenesulfonate.

Test Solution Weigh accurately about 0.1 g of Food Yellow No. 5, and dissolve it in ammonium acetate solution (1.54 in1,000, pH 8.0) to make exactly 100 ml.

Standard Solutions Weigh 0.0100 g each of 4-aminobenzenesulfonic acid, disodium 7-hydroxy-1,3-naphthalenedisulfonate, disodium 3-hydroxy-2,7-naphthalenedisulfonate, monosodium 6-hydroxy-2-naphthalenesulfonate, disodium 6,6′-oxybis(2-naphthalenesulfonate), and disodium 4,4′-(diazoamino)dibenzenesulfonate, dried previously in a vacuum desiccator for 24 hours. Dissolve 4,4′-(diazoamino)-dibenzenesulfonate in sodium hydroxide solution (4 in 1,000) and each of the remaining salts in ammonium acetate solution (1.54 in 1,000, pH 8.0) to prepare standard stock solutions of exactly 100 ml each. Proceed as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions).

Procedure Determine the amounts of these substances in the test solution as directed in the Coloring Matter Tests (Unreacted Raw Materials and Products of Side Reactions), and calculate the total amount.

Operating Conditions
Determination wavelengths
4-Aminobenzenesulfonic acid: 232 nm.
Disodium 7-hydroxy-1,3-naphthalenedisulfonate: 232 nm.
Disodium 3-hydroxy-2,7-naphthalenedisulfonate: 232 nm.
Monosodium 6-hydroxy-2-naphthalenesulfonate: 232 nm.
Disodium 6,6′-oxybis(2-naphthalenesulfonate): 232 nm.
Disodium 4,4′-(diazoamino)dibenzenesulfonic acid: 358 nm.
Mobile phase
A: Ammonium acetate solution (1.54 in 1,000).
B: Acetonitrile.
Concentration gradient (A/B): Run a linear gradient from 100% A to 60% A over 50 minutes.

(7) Unsulfonated primary aromatic amines Not more than 0.01% as aniline (Coloring Matter Tests).

Loss on Drying Not more than 10.0% (135°C, 6 hours).

Assay Weigh accurately about 1.3 g of Food Yellow No. 5, and dissolve it in water to make exactly 250 ml. Measure exactly 50 ml of this solution, use as the test solution, and proceed as directed in Titanium Trichloride Method (i) in the Assay in the Coloring Matter Tests.

Each ml of 0.1 mol/L titanium trichloride = 11.31 mg of $C_{16}H_{10}N_2Na_2O_7S_2$

Food Yellow No. 5 Aluminum Lake

Sunset Yellow FCF Aluminum Lake

食用黄色5号アルミニウムレーキ

Definition Food Yellow No. 5 Aluminum Lake is prepared by adsorbing Food Yellow No. 5 to a solution of aluminum salt that was reacted with alkali. Following lake formulation, the product is filtered, dried, and crushed.

Content Food Yellow No. 5 Aluminum Lake contains the equivalent of not less than 10.0% of disodium 6-hydroxy-5-[(4-sulfonatophenyl)diazenyl]naphthalene-2-sulfonate ($C_{16}H_{10}N_2Na_2O_7S_2$ = 452.37).

Description Food Yellow No. 5 Aluminum Lake occurs as a fine, orange-yellow powder. It is odorless.

Identification

(1) To 0.1 g of Food Yellow No. 5 Aluminum Lake, add 5 ml of sulfuric acid, and heat in a water bath for about 5 minutes with occasional shaking. An orange-red color develops. After cooling, and add 2 to 3 drops of the supernatant to 5 ml of water. An orange-yellow color develops.

(2) To 0.1 g of Food Yellow No. 5 Aluminum Lake, add 5 ml of diluted sulfuric acid (1 in 20), stir well, and add ammonium acetate solution (3 in 2,000) to make 100 ml. If the solution is not clear, centrifuge. Measure 1 to 10 ml of this solution so that the absorbance to be measured is in the range of 0.2 to 0.7, and add ammonium acetate solution (3 in 2,000) to make 100 ml. The solution exhibits absorption maximum at a wavelength of 480–484 nm.

(3) To 0.1 g of Food Yellow No. 5 Aluminum Lake, add 10 ml of diluted hydrochloric acid (1 in 4), heat in a water bath until most of it dissolves, add 0.5 g of active carbon, shake well, and filter. Neutralize the colorless filtrate with sodium hydroxide solution (1 in 10). The solution responds to all tests for Aluminum Salt in the Qualitative Tests.

Purity

(1) Hydrochloric acid- and ammonia-insoluble substances
Not more than 0.5% (Coloring Matter Aluminum Lake Tests).

(2) Heavy metals Not more than 20 μg/g as Pb (Coloring Matter Aluminum Lake Tests, Heavy Metals (3)).

(3) Barium Not more than 500 μg/g as Ba (Coloring Matter Aluminum Lake Tests).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (Coloring Matter Aluminum Lake Tests).

(5) Subsidiary colors
Not more than 5% (when the content of Food Yellow No. 5 used for production is 85.0%) as the total of:
sulfanilic acid azo G salt color,
sulfanilic acid azo R salt color,
sulfanilic acid azo β-naphthol color, and
aniline azo Schaefer's salt color; and
not more than 2% of colors other than sulfanilic acid azo R salt color (when the content of Food Yellow No. 5 used for production is 85.0%).

Test Solution Weigh accurately about 0.1 g of Food Yellow No. 5 Aluminum Lake, add 60 ml of diluted ammonia solution (4 in 100), heat to boil, and concentrate to about 40 ml. Cool and centrifuge the liquid, and then remove the supernatant. To the residue, add 10 ml of water, mix, and centrifuge again. Combine both supernatants, and add ammonium acetate solution (7.7 in 1,000) to make exactly 100 ml. Use this solution as the test solution. Proceed as directed in Purity (5) for Food Yellow No. 5.

Loss on Drying Not more than 30.0% (135°C, 6 hours).

Assay Weigh accurately an amount of Food Yellow No. 5 Aluminum Lake so that the volume of 0.1 mol/L titanium trichloride consumed is about 20 ml, and proceed as directed in Assay (1) in the Coloring Matter Aluminum Lake Tests.

Each ml of 0.1 mol/L titanium trichloride = 11.31 mg of $C_{16}H_{10}N_2Na_2O_7S_2$

Fukuronori Extract

フクロノリ抽出物

Definition Fukuronori Extract is obtained from the whole *fukurofunori* algae, *Gloiopeltis furcata* J. Agardh, and consists manly of polysaccharides. It may contain sucrose, glucose, lactose, dextrin, or maltose.

Description Fukuronori Extract occurs as a white to brown powder or granules. It has little or no odor.

Identification

(1) To 200 ml of water, add 4 g of Fukuronori Extract, and keep at about 80°C in a water bath while stirring until a homogenous viscous liquid is formed. Replenish the lost water, and cool to room temperature. It remains viscous.

(2) To 50 ml of the viscous solution obtained in Identification (1), add 0.2 g of potassium chloride, warm again, stir well, and cool to room temperature. The solution remains viscous.

(3) To 20 ml of water, add 0.1 g of Fukuronori Extract, add 3 ml of barium chloride solution (3 in 25) and 5 ml of diluted hydrochloric acid (2 in 5), and mix well. If necessary, remove any precipitate that has been produced. Boil the mixture for 10 minutes. A white crystalline precipitate is formed.

Purity

(1) Viscosity Not less than 5.0 mPa·s (1.5%, 75°C).

(2) Sulfuric group 5–30%.
Proceed as directed in Purity (4) for Processed Eucheuma Algae.

(3) Acid-insoluble matter Not more than 2.0%.
Proceed as directed in Purity (5) for Processed Eucheuma Algae.

(4) Heavy metals Not more than 40 μg/g as Pb (0.50 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(5) Lead Not more than 10 μg/g as Pb (1.0 g, Method 1).

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50g, Method 3, Apparatus B).

Loss on Drying Not more than 12.0% (105°C, 5 hours).

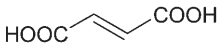
Ash 5–30% (on the dried basis).

Acid-insoluble Ash Not more than 1.0%.

Microbial Limits Proceed as directed in the Microbial Limit Tests. The total bacterial count is not more than 10,000/g, and *Escherichia coli* is negative.

Fumaric Acid

フマル酸



$C_4H_4O_4$ Mol. Wt. 116.07

(2*E*)-But-2-enedioic acid [110-17-8]

Content Fumaric Acid contains not less than 99.0% of fumaric acid ($C_4H_4O_4$).

Description Fumaric Acid occurs as a white crystalline powder. It is odorless and has a characteristic acid taste.

Identification

(1) Heat Fumaric Acid. It sublimates.

(2) Dry Fumaric Acid at 105°C for 3 hours. The melting point is 287–302°C (in sealed tube, decomposition).

(3) To 0.5 g of Fumaric Acid, add 10 ml of water, dissolve by boiling, and add 2–3 drops of bromine TS while hot. The color of the solution disappears.

(4) Place 0.05 g of Fumaric Acid into a test tube, add 2–3 mg of resorcinol and 1 ml of sulfuric acid, and shake. Heat at 120–130°C for 5 minutes, cool, and add water to make 5 ml. To this solution, add sodium hydroxide solution (3 in 10) dropwise while cooling to make alkaline, and add water to make 10 ml. A green-blue fluorescence appears under ultraviolet light.

Purity

(1) Clarity and color of solution Colorless and clear (0.50 g, sodium hydroxide solution (1 in 25) 10 ml).

(2) Sulfate Not more than 0.010% as SO_4.

Sample Solution Weigh 1.0 g of Fumaric Acid, add 30 ml of water, and shake. Add 1 drop of phenolphthalein TS, then add ammonia TS dropwise until the color of the solution changes to a slightly pink color.

Control Solution 0.20 ml of 0.005 mol/L sulfuric acid.

(3) Heavy metals Not more than 10 μg/g as Pb.

Test Solution Weigh 2.0 g of Fumaric Acid, add 30 ml of water, shake, and add 1 drop of phenolphthalein TS. Then add, dropwise, ammonia TS until the color of the solution changes to a slightly pink color. Add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution To 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(4) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.50 g of Fumaric Acid, add 10 ml of water, dissolve by heating, and cool.

Apparatus Use Apparatus B.

Procedure Perform the test using 10 ml of acidic stannous chloride TS and 3 g of arsenic-free zinc.

Residue on Ignition Not more than 0.05% (5 g).

Assay Weigh accurately about 1 g of Fumaric Acid, and dissolve it in water to make exactly 250 ml. Measure exactly 25 ml of this solution, and titrate with 0.1 mol/L sodium hydroxide (indicator: 2 drops of phenolphthalein TS).

Each ml of 0.1 mol/L sodium hydroxide = 5.804 mg of $C_4H_4O_4$

Gardenia Blue

クチナシ青色素

Definition Gardenia Blue is obtained from the fruits of *Gardenia augusta* Merrill or *Gardenia jasminoides* Ellis. It is produced by adding β-glucosidase to a mixture of iridoid glycosides from gardenia fruits and protein degradation products. It may contain dextrin or lactose.

Color Value The Color Value ($E^{10\%}_{1cm}$) of Gardenia Blue is not less than 50 and is in the range of 90–110% of the labeled value.

Description Gardenia Blue occurs as a dark purple to blue powder, lumps, paste, or liquid having a slight characteristic odor.

Identification

(1) Weigh the equivalent of 0.2 g of Gardenia Blue with a Color Value 50, and dissolve it in 100 ml of citrate buffer (pH 7.0). A blue to blue-purple color develops.

(2) A solution of Gardenia Blue in citrate buffer (pH 7.0) exhibits an absorption maximum at a wavelength of 570–610 nm.

(3) Weigh the equivalent of 0.2 g of Gardenia Blue with a Color Value 50, and add water to make 100 ml. To 5 ml of this solution, add 1 to 2 drops of hydrochloric acid, and then add 1 to 3 drops of sodium hypochlorite TS. The solution is immediately decolorized.

(4) Weigh the equivalent of 0.2 g of Gardenia Blue with a Color Value 50, and add water to make 100 ml. To 5 ml of this solution, add 5 ml of sodium hydroxide solution (1 in 25), and heat at 40–43°C for 20 minutes. No definite color change is observed.

Purity

(1) Heavy metals Not more than 40 μg/g as Pb (0.50 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 8.0 μg/g as Pb (1.25 g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50g, Method 3, Apparatus B).

(4) Methanol Not more than 0.10% (on the basis of a Color Value 50).

Test Solution Weigh exactly the equivalent of 1.00 g of Gardenia Blue with a Color Value 50, into a 10-ml volumetric flask, dissolve it in water, and add exactly 2 ml of the internal standard solution and water to volume. Use this solution as the sample solution. Prepare a 500-mg graphite carbon cartridge by pouring 4 ml of ethanol and 10 ml of water and discarding the effluent. To the cartridge, pour exactly 1 ml of the sample solution, and collect the effluent in a 5-ml volumetric flask. Pour water into the cartridge at a flow rate that does not allow the blue color to elute until the total volume of effluent reaches 5 ml.

Control Solution Weigh 0.50 g of methanol into a 100-ml volumetric flask, and add water to volume. Measure exactly 10 ml of this solution into a 100-ml volumetric flask, and add water to make 100 ml. Then measure exactly 2 ml of the second solution into a 50-ml volumetric flask, add exactly 2 ml of the internal standard solution, and add water to volume.

Internal Standard Solution Weigh 0.50 g of 2-propanol into a 100-ml volumetric flask, and add water to volume. Measure exactly 10 ml of this solution into a 100-ml volumetric flask, and add water to volume.

Procedure Analyze 2.0 μl portions of the test solution and the control solution by gas chromatography using the operating conditions given below. The peak area ratio of methanol to 2-propanol for the test solution is not larger than that for the control solution.

Operating Conditions

Detector: Ionization detector.

Column: A glass or stainless steel tube of 3–4 mm internal diameter and 1–2 m length.

Column packing material: 180- to 250-μm styrene-divinylbenzene porous polymer for gas chromatography.

Column temperature: A constant temperature at about 120°C.

Inlet temperature: 160–200°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust so that the retention time of methanol is 2–4 minutes.

Color Value Test Proceed as directed in the Color Value Test, according to the following operating conditions:

Operating Conditions

Solvent: Citrate buffer (pH 7.0).

Wavelength: Maximum absorption wavelength of 570–610 nm.

Gardenia Red

クチナシ赤色素

Definition Gardenia Red is obtained from the fruits of *Gardenia augusta* Merrill or *Gardenia jasminoides* Ellis. It is produced by adding β-glucosidase to a mixture of ester-hydrolysis products of iridoid glycosides from gardenia fruits and protein degradation products. It may contain dextrin or

lactose.

Color Value The color value ($E^{10\%}_{1cm}$) of Gardenia Red is not less than 50 and is in the range of 90–110% of the labeled value.

Description Gardenia Red occurs as a dark red-purple to red powder, lumps, paste, or liquid having a slight characteristic odor.

Identification

(1) Weigh the equivalent of 0.2 g of Gardenia Red with a Color Value 50, and dissolve it in 100 ml of acetate buffer (pH 4.0). A red to purple-red color develops.

(2) A solution of Gardenia Red in acetate buffer (pH 4.0) exhibits an absorption maximum at a wavelength of 520–545 nm.

(3) Weigh the equivalent of 0.2 g of Gardenia Red with a Color Value 50, and add water to make 100 ml. To 5 ml of this solution, add 1 to 2 drops of hydrochloric acid and 1 to 3 drops of sodium hypochlorite TS. The solution is immediately decolorized.

(4) Weigh the equivalent of 0.2 g of Gardenia Red with a Color Value 50, add water to make 100 ml, and use the solution obtained as the test solution. To 5 ml of the test solution, add 5 ml of sodium hydroxide solution (1 in 25) to make it alkaline. The solution may become turbid but no definite color change is observed. To 5 ml of the test solution, add 1 to 3 drops of hydrochloric acid. The solution may become turbid, but no definite color change is observed.

Purity

(1) Heavy metals Not more than 40 μg/g as Pb (0.50 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 8.0 μg/g as Pb (1.25 g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Color Value Test Proceed as directed in the Color Value Test, using the following operating conditions.

Operating Conditions

Solvent: Acetate buffer (pH 4.0).

Wavelength: Maximum absorption wavelength of 520–545 nm.

Gardenia Yellow

クチナシ黄色素

Definition Gardenia Yellow is obtained from the fruits of *Gardenia augusta* Merrill or *Gardenia jasminoides* Ellis and consists mainly of crocin and crocetin. It may contain dextrin or lactose.

Color Value The Color Value ($E^{10\%}_{1cm}$) of Gardenia Yellow is not less than 100 and is in the range of 90–120% of the labeled value.

Description Gardenia Yellow occurs as a yellow to dark red powder, lumps, paste, or liquid having a slight characteristic odor.

Identification

(1) Weigh the equivalent of 0.1 g of Gardenia Yellow with a Color Value 100, and add 100 ml of 0.02 mol/L sodium hydroxide. A yellow color develops.

(2) Weigh the equivalent of 0.1 g of Gardenia Yellow with a Color Value 100, and add 100 ml of 0.02 mol/L sodium hydroxide. Dissolve it with constant shaking while warming in a water bath at 50°C for 20 minutes. The resulting solution exhibits an absorption maximum at a wavelength of 410–425 nm.

(3) Weigh the equivalent of 0.1 g of Gardenia Yellow with a Color Value 100, and if necessary, evaporate to dryness on a water bath. Cool, and add 5 ml of sulfuric acid. A blue color develops, which changes through purple to brown.

(4) Weigh the equivalent of 1 g of Gardenia Yellow with a Color Value 100. Add 100 ml of 0.02 ml/L sodium hydroxide, warm in a water bath at 50°C for 20 minutes, and shake if necessary to dissolve. Use this solution as the test solution. Analyze a 5 μl portion of the test solution by thin-layer chromatography using an 8:7:7 mixture of tetrahydrofuran/acetonitrile/oxalic acid solution (1 in 80) as the developing solvent. No control solution is used. Use a thin-layer plate coated with octadecylsilanized silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the original line, air-dry the plate, and examine. A yellow spot is observed at an R_f value of about 0.4–0.6.

Purity

(1) Heavy metals Not more than 40 μg/g as Pb (0.50 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 8.0 μg/g as Pb (1.25 g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50g, Method 3, Apparatus B).

(4) Geniposide Not more than 0.5% (on the basis of a Color Value 100).

Test Solution Weigh the equivalent of 1.0 g of Gardenia Yellow with a Color Value 100, and add a 17:3 mixture of water/acetonitrile to make exactly 25 ml. Centrifuge if necessary, and use the supernatant as the test solution.

Standard Solutions Weigh accurately about 0.01 g of geniposide for assay, previously dried for 24 hours in a deccicator, and dissolve it in a 17:3 mixture of water/acetonitrile to make exactly 100 ml. Transfer exactly 1 ml, 5 ml, and 10 ml of this solution into separate 100-ml volumetric flasks, and dilute each with a 17:3 mixture of water/acetonitrile to volume.

Procedure Analyze 10 μl portions of the test solution and the standard solutions by liquid chromatography using the operating conditions given below. Measure the peak areas of geniposide for the standard solutions, and prepare a calibration curve. Determine the concentration (μg/ml) of geniposide in the test solution from the calibration curve and the peak area of geniposide for the test solution. Calculate the content of geniposide by the formula:

Content (%) of geniposide in terms of Color Value 100
= concentration (μg/ml) of geniposide × 0.0025

Operating Conditions

Detector: Ultraviolet spectrophotometer (measurement wavelength: 238 nm).

Column: A stainless steel tube of 4–5 mm internal diameter and 15–30 cm length.

Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 40°C.

Mobile phase: A 17:3 mixture of water/acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of geniposide is about 15 minutes.

Color Value Test Weigh accurately the equivalent of about 5 g of Gardenia Yellow with a Color Value 100, add 50 ml of 0.02 mol/L sodium hydroxide, and warm in a water bath at 50°C for 20 minutes to dissolve while shaking if necessary. To this solution, add water to make exactly 100 ml. Measure exactly 1 ml of this solution, add 50% (vol) ethanol to make exactly 100 ml, and use this as the test solution. If necessary, centrifuge the test solution, and use the supernatant for the test. Measure the absorbance (A) in a 1-cm cell at a maximum absorption wavelength of 410–425 nm. Use 50% (vol) ethanol as the reference solution. Calculate the a Color Value by the formula:

$$\text{Color Value} = \frac{\text{A} \times 1{,}000}{\text{Weight (g) of the sample}}$$

Gellan Gum

ジェランガム

[71010-52-1]

Definition Gellan Gum is obtained from the culture fluid of *Sphingomonas elodea* and consists mainly of polysaccharides.

Content Gellan Gum, when dried, contains 85.0–108.0 % of gellan gum .

Description Gellan Gum occurs as a white to brownish powder having a slight, particular odor.

Identification

(1) When dissolved in water, Gellan Gum forms a viscous liquid.

(2) To 1 g of Gellan Gum, add 100 ml of water, and stir for 2 hours. Pipet a small amount of the solution into 10% calcium chloride solution. Immediately a linear gel is formed.

(3) To 90 ml of the solution obtained in Identification (2), add 0.50 g of sodium chloride. Heat the solution to 80°C while stirring, and allow for 1 minutes. Cool to room temperature without stir. A gel is formed.

Purity

(1) Total Nitrogen Not more than 3%.

Weigh accurately about 1 g of Gellan Gum, and proceed as directed in the Kjeldahl Method under Nitrogen Determination.

(2) Lead Not more than 2.0 μg/g as Pb (5.0 g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(4) 2-Propanol Not more than 0.075%.

(i) Apparatus Use the apparatus shown in Purity (9) for Processed Eucheuma Algae.

(ii) Method

Test Solution Weigh accurately about 2 g of Gellan Gum in eggplant-shaped flask A, add 200 ml of water, a few boiling chips, and about 1 ml of silicon resin, and stir well. Place exactly 4 ml of the internal standard solution in volumetric flack E, and moisten the joint parts with water, and set the apparatus. Distill it at a rate of 2 to 3 ml/minute, controlling the heat so that bubbles do not come in delivery tube C, and collect about 90 ml of distillate. To the distillate, add water to make exactly 100 ml. Use *tert*-butanol solution (1 in 1,000) as the internal standard solution.

Standard Solution Weigh accurately about 0.5 g of 2-propanol, and add water to make exactly 50 ml. Measure exactly 5 ml of this solution, and add water to make exactly 50 ml. Then measure exactly 3 ml of the second solution and 8 ml of the internal standard solution in a volumetric flask, and add water to make exactly 200 ml.

Procedure Analyze 2.0 μl portions of the test solution and the standard solution by gas chromatography using the operating conditions below. Obtain the peak area ratios (Q_T and Q_S) of 2-propanol to *tert*-butanol for the test solution and the standard solution, and calculate the content by the formula:

$$\text{Content of 2-propanol (\%)} = \frac{\text{Weight (g) of 2-propanol}}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 0.3$$

Operating Conditions

Detector: Flame-ionization detector.

Column: A glass tube of 3 mm internal diameter and 2 m length.

Column packing material: 180- to 250-μm styrene-divinylbenzene porous polymer for gas chromatography.

Column temperature: A constant temperature at about 120°C.

Injection port: A constant temperature at about 200°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust so that the retention time of 2-propanol is about 10 minutes.

Loss on Drying Not more than 15.0% (105°C, 2.5 hours).

Ash Not more than 16.0% (on the dried basis).

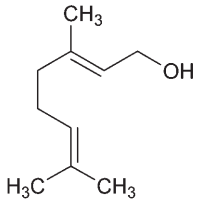
Microbial Limits Proceed as directed in the Microbial Limit Tests. The total bacterial count is not more than 10,000/g, and *Escherichia coli* is negative.

Assay Weigh about 1.0 g of diatomaceous earth for chromatography into a glass filter (1G3), and spread uniformly. Dry the glass with the diatomaceous earth at 105°C for 5 hours, allow to cool in a desiccator, and weigh accurately. Weigh accurately about 0.2 g of dried Gellan Gum, add 50 ml of water, and dissolve while stirring in a water bath. Add 200 ml of 2-propanol, previously warmed at 60–70°C, mix well, and allow to stand over night. Wash down the produced precipitate with 78% (vol) 2-propanol into the glass filter, and filter. Wash the residue 3 times with 20 ml of 78% (vol) 2-peopanol each time, and twice with 10 ml of 78% (vol) 2-propanol each time. Dry the glass filter with residue at 105°C over night, and weigh accurately. Calculate the content by the following formula:

$$\text{Content (\%) of gellan gum} = \frac{\text{Weight (g) of the residue}}{\text{Weight (g) of the sample}} \times 100$$

Geraniol

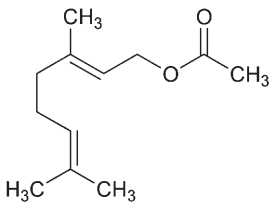
ゲラニオール



$C_{10}H_{18}O$ Mol. Wt. 154.25
(2*E*)-3,7-Dimethylocta-2,6-dien-1-ol [106-24-1]
Content Geraniol contains not less than 85.0% of geraniol ($C_{10}H_{18}O$).
Description Geraniol is a colorless to light yellow, transparent liquid having a characteristic odor.
Identification To 1 ml of Geraniol, add 1 ml of acetic anhydride and 1 drop of phosphoric acid, keep lukewarm for 10 minutes, add 1 ml of water, and shake in warm water for 5 minutes. Cool, and make slightly alkaline with anhydrous sodium carbonate solution (1 in 8). An odor of geranyl acetate is evolved.
Purity
(1) Refractive index n_D^{20}: 1.469–1.478.
(2) Specific gravity 0.870–0.885.
(3) Clarity of solution Clear (1.0 ml, 70% (vol) ethanol 3.0 ml).
(4) Acid value Not more than 1.0 (Flavoring Substances Tests).
(5) Ester value Not more than 3.0 (5.0 g, Flavoring Substances Tests).
(6) Aldehyde Weigh accurately about 5 g of Geraniol, and proceed as directed in Method 2 in the Aldehyde and Ketone Content Test in the Flavoring Substances Tests. In the test, allow the mixture to stand for 15 minutes before titrating. The volume of consumed 0.5 mol/L hydrochloric acid is not more than 0.65 ml.
Assay Proceed as directed in Method 1 in the Alcohol Content Test in the Flavoring Substances Tests, using 1 g of acetylated oil.

Geranyl Acetate

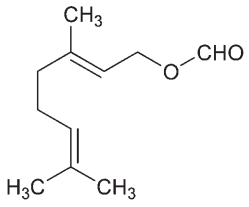
酢酸ゲラニル



$C_{12}H_{20}O_2$ Mol. Wt. 196.29
(2*E*)-3,7-Dimethylocta-2,6-dien-1-yl acetate [105-87-3]
Content Geranyl Acetate contains not less than 90.0% of geranyl acetate ($C_{12}H_{20}O_2$).
Description Geranyl Acetate is a colorless to light yellow, transparent liquid having a characteristic odor.
Identification To 1 ml of Geranyl Acetate, add 5 ml of ethanolic 10% potassium hydroxide TS, and heat in a water bath. The characteristic odor disappears, and an odor of geranyl is evolved. After cooling, add 2 ml of diluted hydrochloric acid (1 in 4) and 2 ml of water. The solution responds to test (3) for Acetate in the Qualitative Tests.
Purity
(1) Refractive index n_D^{20}: 1.457–1.464.
(2) Specific gravity 0.903–0.917.
(3) Clarity of solution Clear (1.0 ml, 80% (vol) ethanol 4.0 ml).
(4) Acid value Not more than 1.0 (Flavoring Substances Tests).
Assay Weigh accurately about 1 g of Geranyl Acetate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.
Each ml of 0.5 mol/L ethanolic potassium hydroxide = 98.14 mg of $C_{12}H_{20}O_2$.

Geranyl Formate

ギ酸ゲラニル



$C_{11}H_{18}O_2$ Mol. Wt. 182.26
(2*E*)-3,7-Dimethylocta-2,6-dien-l-yl formate [105-86-2]
Content Geranyl Formate contains not less than 85.0% of geranyl formate ($C_{11}H_{18}O_2$).
Description Geranyl Formate is a colorless or slightly yellowish, transparent liquid having a characteristic odor.

Identification

(1) To 1 ml of Geranyl Formate, add 10 ml of ethanolic 10% potassium hydroxide TS, and heat in a water bath for 5 minutes while shaking. The characteristic odor disappears, and an odor of geraniol is evolved.

(2) To 1 ml of Geranyl Formate, add 10 ml of sodium hydroxide solution (1 in 25), heat in a water bath for 5 minutes while shaking, and allow to stand. To 1 ml of an aqueous solution of the lower layer, add 1.5 ml of diluted hydrochloric acid (1 in 4), and add 0.02 g of magnesium dust in several divided portions. After effervescence ceases, add 3 ml of diluted sulfuric acid (3 in 5) and 0.010 g of chromotropic acid, shake, and warm in a warm water for 10 minutes. A pink-purple color develops.

Purity

(1) Refractive index n_D^{20}: 1.457–1.466.

(2) Specific gravity 0.909–0.917.

(3) Clarity of solution Clear (1.0 ml, 80% (vol) ethanol 3.0 ml).

(4) Acid value Not more than 1.0 (Flavoring Substances Tests).

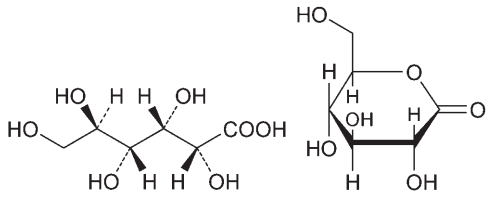
In the test, titrate while cooling in ice water, and continue the titration until a light pink color persists for 10 seconds.

Assay Weigh accurately about 1 g of Geranyl Formate, and perform the tests as directed in the Saponification Value Test and the Acid Value Test, respectively, in the Flavoring Substances Tests. Calculate the content by the formula:

$$\text{Content (\%) of geranyl formate } (C_{11}H_{18}O_2) = \frac{\text{Saponification value} - \text{Acid value}}{561.1} \times 182.3$$

Gluconic Acid

グルコン酸



[526-95-4]

Definition Gluconic Acid is an aqueous solution of gluconic acid and glucono-δ-lactone.

Content Gluconic Acid contains the equivalent of 50.0–52.0% gluconic acid ($C_6H_{12}O_7$ =196.16).

Description Gluconic Acid is a colorless to light yellow, clear syrupy liquid. It is odorless or has a slight odor, and has a sour taste.

Identification

(1) To 1 ml of a solution of Gluconic Acid (1 in 25), add 1 drop of iron(III) chloride solution (1 in 10). A deep yellow color develops.

(2) To 1 ml of Gluconic Acid, add 4 ml of water, and proceed as directed in Identification (2) for Glucono-δ-Lactone.

Purity

(1) Chloride Not more than 0.035% as Cl (0.50 g, Control solution 0.01 mol/L hydrochloric acid 0.50 ml).

(2) Sulfate Not more than 0.024% as SO_4 (1.0 g, Control solution 0.005 mol/L sulfuric acid 0.50 ml).

(3) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of Gluconic Acid, dissolve it in 30 ml of water, add 1 drop of phenolphthalein TS, and add ammonia TS dropwise until a slight pink color develops. Add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

(5) Sucrose or reducing sugars Weigh 1.0 g of Gluconic Acid, and proceed as directed in Purity (6) for Glucono-δ-Lactone.

Residue on Ignition Not more than 0.10% (5 g).

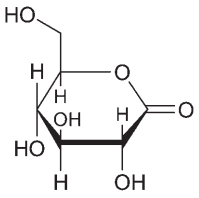
Assay Weigh accurately about 1 g of Gluconic Acid, add 30 ml of water and exactly 40 ml of 0.1 mol/L sodium hydroxide shake, and allow to stand for 20 minutes. Titrate the excess alkali with 0.05 mol/L sulfuric acid (indicator: 3 drops of phenolphthalein TS). Perform a blank test in the same manner.

Each ml of 0.1 mol/L sodium hydroxide = 19.62 mg of $C_6H_{12}O_7$

Glucono-δ-Lactone

Gluconolactone

グルコノデルタラクトン



$C_6H_{10}O_6$ Mol. Wt. 178.14

D-Glucono-1,5-lactone [90-80-2]

Content Glucono-δ-Lactone, when dried, contains not less than 99.0% of glucono-δ-lactone ($C_6H_{10}O_6$).

Description Glucono-δ-Lactone occurs as white crystals or crystalline powder. It is odorless or has a slight odor. It has a sweet taste at first and changes to a slight acid taste.

Identification

(1) To 1 ml of a solution of Glucono-δ-Lactone (1 in 50), add 1 drop of iron(III) chloride solution (1 in 10). A deep yellow color develops.

(2) To 5 ml of a solution of Glucono-δ-Lactone (1 in 10), add 0.7 ml of acetic acid and 1 ml of freshly distilled phenylhydrazine, and heat on a water bath for 30 minutes. After cooling, rub the inner wall with a glass rod. Crystals are deposited. Collect the crystals by filtration, dissolve them in 10 ml of boiling water, add a small amount of active carbon, and filter. After cool-

ing, rub the inner wall with a glass rod, and dry the deposited crystals. The melting point is 192–202°C (decomposition).

Purity

(1) Clarity and color of solution Colorless and almost clear (1.0 g, water 10 ml).

(2) Chloride Not more than 0.035% as Cl (0.50 g, Control solution 0.01 mol/L hydrochloric acid 0.50 ml).

(3) Sulfate Not more than 0.024% as SO_4 (1.0 g, Control solution 0.005 mol/L sulfuric acid 0.50 ml).

(4) Heavy metals Not more than 20 µg/g as Pb.

Test Solution Weigh 1.0 g of Glucono-δ-Lactone, dissolve it in 30 ml of water, add 1 drop of phenolphthalein TS, and add ammonia TS dropwise until a slight pink color develops. Add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(5) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

(6) Sucrose or reducing sugars Weigh 0.50 g of Glucono-δ-Lactone, add 10 ml of water and 2 ml of diluted hydrochloric acid (1 in 4), boil for 2 minutes. Cool, add 5 ml of anhydrous sodium carbonate solution (1 in 8), allow to stand for 5 minutes, and add water to make 20 ml. Measure 5 ml of this solution, add 2 ml of Fehling's TS, and boil for 1 minute. An orange–yellow to red precipitate is not formed immediately.

Loss on Drying Not more than 1.0% (105°C, 2 hours).

Residue on Ignition Not more than 0.10%.

Assay Weigh accurately about 0.3 g of Glucono-δ-Lactone, previously dried, dissolve it in exactly 30 ml of 0.1 mol/L sodium hydroxide allow to stand for 20 minutes, and titrate the excess alkali with 0.05 mol/L sulfuric acid (indicator: 3 drops of phenolphthalein TS). Perform a blank test in the same manner.

Each ml of 0.1 mol/L sodium hydroxide = 17.81 mg of $C_6H_{10}O_6$

α-Glucosyltransferase Treated Stevia

α-グルコシルトランスフェラーゼ処理ステビア

Definition α-Glucosyltransferase Treated Stevia is obtained by glucosylating "Stevia Extract" with α-glucosyltransferase. It consists mainly of α-glucosylstevioside.

Content α-Glucosyltransferase Treated Stevia, when calculated on the dried basis, contains not less than 80.0% of the sum of α-glucosyl steviol glycosides and unreacted steviol glycosides (stevioside, dulcoside A, rebaudioside A, and rebaudioside C), and not less than 65.0% of α-glucosyl steviol glycosides alone.

Description α-Glucosyltransferase Treated Stevia occurs as a white to light yellow powder, flakes, or granules. It is odorless or has a slight characteristic odor. It has a strong sweet taste.

Identification

(1) Dissolve 0.1 g of α-Glucosyltransferase Treated Stevia in 20 ml of water, and use this solution as the test solution. Analyze 10 µl portions of the test solution and the standard solutions by liquid chromatography using the operating conditions specified in the Assay for Stevia Extract in Monographs. More than one peak is observed later than the retention time of either stevioside or rebaudioside A. The standard solutions should be prepared by dissolving 5 mg each of stevioside for assay and rebaudioside A in 10 ml of water.

(2) To the rest of the test solution used in Identification (1), add 20,000 units of glucoamylase, allow to stand at 55°C for about 45 minutes, and cool to room temperature. Analyze 10 µl of the resulting solution by liquid chromatography using the operating conditions specified in the Assay for Stevia Extract in Monographs. The total area of the plural peaks later than the retention time of either stevioside or rebaudioside A is less than that observed in Identification (1), and at least one of the peak areas of stevioside and rebaudioside A is larger than that observed in identification (1).

Purity

(1) Heavy metals Not more than 10 µg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Arsenic Not more than 2.0 µg/g as As_2O_3 (1.0 g, Method 3, Apparatus B).

Loss on Drying Not more than 6.0% (105°C, 2 hours).

Residue on Ignition Not more than 1.0%.

Assay

(1) Total content of α-glucosyl steviol glycosides and unreacted steviol glycosides

The total content of α-glucosyl steviol glycosides and unreacted steviol glycosides is obtained as the sum of the contents of steviol glycosides and α-glucosyl residues.

Steviol glycosides

Test Solution Weigh accurately about 1 g of α-Glucosyltransferase Treated Stevia, and dissolve it in 50 ml of water. Pour this solution into a glass tube (25 mm internal diameter) packed with 50 ml of acrylic acid ester resin or styrene-divinylbenzene resin, allow it to flow through at a rate of less than 3 ml/minute, and wash the resin with 250 ml of water. Then pour 250 ml of 50% (vol) ethanol to allow it to flow through at a rate of 3 ml/minute or less. Evaporate the collected eluate to about 100 ml, and add exactly 40 ml of acetate buffer (pH 4.5) and water to make about 180 ml. Allow this solution to stand at 55°C for about 5 minutes, add 20,000 units of glucoamylase, and allow to stand at 55°C for about 45 minutes. Heat at 95°C for about 30 minutes, cool to room temperature, and add water to make exactly 200 ml.

Stevioside Standard Solution Weigh accurately about 0.1 g of stevioside for assay, previously dried, and dissolve it in water to make exactly 200 ml.

Procedure Analyze 10 µl each of the test solution and the standard solution in accordance with the procedure specified in the Assay for Stevia Extract in the Monographs to determine the content of steviol glycosides.

α-Glucosyl residues

Test Solution Use the test solution prepared for the determination of steviol glycosides.

Blank Test Solution Measure exactly 40 ml of acetate buffer (pH 4.5), add water to make about 180 ml, and allow to stand at 55°C for about 5 minutes. To this solution, add 20,000 units of glucoamylase, and allow to stand at 55°C for about 45 minutes. Next, heat the solution at 95°C for about 30 minutes, cool to room temperature, and add water to make exactly 200 ml.

Standard Solutions Weigh accurately about 1g of glucose, and dissolve it in water to make exactly 100 ml. Transfer exactly 5 ml, 10 ml, 20 ml, and 30 ml of this solution into

separate 100-ml volumetric flasks, and dilute each with water to volume.

Procedure Measure 20 µl of the test solution, add exactly 3 ml of color fixing TS for D-glucose determination, and shake. Allow to stand at 37°C for exactly 5 minutes, and cool to room temperature. Measure the absorbance of the resulting solution at a wavelength of 505 nm against the reference solution prepared as follows: Measure 20 ml of water instead of the test solution, and proceed as directed for the test solution. Perform a blank test by measuring the absorbance of the blank test solution in the same manner as for the test solution, and make any necessary correction. Prepare a calibration curve by measuring the absorbances of the standard solutions in the same manner as for the test solution.

Determine the concentration of D-glucose in the test solution from the calibration curve and the corrected absorbance of the test solution, and calculate the α-glucosyl residue content by the formula:

$$\text{Content (\%) of α-glucosyl residues content} = \frac{\left(\begin{array}{c}\text{Concentration (mg/ml) of}\\ \text{D-glucose in the test solution}\end{array}\right) \times 200}{\text{Dry basis weight (g) of the sample} \times 1{,}000 \times 0.900 \times 100}$$

Calculate the total content of α-glucosyl steviol glycosides and unreacted steviol glycosides from the contents of steviol glycosides and α-glucosyl residues.

Total content (%) of α-glucosyl steviol glycosides and unreacted steviol glycosides
= Content (%) of steviol glycosides
+ Content (%) of α-glucosyl residues

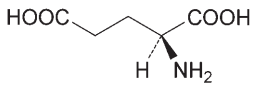
(2) Content of α-glucosyl steviol glycosides

Weigh accurately about 1 g of α-Glucosyltransferase Treated Stevia, and dissolve in water to make exactly 200 ml. Use this solution as the test solution. Measure 10 µl each of the test solution and the stevioside standard solution prepared in section (1), and proceed as directed in the Assay for Stevia Extract in the Monographs to determine the content of steviol glycosides. Consider the content determined to be the content of unreacted steviol glycosides. Finally, calculate the content of α-glucosyl steviol glycosides by the formula:

Content (%) of α-glucosyl steviol glycosides
= Content (%) of steviol glycosides
+ Content (%) of α-glucosyl residues
− Content (%) of unreacted steviol glycosides

L-Glutamic Acid

L-グルタミン酸



$C_5H_9NO_4$ Mol. Wt. 147.13

(2*S*)-2-Aminopentanedioic acid [56-86-0]

Content L-Glutamic Acid, when calculated on the dried basis, contains not less than 99.0% of L-glutamic acid ($C_5H_9NO_4$).

Description L-Glutamic Acid occurs as colorless to white crystals or as a white crystalline powder having a slight, characteristic and acid taste.

Identification To 5 ml of a solution of L-Glutamic Acid (1 in 1,000), add 1 ml of ninhydrin solution (1 in 1,000), and heat for 3 minutes. A purple color develops.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +31.5 to +32.5° (10 g, diluted hydrochloric acid (1 in 6), 100 ml, on the dried basis).

(2) Clarity and color of solution Colorless and clear.

Test Solution Weigh 0.50 g of L-Glutamic Acid, add 50 ml of water, and dissolve by warming.

(3) pH 3.0–3.5 (saturated solution).

(4) Chloride Not more than 0.021% as Cl (0.50 g, Control solution 0.01 mol/L hydrochloric acid 0.30 ml).

(5) Heavy metals Not more than 10 µg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(6) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 2, Apparatus B).

Loss on Drying Not more than 0.20% (105°C, 3 hours).

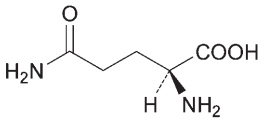
Residue on Ignition Not more than 0.20%.

Assay Weigh accurately about 0.2 g of L-Glutamic Acid, dissolve it in 6 ml of formic acid, and proceed as directed in the Assay for DL-Alanine.

Each ml of 0.1 mol/L perchloric acid = 14.71 mg of $C_5H_9NO_4$

L-Glutamine

L-グルタミン



$C_5H_{10}N_2O_3$ Mol. Wt. 146.14

(2*S*)-2-Amino-4-carbamoylbutanoic acid [56-85-9]

Content L-Glutamine, when calculated on the dried basis, contains 98.0–102.0% of L-glutamine ($C_5H_{10}N_2O_3$).

Description L-Glutamine occurs as white crystals or crystalline powder. It is odorless, and has a very slight character-

istic taste.

Identification

(1) To 5 ml of a solution of L-Glutamine (1 in 1,000), add 1 ml of ninhydrin solution (1 in 50), and heat for 3 minutes in a water bath. A purple color develops.

(2) Proceed as directed in Identification (2) for L-Asparagine.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +6.3 to +7.3°.

Weigh accurately about 4 g of L-Glutamine, add water, and dissolve by warming. Cool rapidly, and add water to make exactly 100 ml. Measure the angular rotation of this solution, and calculate on the dried basis.

(2) Clarity and color of solution Colorless and clear (1.0 g, water 50 ml).

(3) pH 4.5–6.0 (1.0 g, water 50 ml).

(4) Chloride Not more than 0.1% as Cl (0.07 g, Control solution 0.01 mol/L hydrochloric acid 0.20 ml).

(5) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Drying Not more than 0.30% (105°C, 3 hours).

Residue on Ignition Not more than 0.10%.

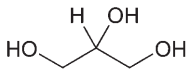
Assay Weigh accurately about 0.3 g of L-Glutamine, and proceed as directed in the Assay for L-Asparagine.

Each ml of 0.1 mol/L perchloric acid = 14.61 mg $C_5H_{10}N_2O_3$

Glycerol

Glycerin

グリセリン



$C_3H_8O_3$ Mol. Wt. 92.09

Propane-1,2,3-triol [56-81-5]

Content Glycerol contains not less than 95.0% of glycerol ($C_3H_8O_3$).

Description Glycerol is a colorless, viscous liquid. It is odorless and has a sweet taste.

Identification To 2–3 drops of Glycerol, add 0.5 g of potassium hydrogen sulfate, and heat. An acrolein–like odor is evolved.

Purity

(1) Specific gravity 1.250–1.264.

(2) Heavy metals Not more than 5.0 μg/g as Pb (5.0 g, Method 1, Control solution Lead Standard Solution 2.5 ml).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 10 g of Glycerol, and add water to make 100 ml. Use 5 ml of this solution as the test solution.

Apparatus Use Apparatus B.

(4) Chlorinated compounds Not more than 0.003% as Cl.

Test Solution Weigh 5.0 g of Glycerol, transfer into a flask equipped with a reflux condenser, add 15 ml of morpholine, heat, and reflux gently for 3 hours. Cool, rinse the reflux condenser with 10 ml of water, add the rinses to the flask, and acidify the contents with nitric acid. Transfer this solution into a Nessler tube, add 0.5 ml of silver nitrate solution (1 in 50), and add water to make 50 ml.

Procedure The test solution is not more turbid than a control solution prepared as follows: Proceed as directed for the test solution, except for the heating and refluxing operation, using 0.40 ml of 0.01 mol/L hydrochloric acid instead of the sample Glycerol.

(5) Reducing substances Measure 3.0 ml of Glycerol, dissolve it in 5 ml of water, add 0.5 ml of ammonia TS, and warm in a water bath at 60°C for 5 minutes. No yellow color develops. Add 0.5 ml of silver nitrate solution (1 in 10), shake, and allow to stand in a dark place for 5 minutes. The solution is not more turbid than a control solution prepared in the same manner as the sample, using a solution of pyrogallol in glycerol (3 in 100,000) instead of Glycerol.

Residue on Ignition Not more than 0.01% (10 g).

Assay Weigh quickly and accurately about 0.5 g of Glycerol, and add water to make exactly 500 ml. Measure exactly 50 ml of this solution, add about 200 ml of water, and adjust the pH to 7.9±0.1 with diluted sulfuric acid (3 in 1,000) or sodium hydroxide solution (1 in 250). Add 50 ml of sodium periodate TS for glycerol, stir gently, cover with a watch glass, and allow to stand in a dark place for 30 minutes. Add 10 ml of a 1:1 mixture of water/ethylene glycol, shake, and allow to stand in a dark place for 20 minutes. Add 5 ml of sodium formate solution (1 in 15), and titrate with 0.1 mol/L sodium hydroxide until the pH becomes 7.9±0.2. Perform a blank test in the same manner. Use only freshly boiled and cooled water for the test.

Each ml of 0.1 mol/L sodium hydroxide = 9.209 mg of $C_3H_8O_3$

Glycerol Esters of Fatty Acids

グリセリン脂肪酸エステル

Definition Glycerol Esters of Fatty Acids are esters of fatty acids and glycerol or polyglycerol and their derivatives. They are categorized into several types: glycerol fatty acid ester, glycerol acetic acid fatty acid ester, glycerol lactic acid fatty acid ester, glycerol citric acid fatty acid ester, glycerol succinic acid fatty acid ester, glycerol diacetyl tartaric acid fatty acid ester, glycerol acetic acid ester, polyglycerol fatty acid ester, and polyglycerol condensed ricinoleic acid ester.

Description Glycerol Esters of Fatty Acids occur as a colorless to brown powder, flakes, or granules, as granular or waxy lumps, or as a semifluid or liquid. They are odorless or have a characteristic odor.

Identification

(1) To about 5 g of Glycerol Esters of Fatty Acids (1.5 g in case of glycerol acetic acid ester), add 50 ml of ethanolic potassium hydroxide TS, heat under a reflux condenser in a water bath for 1 hour, and evaporate the ethanol to almost dryness. Add 50 ml of diluted hydrochloric acid (1 in 10), shake well, separate the produced fatty acid by extracting three times with 40 ml of a 7:1 mixture of petroleum ether/methyl ethyl ketone each time. Stir the aqueous layer well,

add sodium hydroxide solution (1 in 9) until it is almost neutral, and concentrate under reduced pressure in a water bath to obtain residue. Use a solution of the residue in methanol (1 in 10) as test solution. Analyze a 5-μl portion of the test solution by thin-layer chromatography, using a 9:1 mixture of methanol/glycerol as the control solution and a 9:1 mixture of acetone/water as the developing solvent. Use a thin layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point 15 cm above the original line. Air-dry the plate, heat at 110°C for 10 minutes to remove the solvent, and cool. Spray with the thymol–sulfuric acid TS, and heat at 110°C for 20 minutes to develop the color. In the case of glycerol esters, a brown spot is observed at the position corresponding to the spot from the control solution; in the case of polyglycerol ester, a brown spot or a brown band-shaped spot is observed at a position corresponding to or below the spot from the control solution.

(2) This test applies to esters other than glycerol acetic acid ester. Combine the petroleum ether/methyl ethyl ketone layers obtained by separation in Identification (1), and evaporate the solvent. An oily substance or white to yellowish white solid remains. Add 5 ml of diethyl ether to 0.1 g of the residue, and shake. It dissolves.

(3) This test applies to esters other than glycerol fatty acid esters and polyglycerol esters.

Test Solution Dissolve 0.1 g of the residue obtained in Identification (1) in 2 ml of 0.005 mol/L sulfuric acid.

Standard Solutions Prepare standard solutions by separately dissolving the specified amounts of the substances given below in 2 ml of 0.005 mol/L sulfuric acid: 0.01 g of acetic acid for glycerol acetic acid fatty acid ester and glycerol acetic acid ester, 0.02 g of "Sodium Lactate" for glycerol lactic acid fatty acid ester, 0.01 g of citric acid for glycerol citric acid fatty acid ester, 0.01 g of "Succinic Acid" for glycerol succinic acid fatty acid ester, 0.01 g of acetic acid, and 0.01 g of tartaric acid for glycerol diacetyl tartaric acid fatty acid ester.

Procedure Analyze 20 μl portions of the test solution and the corresponding standard solution by liquid chromatography using the operating conditions given below. The peak of the test solution is observed at the same retention time as that of the standard solution.

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube of 8 mm internal diameter and 30 cm length.

Column packing material: Styrene-divinylbenzene strong cation-exchange resin

Column temperature: 60°C.

Mobile phase: 0.005 mol/L sulfuric acid.

Flow rate: 0.7 ml/minute.

(4) In the case of polyglycerol condensed ricinoleic acid ester, combine the petroleum ether/methyl ethyl ketone layers obtained by separation in Identification (1) above, wash this solution twice with 50 ml of water each time, and dehydrate with anhydrous sodium sulfate. Filter the dehydrated liquid and remove the solvent by warming under reduced pressure. Weigh accurately about 1 g of the residue, and proceed as directed in the Hydroxyl Value Test in the Fats and Related Substances Tests. Use about 0.5 g of the residue to measure the acid value. The hydroxyl value is 150–170.

Purity

(1) Acid value

Glycerol fatty acid ester: Not more than 6.0 (Fats and Related Substances Tests).

Glycerol acetic acid fatty acid ester: Not more than 6.0 (Fats and Related Substances Tests).

Glycerol lactic acid fatty acid ester: Not more than 6.0 (Fats and Related Substances Tests).

Glycerol acetic acid ester: Not more than 6.0 (Fats and Related Substances Tests).

Polyglycerol fatty acid ester: Not more than 12 (Fats and Related Substances Tests).

Polyglycerol condensed ricinoleic acid ester: Not more than 12 (Fats and Related Substances Tests).

Glycerol citric acid fatty acid ester: Not more than 100 (Fats and Related Substances Tests).

Glycerol succinic acid fatty acid ester: 60–120 (Fats and Related Substances Tests).

Glycerol diacetyl tartaric acid fatty acid ester: 60–120 (Fats and Related Substances Tests).

(2) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

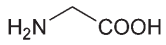
(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(4) Polyoxyethylene Weigh 1.0 g of Glycerol Esters of Fatty Acids, transfer into a 200-ml flask, add 25 ml of ethanolic potassium hydroxide TS, and boil under a ground-glass reflux condenser on a water bath for 1 hour while shaking. Evaporate the ethanol on a water bath or under reduced pressure until it becomes almost dry, add 20 ml of diluted sulfuric acid (3 in 100), and shake well while warming. Add 15 ml of ammonium thiocyanate–cobalt nitrate TS, shake well, add 10 ml of chloroform, shake again, and allow to stand. The color of the chloroform layer does not change to blue.

Residue on Ignition Not more than 1.5%.

Glycine

グリシン



$C_2H_5NO_2$ Mol. Wt. 75.07

Aminoacetic acid [56-40-6]

Content Glycine, when calculated on the dried basis, contains 98.5–101.5% of glycine ($C_2H_5NO_2$).

Description Glycine occurs as white crystals or crystalline powder having a sweet taste.

Identification

(1) To 5 ml of a solution of Glycine (1 in 1,000), add 1 ml of ninhydrin solution (1 in 1,000), and heat for 3 minutes. A purple color develops.

(2) To 5 ml of a solution of Glycine (1 in 10), add 5 drops of diluted hydrochloric acid (1 in 4) and 1 ml of freshly prepared sodium nitrite solution (1 in 10). A colorless gas is evolved. Transfer 5 drops of this solution into a small test tube, boil for a while, and evaporate to dryness on a water bath. After cooling, add 5–6 drops of chromotropic acid TS

to the residue, and heat in a water bath for 10 minutes. A deep purple color develops.

Purity

(1) Clarity and color of solution Colorless and clear (1.0 g, water 10 ml).

(2) pH 5.5–7.0 (1.0 g, water 20 ml).

(3) Chloride Not more than 0.021% as Cl (0.50 g, Control solution 0.01 mol/L hydrochloric acid 0.30 ml).

(4) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 4, Control solution Lead Standard Solution 2.0 ml).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Drying Not more than 0.30% (105°C, 3 hours).

Residue on Ignition Not more than 0.10%.

Assay Weigh accurately about 0.15 g of Glycine, and proceed as directed in the Assay for DL-Alanine.

Each ml of 0.1 mol/L perchloric acid = 7.507 mg of $C_2H_5NO_2$

Grape Skin Extract

Grape Skin Color

ブドウ果皮色素

Definition Grape Skin Extract is obtained from the skins of grapes, *Vitis labrusca* Linné or *Vitis vinifera* Linné, and consists mainly of anthocyanin. It may contain dextrin or lactose.

Color Value The Color Value ($E^{10\%}_{1cm}$) of Grape Skin Extract is not less than 50 and is in the range of 90–120% of the labeled value.

Description Grape Skin Extract occurs as a red to dark red powder, lumps, paste, or liquid having a slightly characteristic odor.

Identification

(1) Weigh the equivalent of 1 g of Grape Skin Extract with a Color Value 50, and dissolve it in 1,000 ml of citrate buffer (pH 3.0). A red to red-purple color develops.

(2) Add sodium hydroxide solution (1 in 25) to the solution obtained in Identification (1) to make alkaline. The solution turns dark green.

(3) A solution of Grape Skin Extract in citrate buffer (pH 3.0) exhibits an absorption maximum at a wavelength of 520–534 nm.

Purity

(1) Heavy metals Not more than 40 μg/g as Pb (0.50g, Method 2, Control solution Lead standard solution 2.0 ml).

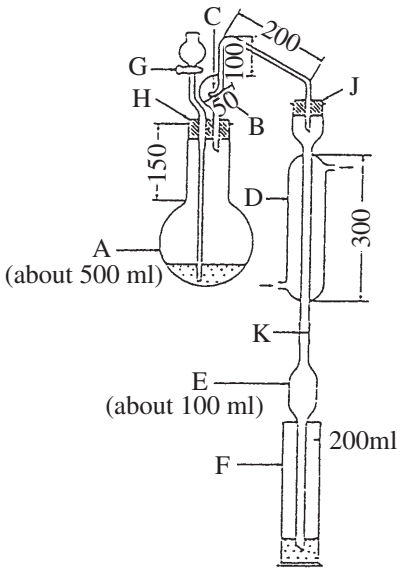
(2) Lead Not more than 10 μg/g as Pb (1.0g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50g, Method 3, Apparatus B).

(4) Sulfur dioxide Not more than 0.005% per Color Value.

(i) Apparatus Use the apparatus as illustrated in the right column. Hard glass-made apparatus should be used. Groundglass may be used for the joint parts.

(ii) Procedure Weigh accurately 1–3 g of Grape Skin Extract, transfer into 500 ml-distillation flask A with the Wagner tube, add 100 ml of water, and connect with distillation apparatus. Place 25 ml of lead acetate solution (1 in 50) in the receiver (measuring cylinder F) as an absorbing solution.



(Unit: mm)

A: Distilling flask
B: Wagner tube with spray trap
C: Small hole
D: Condenser
E: Trap
F: Measuring cylinder
G: Funnel with stopcock
H: Silicon rubber stopper
J: Silicon rubber stopper
K: Silicon rubber tube

In the absorbing solution, immerse the lower end of trap E attached to the condenser. Add 25 ml of phosphoric acid solution (2 in 7) from a funnel fitted with a stopcock, and distil until the liquid in the receiver reaches 100 ml. Take the lower end of the condenser out of the liquid, rinse the end with a little amount of water into receiver F. To the solution in the receiver add 5 ml of hydrochloric acid, and titrate with 0.005 mol/L iodine (indicator: starch TS).

Each ml of 0.005 mol/L iodine = 0.3203 mg of SO_2.

Color Value Test Proceed as directed in the Color Value Test, using the conditions below.

Operating Conditions

Solvent: Citrate buffer (pH 3.0).

Wavelength: Maximum absorption wavelength of 520–534 nm.

Guar Gum

グァーガム

Definition Guar Gum is obtained from the seeds of the guar plant *Cyamopsis tetragonolobus* Taubert and consists mainly of polysaccharides. It may contain sucrose, glucose, lactose, or dextrin.

Description Guar Gum occurs as a white to slightly yellow-

brown powder or granules. It has slight or no odor.

Identification

(1) Proceed as directed in Identification (1) for Carob Bean Gum, a viscous solution is formed. Heat 100 ml of this solution on the water bath for about 10 min., and cool to room temperature, the viscosity of the solution hardly changes after heating.

(2) Proceed as directed in Identification (2) for Carob Bean Gum.

Purity

(1) Protein Not more than 7.0%.

Weigh accurately about 0.15 g of Guar Gum, proceed as directed in the Semi-micro Kjeldahl Method under Nitrogen Determination.

Each ml of 0.005 mol/L sulfuric acid = 0.8754 mg of protein

(2) Acid-insoluble substances Not more than 7.0%.

Proceed as directed in Purity (5) for Processed Eucheuma Algae.

(3) Lead Not more than 2.0 μg/g as Pb (5.0 g, Method 1).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(5) Starch Proceed as directed in Purity (5) for Carob Bean Gum.

(6) 2-Propanol Not more than 1.0%.

Proceed as directed in Purity test (6) for Carob Bean Gum.

Loss on Drying Not more than 14.0% (105ºC, 5 hours).

Ash Not more than 1.5% (800ºC, 3–4 hours).

Microbial Limits Proceed as directed in the Microbial Limit Tests. The total bacterial count is not more than 10,000/g, and *Escherichia coli* is negative.

Gum Arabic

Arabic Gum
Acacia Gum

アラビアガム

Definition Gum Arabic is obtained by drying the exudate of *Acacia senegal* Willdenow or *Acacia seyal* Delile or by desalinating the dried exudate. It mainly consists of polysaccharides.

Description Gum Arabic occurs as a white to light yellow powder or granules or as light yellow to brown lumps. It is odorless.

Identification

(1) To 1 g of Gum Arabic, previously powdered, add 2 ml of water. It almost dissolves, and the solution is acidic.

(2) To 10 ml of a solution of Gum Arabic (1 in 50), add 0.2 ml of diluted basic lead acetate TS (1 in 50). A white fibrous precipitate is formed immediately.

(3) Dissolve 5 g of Gum Arabic in 100 ml of water. If it is turbid, filter with suction through a 0.45-μm membrane filter or centrifuge to remove the contaminants. Measure the specific rotation of the resulting liquid. Solutions from *Acacia senegal* are levorotatory, and solutions from *Acacia seyal* are dextrorotatory.

Purity

(1) Hydrochloric acid-insoluble substances Not more than 1.0%.

Dry a glass filter (1G3) for 30 minutes at 110°C, cool in a desiccator, and weigh the glass filter accurately. Weigh accurately 5 g of a powder of Gum Arabic, dissolve it in about 100 ml of water, add 10 ml of diluted hydrochloric acid (1 in 4), heat gradually and boil for 15 minutes. Filter the solution while warming, using the glass filter described above under the reduced pressure. Wash the precipitate well by warm water, dry 2 hours at 105°C together with the glass filter. Cool in a desiccator, weigh accurately.

(2) Lead Not more than 2.0 μg/g as Pb(5.0 g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(4) Tannin-bearing gums To 10 ml of a solution of Gum Arabic (1 in 50), add 3 drops of iron(III) chloride solution (1 in 10). No dark green color develops.

(5) Starch or Dextrin To 0.2 g of Gum Arabic , add 10 ml of water, and boil. Cool, and add 1 drop of Iodine TS. No blue or red-purple color develops.

Loss on Drying Not more than 17.0% (105°C, 6 hours).

Ash Not more than 4.0%.

Acid-insoluble Ash Not more than 0.50%.

Microbial Limits Proceed as directed in the Microbial Limit Tests. The total bacterial count is not more than 10,000/g, and *Escherichia coli* is negative.

Gum Ghatti

ガティガム

[9000-28-6]

Definition Gum Ghatti is obtained from the exudate of the ghatti tree *Anogeissus latifolia* Wallich and consists mainly of polysaccharides.

Description Gum Ghatti occurs as a gray to reddish-gray powder or granules or as light to dark brown lumps. It is almost odorless.

Identification

(1) To 1 g of Gum Ghatti, add 5 ml of water. A viscous liquid is formed.

(2) To 5 ml of a solution of Gum Ghatti (1 in 100), add 0.2 ml of dilute basic lead acetate TS (1 in 5). A little or no precipitate is formed, but a milk white precipitate is formed on the addition of 0.5 ml of Ammonia TS.

(3) A solution of Gum Ghatti (1 in 50) filtered through diatomaceous earth for chromatography is levorotatory.

Purity

(1) Heavy metals Not more than 40 μg/g as Pb (0.50 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 10 μg/g as Pb (1.0 g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Loss on Drying Not more than 14.0% (105°C, 5 hours).

Ash Not more than 6.0%.

Acid-insoluble Ash Not more than 1.0%.

Microbial Limits Proceed as directed in the Microbial Limit Tests. The total bacterial count is not more than 10,000/g, and *Escherichia coli* is negative.

Haematococcus Algae Color

ヘマトコッカス藻色素

Definition Haematococcus Algae Color is obtained from the whole algae of *Haematococcus* spp. and consists mainly of astaxanthins. It may contain edible fats or oils.

Color Value The Color Value ($E^{10\%}_{1cm}$) of Haematococcus Algae Color is not less than 600 and is in the range of 95–115% of the labeled value.

Description Haematococcus Algae Color occurs as orange to dark brown lumps, paste, or liquid having a slight characteristic odor.

Identification

(1) Weigh the equivalent of 0.4 g with a Color Value 600, and dissolve it in 100 ml of acetone. An orange-yellow to red-orange color develops.

(2) To 0.1 ml of the solution prepared in Identification (1), add 5 ml of sulfuric acid. A blue-green to dark blue color develops.

(3) A solution of Haematococcus Algae Color in acetone exhibits an absorption maximum at a wavelength of 460–480 nm.

(4) Weigh the equivalent of 0.4 g with a Color Value 600, dissolve it in 10 ml of acetone, and use this solution as the test solution. Analyze a 5-µl portion of the sample solution by thin-layer chromatography using a 7:3 mixture of hexane/acetone as the developing solvent. No control solution is used. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the original line, and air-dry. A red-orange spot is observed at an R_f value of about 0.4–0.6. Its color immediately disappears when the spot is sprayed with 5% sodium nitrite solution followed by 0.5 mol/L sulfuric acid.

Purity

(1) Heavy metals Not more than 40 µg/g as Pb (0.50 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 8.0 µg/g as Pb (1.25 g, Method 1).

(3) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50g, Method 3, Apparatus B).

Color Value Test Perform the test as directed in the Color Value Test, according to the following operating conditions.

Solvent: Acetone.

Wavelength: Maximum absorption wavelength of 460–480 nm.

Heme Iron

ヘム鉄

Definition Heme Iron is obtained by isolation from protease-treated hemoglobin. It consists mainly of heme iron.

Content Heme Iron, when calculated on the dried basis, contains 1.0–2.6% of iron (Fe = 55.85).

Description Heme Iron occurs as a brown to blackish brown powder or granules. It is odorless or has a little characteristic odor.

Identification

(1) To 0.010 g of Heme Iron, add 1 ml of diluted sulfuric acid (1 in 20) and 1 ml of nitric acid to dissolve, and evaporate on a water bath to dryness. Dissolve the residue in 10 ml of diluted hydrochloric acid (1 in 2), and add ammonium thiocyanate solution (2 in 25). A red color develops.

(2) Dissolve 5 mg of Heme Iron in 10 ml of pyridine-sodium hydroxide TS, and add 0.1 g of sodium sulfide. A red color develops.

(3) To 0.010 g of Heme Iron, add 5 ml of nitric acid, and heat. A yellow color develops. After cooling, make the solution alkaline with ammonia solution. The color changes to orange-yellow.

Purity

(1) Heavy metals Not more than 20 µg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Loss on Dryness Not more than 5.0% (105°C, 5 hours).

Residue on Ignition Not more than 12.0%.

Assay Weigh accurately about 10 g of Heme Iron, moisten with 5 ml of diluted sulfuric acid (1 in 20) and 5 ml of nitric acid, heat carefully until white fumes are no longer evolved, and incinerate at 450–550°C. To the residue, add 10 ml of diluted hydrochloric acid (1 in 2), boil until there is almost no insoluble matter, add 20 ml of water, and filter. Wash the insoluble residue on the filter paper, and combine the washing with the filtrate. To the combined solution, add water to make exactly 100 ml. Measure exactly 25 ml of the resulting solution into a stoppered flask, add 2 g of potassium iodide, immediately stopper tightly, and allow to stand in a dark place for 15 minutes. Add 100 ml of water, and titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: starch TS). Separately, perform a blank test to make any necessary correction, and calculate on the dried basis.

Each ml of 0.1 mol/L sodium thiosulfate solution = 5.585 mg of Fe

Hexane

ヘキサン

Definition Hexane mainly contains *n*-hexane (C_6H_{14}).

Description Hexane is a clear, colorless, volatile liquid having a characteristic odor.

Purity

(1) Refractive index n_D^{20}: 1.374–1.386.

(2) Specific gravity 0.659–0.687.

(3) Distillate Not less than 95% (vol) of Hexane is distilled at 64–70°C (Method 2).

(4) Sulfur compounds Measure 5 ml of Hexane, add 5 ml of silver nitrate–ammonia TS, and heat at 60°C for 5 minutes with protection from light, while shaking well. No brown color develops.

(5) Benzene Not more than 0.25% (vol) as benzene.

Test Solution Measure exactly 50 ml of Hexane, add exactly 50 ml of the internal standard solution, and mix. As the internal standard solution, use a solution prepared by adding

hexane for ultraviolet absorption spectrum measurement to 0.5 ml of 4-methyl-2-pentanone to make 100 ml.

Control Solution Measure exactly 0.25 ml of benzene, and add hexane for ultraviolet absorption spectrum measurement to make exactly 100 ml. To exactly 50 ml of this solution, add exactly 50 ml of the internal standard solution, and mix.

Procedure Analyze the test solution and the control solution by gas chromatography using the conditions given below. The ratio (Q_T) of the height of the peak corresponding to benzene to the height of the peak of the 4-methyl-2-pentanone in the test solution does not exceed the ratio (Q_S) of the height of the peak corresponding to benzene to the height of the peak of 4-methyl-2-pentanone in the control solution.

Operating Conditions

Detector: Flame ionization detector.

Column: A stainless-steel or glass tube of 3–4 mm internal diameter and 2–3 m length.

Column packing material

Liquid phase: 10% polyethylene glycol 6,000 of the amount of support.

Support: 177- to 250-μm diatomaceous earth for gas chromatography.

Column temperature: A constant temperature of 50–70°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the benzene peak appears about 5 minutes after the injection of the sample.

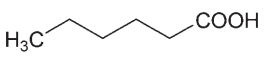
(6) Residue on evaporation Not more than 0.0013% (w/v). Measure 150 ml of Hexane, evaporate carefully, and dry at 105°C for 2 hours. Weigh the residue.

(7) Readily carbonizable substances Perform the test with 5 ml of Hexane, using Matching Fluid B.

Hexanoic Acid

Caproic Acid

ヘキサン酸



$C_6H_{12}O_2$ Mol. Wt. 116.16

Hexanoic acid [142-62-1]

Content Hexanoic Acid contains not less than 98.0% of hexanoic acid ($C_6H_{12}O_2$).

Description Hexanoic Acid is a colorless to light yellow, transparent liquid having a characteristic odor.

Identification

(1) Dissolve 2 ml of Hexanoic Acid in 6 ml of 50% (vol) ethanol. The solution is slightly acidic.

(2) To 1 ml of Hexanoic Acid, add 1 ml of ethanol and 3 drops of sulfuric acid, and warm in warm water for 5 minutes. An odor of ethyl hexanoate is evolved.

Purity

(1) Refractive index n_D^{20}: 1.415–1.418.

(2) Specific gravity 0.926–0.931.

(3) Alkali-insoluble substances Not more than 10%.

Measure 5.0 ml of Hexanoic Acid, transfer into a 150-ml Cassia flask, add 75 ml of sodium hydrogen carbonate solution (1 in 20) in three portions while shaking well, and shake well for an additional 5 minutes. Allow to stand for 30 minutes, and gradually add water to raise the insoluble oil to the scaled portion of the Cassia flask. Allow to stand for 1 hour, and measure the volume of the insoluble oil.

Assay Weigh accurately about 1 g of Hexanoic Acid, dissolve it in 10 ml of neutralized ethanol, and titrate with 0.5 mol/L ethanolic potassium hydroxide (indicator: 2 drops of phenolphthalein TS).

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 58.08 mg of $C_6H_{12}O_2$

High-Test Hypochlorite

高度サラシ粉

Content High-Test Hypochlorite contains not less than 60.0% of available chlorine.

Description High-Test Hypochlorite occurs as a white to whitish powder or granules having an odor of chlorine.

Identification

(1) To 0.5 g of High-Test Hypochlorite, add 5 ml of water, shake, and dip a red litmus paper into the solution. Its color changes to blue and then fades.

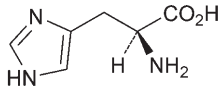
(2) To 0.1 g of High-Test Hypochlorite, add 2 ml of diluted acetic acid (1 in 4). It dissolves with evolution of gas. To the solution, add 5 ml of water, and filter. The solution responds to all tests for Calcium Salt in the Qualitative Tests.

Assay Weigh accurately an amount of High-Test Hypochlorite equivalent to 0.7–1.3 g of available chlorine. Add about 50 ml of water, grind well in a mortar, and add water to make exactly 500 ml. Shake well, measure exactly 50 ml of the solution, and add 2 g of potassium iodide and 10 ml of diluted acetic acid (1 in 2). Stopper immediately, allow to stand in a dark place for 15 minutes, and titrate liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: starch TS). Perform a blank test in the same manner, and make any necessary correction.

Each ml of 0.1 mol/L sodium thiosulfate = 3.545 mg of Cl

L-Histidine

L-ヒスチジン



$C_6H_9N_3O_2$ Mol. Wt. 155.15

(2*S*)-2-Amino-3-(1*H*-imidazol-4-yl)propanoic acid [71-00-1]

Content L-Histidine, when calculated on the dried basis,

contains 98.0–102.0% of L-histidine ($C_6H_9N_3O_2$).

Description L-Histidine occurs as white crystals or crystalline powder. It is odorless, and has a slight bitter taste.

Identification

(1) To 5 ml of a solution of L-Histidine (1 in 1,000), add 1 ml of ninhydrin solution (1 in 50), and heat for 3 minutes in a water bath. A purple color develops.

(2) To 5 ml of a solution of L-Histidine (1 in 100), add 2 ml of bromine TS. A yellow color develops. When gently heated, the solution turns colorless then reddish brown, and forms a blackish precipitate.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +11.5 to +13.5°.

Weigh accurately about 11 g of L-Histidine, and dissolve it in 6 mol/L hydrochloric acid to make exactly 100 ml. Measure the angular rotation of this solution and calculate on the dried basis.

(2) Clarity and color of solution Colorless and clear (1.0 g, water 40 ml).

(3) pH 7.0–8.5 (1.0 g, water 50 ml).

(4) Chloride Not more than 0.1% as Cl (0.07 g, Control solution 0.01 mol/L hydrochloric acid 0.20 ml).

(5) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of L-Histidine, and dissolve it in about 20 ml of water. Add 1 drop of phenolphthalein TS, neutralize with diluted hydrochloric acid (1 in 4), and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Use 2.0 ml of Lead Standard Solution.

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.5 g, Method 1, Apparatus B).

Loss on Drying Not more than 0.30% (105°C, 3 hours).

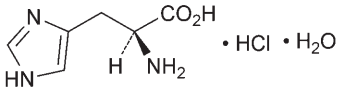
Residue on Ignition Not more than 0.20%.

Assay Weigh accurately about 0.3 g of L-Histidine, and proceed as directed in the Assay for L-Asparagine.

Each ml of 0.1 mol/L perchloric acid = 15.52 mg of $C_6H_9N_3O_2$

L-Histidine Monohydrochloride

L-ヒスチジン塩酸塩



$C_6H_9N_3O_2 \cdot HCl \cdot H_2O$ Mol. Wt. 209.63

(2*S*)-2-Amino-3-(1*H*-imidazol-4-yl)propanoic acid monohydrochloride monohydrate [7048-02-4]

Content L-Histidine Monohydrochloride, when dried, contains 98.0–101.0% of L-histidine monohydrochloride ($C_6H_9N_3O_2 \cdot HCl \cdot H_2O$).

Description L-Histidine Monohydrochloride occurs as white crystals or crystalline powder. It is odorless and has a bitter and slight acid taste.

Identification

(1) To 5 ml of a solution of L-Histidine Monohydrochloride (1 in 1,000), add 1 ml of ninhydrin solution (1 in 1,000), and heat for 3 minutes. A purple color develops.

(2) To 5 ml of a solution of L-Histidine Monohydrochloride (1 in 100), add 2 ml of bromine TS. A yellow color develops. When heated gently, the solution turns colorless then red-brown, and forms a blackish precipitate.

(3) To a solution of L-Histidine Monohydrochloride (1 in 10), add sodium hydroxide solution (1 in 5) to make alkaline. The resulting solution is levorotatory. When acidified with hydrochloric acid, it is dextrorotatory.

(4) L-Histidine Monohydrochloride responds to all tests for Chloride in the Qualitative Tests.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +8.5 to +10.5° (5.5 g, diluted hydrochloric acid (1 in 2), 50 ml, on the dried basis).

(2) Clarity and color of solution Colorless and almost clear (1.0 g, water 10 ml).

(3) pH 3.5–4.5 (1.0 g, water 20 ml).

(4) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Drying Not more than 0.30% (105°C, 3 hours).

Residue on Ignition Not more than 0.10% .

Assay Weigh accurately about 0.1 g of L-Histidine Monohydrochloride, previously dried, and dissolve it in 2 ml of formic acid. Add exactly 15 ml of 0.1 mol/L perchloric acid, and heat on a water bath for 30 minutes. After cooling, add acetic acid to make 60 ml, and titrate the excess perchloric acid with 0.1 mol/L sodium acetate. The endpoint is usually confirmed by a potentiometer. When 1 ml of crystal violet–acetic acid TS is used as the indicator, the endpoint is when the color of the solution changes from yellow through yellow-green to blue-green. Perform a blank test in the same manner.

Each ml of 0.1 mol/L perchloric acid = 10.48 mg of $C_6H_9N_3O_2 \cdot HCl \cdot H_2O$

Hydrochloric Acid

塩酸

Hydrochloric acid [7647-01-0]

Content Hydrochloric Acid contains 90–120% of the labeled content of hydrogen chloride (HCl = 36.46).

Description Hydrochloric Acid is a colorless to light yellow liquid having a pungent odor.

Identification

(1) A solution of Hydrochloric Acid (1 in 100) is strongly acidic.

(2) Hydrochloric Acid responds to all tests for Chloride in the Qualitative Tests.

Purity

(1) Sulfate Not more than 0.48% (w/v) as SO_4.

Sample Solution Measure 1.0 ml of Hydrochloric Acid, and add water to make 100 ml. Measure 5.0 ml of this solution, add 20 ml of water, and neutralize with ammonia TS.

Control Solution 0.50 ml of 0.005 mol/L sulfuric acid.

(2) Heavy metals Not more than 10 μg/ml as Pb.

Test Solution Measure 2.0 ml of Hydrochloric Acid, add 20 ml of water, and neutralize with ammonia TS. Add 2 ml

of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(3) Iron Not more than 30 μg/ml as Fe (1.0 ml, Method 1, Control solution Iron Standard Solution 3.0 ml).

(4) Arsenic Not more than 2.0 μg/ml as As_2O_3 (1.0 ml, Method 1, Apparatus B).

Residue on Ignition Not more than 0.020% (100 g).

Assay Transfer 20 ml of water into a flask with a ground-glass stopper, weigh accurately, add about 3 ml of Hydrochloric Acid, and weigh accurately again. Add 25 ml of water, and titrate with 1 mol/L sodium hydroxide (indicator: 3–5 drops of bromothymol blue TS).

Each ml of 1 mol/L sodium hydroxide = 36.46 mg of HCl

Hydrogen Peroxide

過酸化水素

Hydrogen peroxide [7722-84-1]

Content Hydrogen Peroxide contains 35.0–36.0% of hydrogen peroxide (H_2O_2 = 34.01).

Description Hydrogen Peroxide is a colorless, clear liquid. It is odorless or has a slight odor.

Identification

(1) To 1 ml of a dilute solution of Hydrogen Peroxide (1 in 10), add 5 ml of diluted sulfuric acid (1 in 20) and 1 ml of potassium permanganate solution (1 in 300). The solution effervesces, and the color disappears.

(2) Hydrogen Peroxide responds to the test for Peroxide.

Purity

(1) Free acid Measure exactly 3 ml of Hydrogen Peroxide, add 50 ml of freshly boiled and cooled water and 2 drops of methyl red TS, and titrate with 0.02 mol/L sodium hydroxide. The consumed volume is not more than 1.0 ml.

(2) Phosphate Not more than 62.5 μg/ml as PO_4.

Test Solution Measure exactly 8 ml of Hydrogen Peroxide, add 10 ml of water and 3 ml of hydrochloric acid, and evaporate to dryness while gradually heating on a water bath. Dissolve the residue in about 30 ml of warm water, cool, and add water to make 50 ml. Transfer exactly 5 ml of the this solution into a Nessler tube.

Procedure To the test solution, add 4 ml of diluted sulfuric acid (1 in 6) and 1 ml of ammonium molybdate solution (1 in 20), shake well, and allow to stand for 3 minutes. Add 1 ml of 1-amino-2-naphthol-4-sulfonic acid TS, shake, warm in a water bath at 60°C for 10 minutes, and cool with running water. The blue color of the test solution is not deeper than that of a control solution prepared as follows: Place 5.0 ml of Phosphate Standard Solution into a Nessler tube, and treat in the same manner as the test solution.

(3) Heavy metals Not more than 10 μg/ml as Pb.

Test Solution Measure exactly 2 ml of Hydrogen Peroxide, add 10 ml of water, and transfer the solution in small portions into a platinum crucible. Warm gently on a water bath until the effervescence ceases, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(4) Arsenic Not more than 4.0 μg/ml as As_2O_3.

Test Solution Measure 0.5 ml of Hydrogen Peroxide, add water to make 10 ml, and transfer the solution in small portions into a platinum crucible. Evaporate to dryness by heating gradually on a water bath, and dissolve the residue by adding a small amount of water.

Apparatus Use Apparatus B.

(5) Residue on evaporation Not more than 0.030%.

Measure 10 ml of Hydrogen Peroxide, add about 20 ml of water, and transfer the solution in small portions into a platinum crucible. Evaporate to dryness while gradually heating on a water bath, and dry the residue at 105°C for 1 hour. Weigh the residue.

Assay Weigh accurately about 1 g of Hydrogen Peroxide, and add water to make exactly 250 ml. Measure exactly 25 ml of this solution, add 10 ml of diluted sulfuric acid (1 in 20), and titrate with 0.02 mol/L potassium permanganate.

Each ml of 0.02 mol/L potassium permanganate = 1.701 mg of H_2O_2

Hydroxycitronellal

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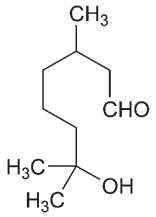

$C_{10}H_{20}O_2$ Mol. Wt. 172.26

7-Hydroxy-3,7-dimethyloctanal [107-75-5]

Content Hydroxycitronellal contains not less than 95.0% of hydroxycitronellal ($C_{10}H_{20}O_2$).

Description Hydroxycitronellal is a colorless to light yellow, transparent liquid having a lily of the valley-like odor.

Identification To 1 ml of Hydroxycitronellal, add 5 ml of sodium hydrogen sulfite TS, and shake. It produces heat, and dissolves. When the solution is cooled, crystalline lumps are formed.

Purity

(1) Refractive index n_D^{20}: 1.447–1.450.

(2) Specific gravity 0.921–0.926.

(3) Clarity of solution Clear (2.0 ml, 50% (vol) ethanol 3.0 ml).

(4) Acid value Not more than 5.0 (Flavoring Substances Tests).

Assay Weigh accurately about 1 g of Hydroxycitronellal, and proceed as directed in Method 2 in the Aldehyde and Ketone Content Test in the Flavoring Substances Tests. Allow the mixture to stand for 1 hour before titrating.

Each ml of 0.5 mol/L hydrochloric acid = 86.13 mg of $C_{10}H_{20}O_2$

Hydroxycitronellal Dimethylacetal

Hydroxycitronellal Dimethyl Acetal
1,1-Dimethoxy-3,7-dimethyloctan-7-ol

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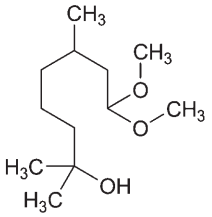


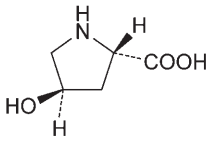
$C_{12}H_{26}O_3$ Mol. Wt. 218.33
8,8-Dimethoxy-2,6-dimethyloctan-2-ol [141-92-4]
Content Hydroxycitronellal Dimethylacetal contains not less than 95.0% of hydroxycitronellal dimethylacetal ($C_{12}H_{26}O_3$).
Description Hydroxycitronellal Dimethylacetal is a colorless or slightly yellowish, transparent liquid having a weak, lily of the valley-like odor.
Identification To 1 ml of Hydroxycitronellal Dimethylacetal, add 1 ml of ethanol and 1 ml of 0.25 mol/L sulfuric acid, and heat in a water bath for about 3 minutes while shaking. An odor of hydroxycitronellal is evolved.
Purity
(1) Refractive index n_D^{20}: 1.441–1.444.
(2) Specific gravity 0.928–0.934.
(3) Clarity of solution Clear (2.0 ml, 50% (vol) ethanol 4.0 ml).
(4) Acid value Not more than 1.0 (Flavoring Substances Tests).
(5) Hydroxycitronellal Weigh accurately about 5 g of Hydroxycitronellal Dimethylacetal, and proceed as directed in Method 2 in the Aldehyde and Ketone Content Test in the Flavoring Substances Tests. In the test, allow the mixture to stand for 1 hour before titrating. The volume of 0.5 mol/L hydrochloric acid consumed per 1 g of the sample is not more than 0.60 ml.
Assay Weigh accurately about 1.5 g of Hydroxycitronellal Dimethylacetal, and proceed as directed in Method 1 in the Aldehyde and Ketone Content Test in the Flavoring Substances Tests. In the test, boil the mixture for 5 minutes before titrating. Calculate the content by the formula:

$$\text{Content (\%) of hydroxycitronellal dimethylacetal } (C_{12}H_{26}O_3) = \frac{(a-b)\times 109.2}{1{,}000}\times 100$$

a = the volume (ml) of 0.5 mol/L ethanolic potassium hydroxide consumed per 1 g of the sample,
b = the volume (ml) of 0.5 mol/L hydrochloric acid consumed per 1 g of the sample obtained in Purity (5).

L-Hydroxyproline

L-ヒドロキシプロリン



$C_5H_9NO_3$ Mol. Wt. 131.13
(2*S*,4*R*)-4-Hydroxypyrrolidine-2-carboxylic acid [51-35-4]
Content L-Hydroxyproline, when calculated on the dried basis, contains 98.0–102.0% of L-hydroxyproline ($C_5H_9NO_3$).
Description L-Hydroxyproline occurs as white crystals or crystalline powder. It is odorless or has a very slight characteristic odor. It has a very slight sweet taste.
Identification To 5 ml of solution of L-Hydroxyproline (1 in 1,000), add 1 ml of ninhydrin solution (1 in 50), and heat for 3 minutes in a water bath. A yellow color develops.
Purity
(1) Specific rotation $[\alpha]_D^{20}$: –74.0 to –77.0°.
Weigh accurately about 4 g of L-Hydroxyproline, and dissolve it in water to make exactly 100 ml. Measure the angular rotation of this solution, and calculate on the dried basis.
(2) Clarity and color of solution Colorless and almost clear (1.0 g, water 10 ml).
(3) pH 5.0–6.5 (1.0 g, water 10 ml).
(4) Chloride Not more than 0.1% as Cl (0.070 g, Control solution 0.01 mol/L hydrochloric acid 0.20 ml).
(5) Heavy metals Not more than 20 µg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).
(6) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).
Loss on Drying Not more than 0.30% (105°C, 3 hours).
Residue on Ignition Not more than 0.20%.
Assay Weigh accurately about 0.3 g of L-Hydroxyproline, and proceed as directed in the Assay for L-Asparagine.
Each ml of 0.1 mol/L perchloric acid = 13.11 mg of $C_5H_9NO_3$

Hydroxypropyl Cellulose

ヒドロキシプロピルセルロース

2-Hydroxypropyl ether cellulose [9004-64-2]
Definition Hydroxypropyl Cellulose is hydroxypropyl ether of cellulose.
Content Hydroxypropyl Cellulose, when dried, contains not more than 80.5% of the hydroxypropoxy group ($-OC_3H_6OH$ = 75.09).
Description Hydroxypropyl Cellulose occurs as a white to yellowish white powder or granules. It is odorless. When water is added, it swells and produces a clear or slightly turbid, viscous liquid.
Identification
(1) Vigorously shake a solution of Hydroxypropyl Cellu-

lose (1 in 1,000). Effervescence occurs.

(2) To 5 ml of a solution of Hydroxypropyl Cellulose (1 in 500), add 5 ml of cupper sulfate solution (1 in 20). No precipitate is produced.

Purity

(1) pH: 5.0–8.0 (1.0 g, water 100 ml).

(2) Propylene chlorohydrin Not more than 1.0 μg/g.

Test Solution Weigh 1.0 g of Hydroxypropyl Cellulose, add exactly 5 ml of diethyl ether, and stopper. Sonicate for 10 minutes. Centrifuge the mixture, and use the supernatant for the test solution.

Standard Solution Weigh 0.030 g of propylene chlorohydrin, add diethyl ether to make exactly 100 ml. Perform serial dilutions of this solution. To exactly 1 ml of the solution, add diethyl ether to make exactly 50 ml. Next, to exactly 1 ml of the diluted solution, add diethyl ether to make exactly 20 ml.

Procedure Analyze 1 μl portions of the test solution and the standard solution by gas chromatography using the operating conditions given below. Measure the peak area of propylene chlorohydrin for each solution. The peak area for the test solution is not larger than that for the standard solution.

Operating Conditions

Detector: Hydrogen flame-ionization detector.

Detector temperature: 230ºC.

Column: A silicate glass capillary tube (0.25 mm internal diameter and 30 m length) coated with a 0.25-μm thick layer of polyethylene glycol for gas chromatognaphy.

Column temperature: Maintain the temperature at 40ºC for 2 minutes, raise to 80ºC at a rate of 5ºC/minute, and maintain at 80ºC for 8 minutes. Then raise up to 230ºC at a rate of 25ºC/minute, and maintain for 5 minutes.

Injection port temperature: 150ºC.

Injection method: Splitless.

Carrier gas: Nitrogen.

Flow rate: The flow rate should be adjusted so that the peak of propylene chlorohydrin appears 15 minute after injection.

(3) Lead Not more than 2.0 μg/g as Pb (5.0g, Method 1).

Loss on Drying Not more than 5.0% (105ºC, 4 hours).

Residue on Ignition Not more than 0.50%.

Assay

(i) Apparatus *Reaction flask* A 5-ml screw-cap pressure-tight glass bottle having an inverted conical bottom, a 20 mm external diameter, 50 mm high bottle-neck, and 2 ml capacity at the height of about 30 mm. The cap is made of heat-resistant resin and equipped with a fluoroplastic inside stopper or sealer. Confirm that the contents will not leak when heated.

Heater A 60–80 mm-thick, square-shaped aluminum block with holes 20.6 mm in diameter and 32 mm in depth that is capable of maintaining the inside temperature within ± 1ºC.

(ii) Method

Test Solution Weigh accurately about 0.065 g of Hydroxypropyl Cellulose, previously dried, transfer it to the reaction flask, add 0.065 g of adipic acid, 2.0 ml of the internal standard solution, and 2.0 ml of hydriodic acid. Stopper the flask tightly, and weigh the reaction flask with the mixture accurately. Use a solution of octane in *o*-xylene (1 in 25) as the internal standard solution. Shake the flask for 30 seconds, heat on the heater at 150ºC for 30 minute with repeated shaking at 5 minute intervals, and continue heating for an additional 30 minutes. Allow to cool, and then again weigh the flask accurately. Confirm that the weight loss is not more than 0.010 g, and use the upper layer of the mixture in the flask as the test solution.

Standard Solution Place 0.065 g of adipic acid, 2.0 ml of the internal standard solution, and 2.0 ml of iodine hydriodic acid in another reaction flask. Stopper the flask tightly, and weigh accurately. Add 50 μl of isopropyl iodide for assay, stopper tightly, and weigh accurately. Shake the flask for 30 seconds, and use the upper layer of the mixture as the standard solution.

Procedure Analyze 1 μl portions of the test solution and the standard solution by gas chromatography using the operating conditions given below. Determine the peak area ratio of isopropyl iodide to octane for each of the test solution and the standard solution, and express as Q_T for the test solution and as Q_S for the standard solution. Calculate the hydroxypropoxy group content by the formula:

$$\text{Content (\%) of hydroxypropoxy group (-OC}_3\text{H}_6\text{OH)} = \frac{W_S}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 44.17$$

Ws = amount (g) of isopropyl iodide in the standard solution.

Operating Conditions

Detector: Hydrogen flame ionization detector.

Column: A glass tube of 3 mm internal diameter and 30 m length.

Column packing material

Liquid phase: 20% Methyl silicone polymer of the support.

Support: 180- to 250-μm diatomaceous earth for gas chromatography.

Column temperature: A constant temperature at about 100ºC.

Carrier gas: Helium.

Flow rate: Adjust so that the peak of octane appears about 10 minute after injection.

Column selection: Use a column capable of producing well-resolved peaks of isopropyl iodide and octane, in that order, when 1 μl of the standard solution is chromatographed under the above operating conditions.

Hydroxypropyl Methylcellulose

ヒドロキシプロピルメチルセルロース

A mixed methyl and 2-hydroxypropyl ether of cellulose
[9004-65-3]

Definition Hydroxypropyl Methylcellulose is a mixed ether of methyl and hydroxypropyl cellulose.

Content Hydroxypropyl Methylcellulose, when dried, contains 19.0–30.0% of the methoxy group (-OCH_3 = 31.03) and 3.0–12.0% of the hydroxypropoxy group (-OC_3H_6OH = 75.09).

Description Hydroxypropyl Methylcellulose occurs as a white to yellowish white powder or granules. It is odorless

or has a slight characteristic odor. When water is added, it swells and produces a clear or slightly turbid viscous liquid.

Identification

(1) To 1 g of Hydroxypropyl Methylcellulose, add 100 ml of hot water, and cool to room temperature while stirring. Use this solution as the sample solution. Add anthrone TS gently to 5 ml of the sample solution. The boundary surface of both solutions turns blue to blue-green .

(2) To 0.1 ml of the sample solution obtained in Identification (1), add 9 ml of diluted sulfuric acid (9 in 10), shake, heat in a water bath for exactly 3 minutes, and immediately cool in an ice bath. To this solution, carefully add 0.6 ml of a ninhydrin solution (1 in 50), shake, and allow to stand 25°C. A red color develops , and then the color changes to purple within 100 minutes.

(3) Proceed as directed in the Potassium Bromide Disk Method under Infrared Spectrophotometry. Hydroxypropyl Methylcellulose exhibits absorption bands at about 3465 cm^{-1}, 2902 cm^{-1}, 1375 cm^{-1}, and 1125 cm^{-1}.

Purity

(1) pH 5.0–8.0 (1.0 g, hot water 100 ml).

(2) Chloride Not more than 0.28% as Cl.

Test Solution To 1.0 g of Hydroxypropyl Methylcellulose, add 30 ml of hot water, shake well, heat on a water bath for 10 minutes, and filter by decantation while hot. Wash the residue well with hot water, combine the washings with the filtrate, and cool. To the obtained solution, add water to make 100 ml. To 5 ml of this solution, add 6 ml of diluted nitric acid and water to make 50 ml.

Control Solution Use 0.40 ml of 0.01 mol/L hydrochloric acid.

(3) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 2.0 μg/g as As_2O_3 (1.0 g, Method 3, Apparatus B).

Loss on Drying Not more than 8.0% (105°C, 1 hour).

Residue on Ignition Not more than 1.5 % (on the dried basis).

Assay

(i) Apparatus *Reaction flask* A 5-ml screw-cap pressure-tight glass bottle having an inverted conical bottom, a 20 mm external diameter, 50 mm high bottle neck, and 2 ml capacity at the height of 30 mm. The cap is made of heat-resistant resin and equipped with a fluoroplastic inside stopper or seal. Confirm that the contents will not leak when heated.

Heater A 60 to 80-mm square-shaped aluminum block with holes 20.6 mm in diameter and 32 mm in depth that is capable of maintaining the inside temperature within±1℃.

(ii) Method

Test Solution Weigh accurately about 0.065 g of Hydroxypropyl Methylcellulose, previously dried, transfer to a reaction flask, and add 0.065 g of adipic acid, 2.0 ml of the internal standard solution, and 2.0 ml of hydriodic acid. Stopper the flask tightly, and accurately weigh the flask with the mixture. Use a solution of octane in *o*-xylene (1 in 25) as the internal standard solution. Shake the flask for 30 seconds, heat on the heater at 150℃ for 30 minutes with repeated shaking at 5 minute intervals, and continue heating for an additional 30 minutes. Allow to cool, and then again weigh accurately. Confirm that the weight loss is not more than 0.010 g, and use the upper layer of the mixture in the flask as the test solution.

Standard Solution Place 0.065 g of adipic acid, 2.0 ml of the internal standard solution, and 2.0 ml of hydriodic acid in another reaction flask. Stopper the flask tightly, and weigh accurately. To the flask, add 15 μl of isopropyl iodide for assay, stopper, and weigh accurately. Add 45 μl of methyl iodide for assay in the same manner, and again weigh accurately. Shake the reaction flask for 30 seconds and use the upper layer of the mixture as the standard solution.

Procedure Analyze 2 μl portions of the test solution and the standard solution by gas chromatography using the conditions below. Determine the peak area ratio of each of methyl iodide and isopropyl iodide to octane for the test solution, and express as Q_{Ta} and Q_{Tb}, respectively, and also calculate the peak area ratio for the standard solution in the same manner, and express them as Q_{Sa} and Q_{Sa}, respectively. Calculate the contents of methoxy and hydroxypropoxy groups by the following formulae:

$$\text{Content (\%) of methoxy group (-CH}_3\text{O)} = \frac{W_{Sa}}{\text{Weight (g) of the sample}} \times \frac{Q_{Ta}}{Q_{Sa}} \times 21.86$$

$$\text{Content (\%) of hydroxypropoxy group (-C}_3\text{H}_7\text{O}_2\text{)} = \frac{W_{Sb}}{\text{Weight (g) of the sample}} \times \frac{Q_{Tb}}{Q_{Sb}} \times 44.17$$

W_{Sa} = amount (g) of methyl iodide in the standard solution,

W_{Sb} = amount (g) of isopropyl iodide in the standard solution.

Operating Conditions

Detector: Hydrogen flame-ionization detector.

Column: A glass column about 3 mm internal diameter and 3 m length.

Column packing material

Liquid phase: 20% Methyl silicon polymer of the amount of the support.

Support: 180- to 250-μm diatomaceous earth for gas chromatography.

Column temperature: A constant temperature about 100°C.

Carrier gas: Helium gas.

Flow rate: Adjust so that the peak of octane appears about 10 minutes after injection.

Column Selection: Use a column capable of producing well-resolved peaks of methyl iodide, isopropyl iodide, and octane, in that order listed, when 2 μl of the standard solution is chromatographed under the above conditions.

Hypochlorous Acid Water

次亜塩素酸水

Definition Hypochlorous Acid Water is an aqueous solution consisting mainly of hypochlorous acid. It is obtained by electrolyzing hydrochloric acid or a saline solution. There are two types of solutions: Strongly Acidic Hypochlorous Acid Water and Slightly Acidic Hypochlorous Acid Water.

Strongly Acidic Hypochlorous Acid Water is produced from the anode by electrolyzing sodium chloride solution of not more

than 0.2% in an electrolytic cell with a septum ("electrolytic cell with a septum" refers to a cell consisting of an anode and a cathode separated by a septum). Slightly Acidic Hypochlorous Acid Water is produced from the cathode by electrolyzing 2–6% hydrochloric acid in an electrolytic cell without a septum ("electrolytic cell without a septum" refers to a cell consisting of an anode and a cathode not separated by a septum).

Content

Strongly Acidic Hypochlorous Acid Water includes 20–60 mg/kg of available chlorine.

Slightly Acidic Hypochlorous Acid Water includes 10–30 mg/kg of available chlorine.

Description Hypochlorous Acid Water is a colorless liquid. It has little or no odor of chlorine.

Identification

(1) To 5 ml of Hypochlorous Acid Water, add 1 ml of a sodium hydroxide solution (1 in 2,500) and 0.2 ml of potassium iodide TS. A yellow color is produced, which changes to deep blue on the addition of 0.5 ml of starch TS.

(2) To 5 ml of Hypochlorous Acid Water, add 0.1 ml of a potassium permanganate solution (1 in 300) and then 1 ml of diluted sulfuric acid (1 in 20). A reddish purple color of the solution does not fade.

(3) To 90 ml of Hypochlorous Acid Water, add 10 ml of a sodium hydroxide solution (1 in 5). The solution exhibits an absorption maximum at a wavelength of 290–294 nm.

Purity

(1) pH

Strongly Acidic Hypochlorous Acid Water: Not more than 2.7.

Slightly Acidic Hypochlorous Acid Water: 5.0–6.5.

(2) Residue on drying (evaporating) Not more than 0.25 %.

Weigh and evaporate 20.0 g of Hypochlorous Acid Water. Then dry it at 110°C for 2 hours, and weigh the residue.

Assay

(1) Strongly Acidic Hypochlorous Acid Water Weigh accurately about 200 g of Hypochlorous Acid Water, add 2 g of potassium iodide and 10 ml of diluted acetic acid (1 in 4), immediately stopper the container, and allow to stand in a dark place for 15 minutes. Titrate the liberated iodine with 0.01 mol/L sodium thiosulfate, using starch TS as the indicator. Separately, perform a blank test in the same manner to make any necessary correction.

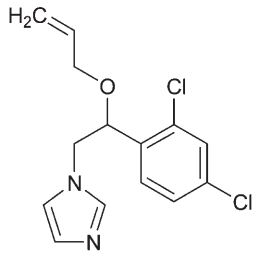
Each ml of 0.01 mol/L sodium thiosulfate = 0.3545 mg of Cl

(2) Slightly Acidic Hypochlorous Acid Water Weigh accurately about 200 g of Hypochlorous Acid Water, add 2 g of potassium iodide and 10 ml of diluted acetic acid (1 in 4), quickly stopper the container, and allow to stand in a dark place for 15 minutes. Titrate the liberated iodine with 0.005 mol/L sodium thiosulfate, using starch TS as the indicator. Separately, perform a blank test in the same manner to make any necessary correction.

Each ml of 0.005 mol/L sodium thiosulfate = 0.1773 mg of Cl

Imazalil

イマザリル



$C_{14}H_{14}Cl_2N_2O$ Mol. Wt. 297.18

1-[(2*RS*)-2-(Allyloxy)-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole [35554-44-0]

Content Imazalil contains not less than 97.5% of imazalil ($C_{14}H_{14}Cl_2N_2O$).

Description Imazalil occurs as a light yellow to light brown powder or granules. It is odorless.

Identification Dissolve 0.04 g of Imazalil in 10 ml of 0.1 mol/L hydrochloric acid, add 2-propanol to make 100 ml. This solution exhibits absorption maxima at wavelengths of 263–267 nm, 270–274 nm, and 278–282 nm.

Purity

(1) Melting point 49–54°C.

(2) Heavy metals Not more than 10 μg/g as Pb (Powderform 1.0 g, Method 2, Control solution Lead Standard Solution 1.0 ml).

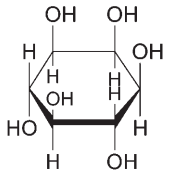
Residue on Ignition Not more than 0.10%.

Assay Weigh accurately about 0.7 g of Imazalil, and add a 7:3 mixture of methyl ethyl ketone/acetic acid to dissolve. Titrate this solution with 0.1 mol/L of perchloric acid (indicator: α-naphtholbenzein TS). The endpoint is when the color of solution changes from orange to green. Perform a blank test to make any necessary correction.

Each ml of 0.1 mol/L of perchloric acid = 29.72 mg of $C_{14}H_{14}Cl_2N_2O$

myo-Inositol

myo-イノシトール



$C_6H_{12}O_6$ Mol. Wt. 180.16

(1*R*,2*s*,3*S*,4*R*,5*r*,6*S*)-Cyclohexane-1,2,3,4,5,6-hexol [87-89-8]

Definition *myo*-Inositol is one of the inositol isomers and

consists mainly of *myo*-inositol. It is produced by decomposing phytic acid obtained from the seed bran of the rice plant *Oryza sativa* Linné or the seeds of the corn plant *Zea mays* Linné. It is also produced by isolation from the juice of the sugar beet, *Beta vulgaris* Linné, or molasses.

Content *myo*-Inositol, when dried, contains not less than 97.0% of *myo*-inositol ($C_6H_{12}O_6$).

Description *myo*-Inositol occurs as white crystals or crystalline powder. It is odorless and has a sweet taste.

Identification Determine the absorption spectrum of *myo*-Inositol as directed in the Potassium Bromide Disk Method under Infrared Spectrophotometry. The spectrum exhibits absorption bands at about 3380 cm^{-1}, 3220 cm^{-1}, 1446 cm^{-1}, 1147 cm^{-1}, 1114 cm^{-1}, and 1049 cm^{-1}.

Purity

(1) Melting point 223–227°C.

(2) Clarity and color of solution Colorless and clear (1.0 g, water 10 ml).

(3) Chloride Not more than 0.005% as Cl (2.0 g, Control solution 0.01 mol/L Hydrochloric acid 0.30 ml).

(4) Sulfate Not more than 0.006% SO_4 (4.0 g, Control solution 0.005 mol/L sulfuric acid 0.50 ml).

(5) Heavy metals Not more than 25 μg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 2.5 ml).

(6) Iron Not more than 5.0 μg/g as Fe (1.0 g, Method 1, Control solution Iron Standards Solution 0.5 ml).

(7) Calcium Dissolve 1.0 g of *myo*-Inositol in 10 ml of water, add 1 ml of ammonium oxalate (1 in 30), and allow to stand for 1 minute. The solution is clear.

(8) Arsenic Not more than 2.0 μg/g as As_2O_3 (1.0 g, Method 1, Apparatus B).

(9) Reducing substance Dissolve 0.50 g of *myo*-Inositol in 10 ml of water, add 5 ml of Fehling's TS, heat for 3 minutes, and allow to stand for 30 minutes. A yellowish orange to red precipitate is not formed.

Loss on Drying Not more than 0.50% (105°C, 4 hours).

Residue on Ignition Not more than 0.10%.

Assay

Test Solution and Standard Solution Weigh accurately about 0.2 g each of *myo*-Inositol and *myo*-inositol for assay, previously dried. To each, add exactly 30 ml of water and exactly 5 ml of 1-propanol solution (3 in 25), and then add water to make 2 solutions of exactly 50 ml each. Use these solutions as the test solution and standard solution, respectively.

Procedure Analyze 10 μl portions of the test solution and the standard solution by liquid chromatography using the operating conditions below. Determine the peak area ratios (Q_T and Q_S) of *myo*-inositol to 1-propanol for the test solution and the standard solution, and calculate the myo-inositol content by the formula:

$$\text{Content (\%) of } myo\text{-inositol } (C_6H_{12}O_6) = \frac{\text{Weight (g) of } myo\text{-inositol for assay}}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 100$$

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube of 8 mm internal diameter and 30 cm length.

Column packing material: 8-μm strongly acidic cation-exchange resin for liquid chromatography.

Column temperature: A constant temperature at about 65°C.

Mobile phase: Water.

Flow rate: Adjust so that the retention time of *myo*-inositol is about 9 minutes.

Ion Exchange Resins

イオン交換樹脂

Definition Ion Exchange Resins occur as granules, powders, and suspensions called Ion Exchange Resin (granule), Ion Exchange Resin (powder), and Ion Exchange Resin (suspension), respectively.

Ion Exchange Resin (granule)

イオン交換樹脂（粒状）

Description Ion Exchange Resin (granule) occurs as a black, brown, light red-brown, or white, spherical, massive, or granular substance. It is almost odorless.

Identification Perform test (I) or (II) whichever is appropriate to identify cation exchange resin or anion exchange resin.

(I) Cation exchange resin Prepare a resin column by pouring 5 ml of Ion Exchange Resin (granule) with water into a glass tube for chromatography (about 1-cm internal diameter). Run 25 ml of diluted hydrochloric acid (1 in 10) through the column at a rate of about 5 ml per minute, and wash the resin by running 100 ml of water through at the same rate. Then run 25 ml of potassium hydroxide solution (1 in 15) through at the same rate, and wash again by running 75 ml of water through at the same rate. To 5 ml of the last washing, add 2 ml of diluted acetic acid (1 in 20) and then 3 drops of sodium cobaltinitrite TS. No yellow turbidity appears. Transfer 2 ml of the resin in the column to a test tube, add 5 ml of diluted hydrochloric acid (1 in 10), shake well for 5 minutes, and filter. Wash the resin on the filter paper with water, and combine the filtrate and the washings to make about 5 ml. Add 4 ml of sodium hydroxide solution (1 in 25) to the solution, shake, add 2 ml of diluted acetic acid (1 in 20), and add 3 drops of sodium cobaltinitrite TS. A yellow precipitate is formed.

(II) Anion exchange resin Prepare a resin column pouring 5 ml of Ion Exchange Resin (granule) with water into a glass tube for chromatography (about 1-cm internal diameter). Run 25 ml of diluted hydrochloric acid (1 in 10) through the column at a rate of about 5 ml per minute, and wash the resin by running 100 ml of water through at the same rate. To 5 ml of the last washing, add 1 ml of diluted nitric acid (1 in 10) and 3 drops of silver nitrate solution (1 in 50). No white turbidity appears. Transfer 1 ml of the resin in the column to a test tube, add 3 ml of sodium hydroxide solution (1 in 25), and shake well for 5 minutes, and filter. Wash the resin on the filter paper with water, and combine

the filtrate and the washings to make about 5 ml. To this solution, add 3 ml of diluted nitric acid (1 in 10) and then 3 drops of silver nitrate solution (1 in 50). A white precipitate is formed.

Purity Prepare the sample by procedure (I) for cation exchange resin or procedure (II) for anion exchange resin, given below, immerse thoroughly in water, and blot the adhering water with a filter paper. Use the prepared resin as the test sample.

(I) Cation exchange resin Prepare the sample (H form) as follows: Measure 30 ml of Ion Exchange Resin (granule), transfer into a glass tube for chromatography (about 3 cm in internal diameter), and run 1,000 ml of diluted hydrochloric acid (1 in 10) through at a rate of 15–20 ml per minute. Then wash the resin by running water through at the same rate. Measure 10 ml of the washings, and perform the test for Chloride. Wash the resin repeatedly with water until the chloride amount is not more than the equivalent of 0.3 ml of 0.01 mol/L hydrochloric acid.

(II) Anion exchange resin Prepare the sample (OH form) as follows: Measure 30 ml of Ion Exchange Resin (granule), transfer it into a glass tube for chromatography (about 3 cm in internal diameter), run 1,000 ml of sodium hydroxide solution (1 in 25) through at a rate of 15–20 ml per minute, and wash the resin by running water through at the same rate. Wash the resin with water until the washings become neutral with phenolphthalein TS.

(1) Solids Not less than 25%.

Weigh 10.0 g of the test sample. Dry it at 100°C for 12 hours in the case of the cation exchange resin or at 40°C for 12 hours in a vacuum desiccator at 4 kPa in the case of the anion exchange resin. Then weigh it again.

(2) Water-soluble substances Not more than 0.50%.

Weigh 10.0 g of the test sample, transfer into a cylindrical filter (28 mm in internal diameter, 10 cm in length), suspend the filter with the sample in 1,000 ml of water, and extract for 5 hours with occasional shaking. Measure 50 ml of the extract, evaporate carefully, and dry at 110°C for 3 hours. Weigh the residue. Perform a blank test in the same manner, and make any necessary correction.

(3) Heavy metals Not more than 20 μg/g as Pb (test sample 1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (test sample 0.50 g, Method 3, Apparatus B).

Total Ion Exchange Capacity Perform the test for cation exchange resin using procedure (I) or for anion exchange resin using procedure (II).

(I) Cation exchange resin Not less than 1.0 milliequivalent/g.

Weigh accurately about 5 g of the test sample prepared for the Purity Tests. Add exactly 500 ml of 0.2 mol/L sodium hydroxide, and allow to stand for 12 hours with occasional shaking. Measure exactly 10 ml of the supernatant, and titrate with 0.05mol/L sulfuric acid (indicator: 3 drops of methyl orange TS). Perform a blank test in the same manner, and calculate the total ion exchange capacity by the formula:

$$\text{Total ione exchange capacity (milliequivalent/g)} = \frac{\left(\begin{array}{l}\text{Volume (ml) of}\\ \text{0.05 mol/L sulfuric acid}\\ \text{consumed in the blank test}\end{array}\right) - \left(\begin{array}{l}\text{Volume (ml) of}\\ \text{0.05 mol/L sulfuric acid}\\ \text{consumed in the test}\end{array}\right)}{\text{Weight (g) of the test sample} \times \frac{\text{Solid (\%)}}{100}} \times 5$$

(II) Anion exchange resin Not less than 1.0 milliequivalent/g.

Weigh accurately about 5 g of the test sample prepared for the Purity Tests. Add exactly 500 ml of 0.2 mol/L hydrochloric acid, and allow to stand for 12 hours with occasional shaking. Measure exactly 10 ml of the supernatant, and titrate with 0.1 mol/L sodium hydroxide (indicator: 3 drops of phenolphthalein TS). Perform a blank test in the same manner, and calculate the total ion exchange capacity by the formula:

$$\text{Total ione exchange capacity (milliequivalent/g)} = \frac{\left(\begin{array}{l}\text{Volume (ml) of}\\ \text{0.1 mol/L sodium hydroxide}\\ \text{consumed in the blank test}\end{array}\right) - \left(\begin{array}{l}\text{Volume (ml) of}\\ \text{0.1 mol/L sodium hydroxide}\\ \text{consumed in the test}\end{array}\right)}{\text{Weight (g) of the test sample} \times \frac{\text{Solid (\%)}}{100}} \times 5$$

Ion Exchange Resin (powder)

イオン交換樹脂（粉状）

Description Ion Exchange Resin (powder) occurs as a black, brown, light red-brown, or white, powdery substance. It is almost odorless.

Identification Perform test (I) or (II) whichever is appropriate to identify cation exchange resin or anion exchange resin.

(I) Cation exchange resin Prepare a resin layer by pouring 2 g of Ion Exchange Resin (powder) with water into a pressure filter (about 7.5-cm internal diameter) equipped with a membrane filter (1-μm pore diameter). Run 25 ml of diluted hydrochloric acid (1 in 10) through at a rate of about 5 ml per minute, and wash the resin by running 100 ml of water through at the same rate. Next, run 25 ml of potassium hydroxide solution (1 in 15) through at the same rate, and wash the resin again by running 75 ml of water through at the same rate. To 5 ml of the last washings, add 2 ml of diluted acetic acid (1 in 20) and then 3 drops of sodium cobaltinitrite TS. No yellow turbidity appears. Transfer 0.5 g of the resin layer to a test tube, add 5 ml of diluted hydrochloric acid (1 in 10), shake well for 5 minutes, and filter. Wash the resin on the filter paper with water, and combine the filtrate and the washings to make about 5 ml. Add 4 ml of sodium hydroxide solution (1 in 25) to the solution, and shake. Add 2 ml of diluted acetic acid (1 in 20) and then 3 drops of sodium cobaltinitrite TS. A yellow precipitate is formed.

(II) Anion exchange resin Prepare a resin layer by pouring 2 g of Ion Exchange Resin (powder) with water into a pressure filter (about 7.5-cm internal diameter) equipped with a membrane filter (1μm in pore diameter). Run 25 ml of diluted hydrochloric acid (1 in 10) through at a rate of

about 5 ml per minute, and wash the resin by running 100 ml of water through at the same rate. To 5 ml of the last washing, add 1 ml of diluted nitric acid (1 in 10) and then 3 drops of silver nitrate solution (1 in 50). No white turbidity appears. Transfer 0.5 g of the resin layer to a test tube, add 3 ml of sodium hydroxide solution (1 in 25), shake well for 5 minutes, and filter. Wash the resin on the filter paper with water, and combine the filtrate and the washings to make about 5 ml. To the solution, add 3 ml of diluted nitric acid (1 in 10) and then 3 drops of silver nitrate solution (1 in 50). A white precipitate is formed.

Purity Prepare the sample by procedure (I) for cation exchange resin or by procedure (II) for anion exchange resin, given below, immerse thoroughly in water, and blot the adhering water with a filter paper. Use the prepared resin as the test sample.

(I) Cation exchange resin Prepare the sample (H form) as follows: Weigh 30 g of Ion Exchange Resin (powder), transfer into a pressure filter (7.5 cm in internal diameter) equipped with a membrane filter (1 μm in pore diameter), run 1,000 ml of diluted hydrochloric acid (1 in 10) through at a rate of 15–20 ml per minute, and wash the resin by running water through at the same rate. Measure 10 ml of the washings, and perform the test for Chloride. Wash the resin repeatedly with water until the chloride amount is not more than the equivalent of 0.3 ml of 0.01 mol/L hydrochloric acid.

(II) Anion exchange resin Prepare the sample (OH form) as follows: Weigh 30 g of Ion Exchange Resin (powder), transfer into a pressure filter (about 7.5 cm in internal diameter) equipped with a membrane filter (1 μm in pore diameter), run 1,000 ml of sodium hydroxide solution (1 in 25) through at a rate of 15–20 ml per minute, and wash the resin by running water through at the same rate. Wash the resin with water until the washings become neutral with phenolphthalein TS.

(1) Solids Not less than 25%.

Proceed as directed in Purity (1) in Ion Exchange Resin (granule).

(2) Water-soluble substances Not more than 0.50%.

Weigh 10.0 g of the test sample, make a suspension by adding 1,000 ml of water, and extract for 5 hours with occasional stirring. Filter the suspension through a pressure filter (about 7.5 cm in internal diameter) equipped with a membrane filter (1 μm in pore diameter). Measure 50 ml of the filtrate, evaporate carefully, and dry at 110°C for 3 hours. Weigh the residue.

(3) Heavy metals Not more than 20 μg/g as Pb (test sample 1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (test sample 0.50 g, Method 3, Apparatus B).

Total Ion Exchange Capacity Perform the test for cation exchange resin using procedure (I) or for anion exchange resin using procedure (II).

(I) Cation exchange resin Not less than 1.0 milliequivalent/g.

Weigh accurately about 5 g of the test sample prepared for the Purity Tests. Add exactly 500 ml of 0.2 mol/L sodium hydroxide, and allow to stand for 12 hours with occasional shaking. Filter the suspension through a pressure filter (7.5 cm in internal diameter) equipped with a membrane filter (1 μm in pore diameter). Measure exactly 10 ml of the filtrate, and titrate with 0.05 mol/L sulfuric acid (indicator: 3 drops of methyl orange TS). Perform a blank test in the same manner, and calculate the total ion capacity by the formula:

$$\text{Total ione exchange capacity (milliequivalent/g)} = \frac{\left(\begin{array}{l}\text{Volume (ml) of}\\ \text{0.05 mol/L sulfuric acid}\\ \text{consumed in the blank test}\end{array}\right) - \left(\begin{array}{l}\text{Volume (ml) of}\\ \text{0.05 mol/L sulfuric acid}\\ \text{consumed in the test}\end{array}\right)}{\text{Weight (g) of the test sample} \times \frac{\text{Solid (\%)}}{100}} \times 5$$

(II) Anion exchange resin Not less than 1.0 milliequivalent/g.

Weigh accurately about 5 g of the test sample prepared for the Purity Tests. Add exactly 500 ml of 0.2 mol/L hydrochloric acid, and allow to stand for 12 hours with occasional shaking. Filter the suspension through a pressure filter (7.5 cm in internal diameter) equipped with a membrane filter (1 μm in pore diameter). Measure exactly 10 ml of the filtrate, and titrate with 0.1 mol/l sodium hydroxide (indicator: 3 drops of phenolphthalein TS). Perform a blank test in the same manner, and calculate the total ion capacity by the formula:

$$\text{Total ione exchange capacity (milliequivalent/g)} = \frac{\left(\begin{array}{l}\text{Volume (ml) of}\\ \text{0.1 mol/L sodium hydroxide}\\ \text{consumed in the blank test}\end{array}\right) - \left(\begin{array}{l}\text{Volume (ml) of}\\ \text{0.1 mol/L sodium hydroxide}\\ \text{consumed in the test}\end{array}\right)}{\text{Weight (g) of the test sample} \times \frac{\text{Solid (\%)}}{100}} \times 5$$

Ion Exchange Resin (suspension)

イオン交換樹脂（懸濁液）

Description Ion Exchange Resin (suspension) is a brown, light red-brown, or white suspension. It is almost odorless.

Identification Perform test (I) or (II) whichever is appropriate to identify whether the resin is cation exchange resin or anion exchange resin.

(I) Cation exchange resin To 0.5 ml of Ion Exchange Resin (suspension), add 5 ml of water and 1 ml of a strongly acidic cation-exchange resin, react for 1 hour with occasional shaking, and filter through absorbent cotton on a funnel. To the filtrate, add 0.3 g of sodium chloride, shake for 3 minutes, add 1 drop of methyl red TS, and shake. A red color develops.

(II) Anion exchange resin To 0.5 ml of Ion Exchange Resin (suspension), add 5 ml of water and 1 ml of a strongly basic anion-exchange resin, react for 1 hour with occasional shaking, and filter through absorbent cotton on a funnel. To the filtrate, add 0.3 g of sodium chloride, shake for 3 minutes, add 1 drop of phenolphthalein TS, and shake. A pink color develops.

Purity

(1) Solids Not less than 4.0%.

Weigh 1.0 g of Ion Exchange Resin (suspension), dry at 105°C for 5 hours, and weigh again.

(2) Water-soluble substances Not more than 0.50% (w/v).

Measure 100 ml of Ion Exchange Resin (suspension), and filter through a pressure filter (about 7.5 cm in internal diameter) equipped with a membrane filter (0.05 μm in pore diameter). Measure 10 ml of the filtrate, evaporate carefully, and dry at 105°C for 3 hours. Weigh the residue.

(3) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Total Ion Exchange Capacity Perform the test for cation exchange resin using procedure (I) or for anion exchange resin using procedure (II).

(I) Cation exchange resin Not less than 1.0 milliequivalent/g.

Weigh accurately an amount of Ion Exchange Resin (suspension) equivalent to about 0.2 g of the solid. Pour it into a glass tube for chromatography (about 1 cm in internal diameter) previously packed with 10 ml of strongly acidic cation-exchange resin to allow the liquid in the suspension to run through at a rate of about 2 ml per minute. Then run about 20 ml of water through at the same rate. Wash the resin again by running about 80 ml of water through at a rate of 15–20 ml per minute. Collect all of the effluent and washings into a beaker, and add about 1 g of sodium chloride. Titrate with 0.1 mol/L sodium hydroxide to a pH of 7.0, using a pH meter. Perform a blank test in the same manner, make any necessary correction, and calculate the total ion exchange capacity by the formula:

$$\text{Total ione exchange capacity (milliequivalent/g)} = \frac{\left(\begin{array}{l}\text{Volume (ml) of}\\ \text{0.1 mol/L sodium hydroxide}\\ \text{consumed in the blank test}\end{array}\right) - \left(\begin{array}{l}\text{Volume (ml) of}\\ \text{0.1 mol/L sodium hydroxide}\\ \text{consumed in the test}\end{array}\right)}{\text{Weight (g) of the sample} \times \dfrac{\text{Solid (\%)}}{100}} \times 5$$

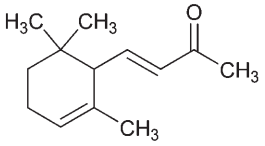
(II) Anion exchange resin Not less than 1.0 milliequivalent/g.

Weigh accurately an amount of Ion Exchange Resin (suspension) equivalent to about 0.2 g of the solid. Pour it into a glass tube for chromatography (about 1 cm in internal diameter) packed with 10 ml of a strongly basic anion-exchange resin to allow the liquid in the suspension to run through at a rate of about 2 ml per minute, and run about 20 ml of water through at the same rate. Wash the resin again by running about 80 ml of water through at a rate of 15–20 ml per minute. Collect all of the effluent and the washings into a beaker, and add about 1 g of sodium chloride. Titrate with 0.1 mol/l hydrochloric acid to a pH of 7.0, using a pH meter. Perform a blank test in the same manner, make any necessary correction, and calculate the total ion exchange capacity by the formula:

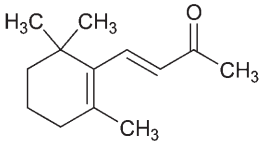
$$\text{Total ione exchange capacity (milliequivalent/g)} = \frac{\left(\begin{array}{l}\text{Volume (ml) of}\\ \text{0.1 mol/L hydrochloric acid}\\ \text{consumed in the blank test}\end{array}\right) - \left(\begin{array}{l}\text{Volume (ml) of}\\ \text{0.1 mol/L hydrochloric acid}\\ \text{consumed in the test}\end{array}\right)}{\text{Weight (g) of the sample} \times \dfrac{\text{Solid (\%)}}{100}} \times 5$$

Ionone

イオノン



α-Ionone



β-Ionone

$C_{13}H_{20}O$ Mol. Wt. 192.30

Mixture of (3*E*)-4-(2,6,6-trimethylcyclohex-2-en-1-yl)but-3-en-2-one (α-ionone) and (3*E*)-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-en-2-one (β-ionone) [8013-90-9]

Content Ionone contains not less than 90.0% of ionone ($C_{13}H_{20}O$).

Description Ionone is a colorless to light yellow transparent liquid having a characteristic odor.

Identification Proceed as directed in the Liquid Film Method under Infrared Spectrophotometry. Ionone exhibits absorption bands at about 2960 cm^{-1}, 1696 cm^{-1}, 1674 cm^{-1}, 1363 cm^{-1}, 1255 cm^{-1}, and 982 cm^{-1}.

Purity

(1) Refractive index n_D^{20}: 1.497–1.522.

(2) Specific gravity 0.930–0.948.

(3) Clarity of solution Clear (1.0 ml, 70% (vol) ethanol 4.0 ml).

Assay Weigh accurately about 1.3 g of Ionone, and proceed as directed in Method 2 in the Aldehyde and Ketone Content Test in the Flavoring Substances Tests. In the test, boil the mixture for 1 hour before titrating.

Each ml of 0.5 mol/L hydrochloric acid = 96.15 mg of $C_{13}H_{20}O$

Iron Lactate

乳酸鉄

Content Iron Lactate contains 15.5–20.0% of iron (Fe = 55.85).

Description Iron Lactate occurs as a greenish white to yellow-brown powder or lumps having a slight, characteristic odor.

Identification

(1) Ignite 0.5 g of Iron Lactate at 450–550°C for 1 hour. To the residue, add 3 ml of diluted hydrochloric acid (1 in 2), and dissolve by heating. The solution responds to all tests for Ferric Salt in the Qualitative Tests.

(2) Iron Lactate responds to all tests for Lactate in the Qualitative Tests.

Purity

(1) Clarity of solution Almost clear.

Test Solution Weigh 1.0 g of Iron Lactate, add 20 ml of water, and dissolve by heating in a water bath.

(2) Chloride Not more than 0.071% as Cl (0.10 g, Control

solution 0.01 mol/L hydrochloric acid 0.20 ml).

(3) Sulfate Not more than 0.48% as SO_4.

Sample Solution Weigh 0.20 g of Iron Lactate, dissolve it in 5 ml of water, and add water to make 10 ml. Use 2.0 ml of this solution as the sample solution.

Control Solution 0.40 ml of 0.005 mol/L sulfuric acid.

(4) Heavy metals Not more than 50 μg/g as Pb.

Test Solution Weigh 0.40 g of Iron Lactate in a porcelain dish, dissolve by adding 3 ml of aqua regia, and evaporate to dryness in a water bath. Dissolve the residue in 5 ml of diluted hydrochloric acid (1 in 2), and transfer into a separating funnel. Wash the porcelain dish twice with 5 ml of diluted hydrochloric acid (1 in 2) each time, and add the washings to the separating funnel. Wash the aqueous layer three times by shaking with diethyl ether (two 40-ml portions followed by one 20-ml portion). After each washing, allow to stand, and discard the diethyl ether layer. Dissolve 0.05 g of hydroxylamine hydrochloride in the aqueous layer, heat in a water bath for 10 minutes, add 1 drop of phenolphthalein TS, and add ammonia solution until a pink color develops. After cooling, add diluted hydrochloric acid (1 in 2) dropwise until the solution is almost colorless, then add 4 ml of diluted acetic acid (1 in 20), shake well, and add water to make 50 ml.

Control Solution Measure exactly 2.0 ml of Lead Standard Solution, transfer into a porcelain dish, add 3 ml of aqua regia, and then proceed as directed for the test solution.

(5) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 1.0 g of Iron Lactate, dissolve it in 25 ml of water, add 1 ml of sulfuric acid and 10 ml of sulfurous acid, evaporate to about 2 ml, and add water to make 10 ml. Use 5 ml of this solution as the test solution.

Apparatus Use Apparatus B.

(6) Readily carbonizable substances and butyrate

Weigh 0.5 g of powdered Iron Lactate, and mix with 1 ml of sulfuric acid. No color develops, and no butyric acid-like odor is evolved.

Assay Weigh accurately about 1 g of Iron Lactate, carbonize by heating gradually, add 1 ml of nitric acid, evaporate to dryness, taking care to prevent the solution from splattering, and ignite. To the residue, add 10 ml of hydrochloric acid (1 in 2), boil until the insoluble substance almost disappears, add 20 ml of water, and filter. Wash the insoluble substance with water, combine the filtrate and the washings, and add water to make exactly 100 ml. Measure exactly 25 ml of this solution, transfer into a flask with a ground-glass stopper, add 2 g of potassium iodide, immediately stopper tightly, and allow to stand in a dark place for 15 minutes. Add 100 ml of water, and titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: starch TS). Perform a blank test in the same manner.

Each ml of 0.1 mol/L sodium thiosulfate = 5.585 mg of Fe

Iron Sesquioxide

Diiron Trioxide
Iron Oxide Red

三二酸化鉄

Fe_2O_3 Mol. Wt. 159.69

Iron(III) oxide [1309-37-1]

Content Iron Sesquioxide contains not less than 98.0% of iron(III) oxide (Fe_2O_3).

Description Iron Sesquioxide occurs as a red to yellow-brown powder.

Identification To 1 g of Iron Sesquioxide, add 3 ml of diluted hydrochloric acid (1 in 2), and dissolve by heating. The solution responds to all tests for Ferric Salt in the Qualitative Tests.

Purity

(1) Water-soluble substances Not more than 0.75%.

Weigh 5.0 g of Iron Sesquioxide, add 200 ml of water, and boil for 5 minutes. Cool, add water to make 250 ml, and filter. Discard about 50 ml of the initial filtrate, measure exactly 100 ml of the subsequent filtrate, and evaporate to dryness on a water bath. Dry the residue at 105–110°C for 2 hours, and weigh.

(2) Heavy metals Not more than 40 μg/g as Pb.

Test Solution Weigh 1.0 g of Iron Sesquioxide, transfer into a porcelain dish, add 20 ml of diluted hydrochloric acid (1 in 2), and dissolve while heating. Evaporate to about 1 ml, add 6 ml of aqua regia, and evaporate to dryness on a water bath. Dissolve the residue in 5 ml of diluted hydrochloric acid (1 in 2), and transfer into a separating funnel. Wash the porcelain dish twice with 5 ml of diluted hydrochloric acid (1 in 2) each time, and add the washings to the separating funnel. Add 40 ml of diethyl ether, shake, allow to stand, and discard the diethyl ether layer. Repeat this procedure two more times with 40 ml of diethyl ether, followed by with 20 ml of diethyl ether. Dissolve 0.05 g of hydroxylamine hydrochloride in the aqueous layer, heat on a water bath for 10 minutes, add one drop of phenolphthalein TS, and add ammonia solution until a pink color develops. Cool, add diluted hydrochloric acid (1 in 2) dropwise until the solution is almost colorless, then add 4 ml of diluted acetic acid (1 in 20), shake well, and filter if necessary. Add water to the resulting solution to make 50 ml.

Control Solution To 4.0 ml of the Lead Standard Solution, add 20 ml of diluted hydrochloric acid (1 in 2), and proceed as directed for the test solution.

(3) Arsenic Not more than 2.0 μg/g as As_2O_3.

Test Solution Weigh 1.0 g of Iron Sesquioxide, add 30 ml of diluted hydrochloric acid (1 in 2) and 1 ml of nitric acid, dissolve while heating, evaporate to about 5 ml on a water bath, add 15 ml of water, and filter. Wash the insoluble substances on the filter paper three times with 5 ml of hot water each time, and combine the filtrate and the washings. Add 1 ml of sulfuric acid to this solution, and evaporate until white fumes are no longer evolved. Add 10 ml of sulfurous acid, evaporate to about 2 ml, and add water to make 5 ml.

Apparatus Use Apparatus B.

Assay Weigh accurately about 0.2 g of Iron Sesquioxide in an iodine bottle, add 5 ml of hydrochloric acid, and heat on

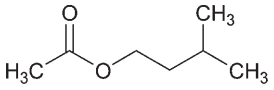
the water bath to make a solution. Add 25 ml of water and 3 g of potassium iodate, put a stopper tightly, and allow to stand for 15 minutes in a dark place. Add 100 ml of water, and titrate the liberated iodine with 0.1 mol/L sodium thiosulfate. Add 3 ml of starch TS when the color of the solution changes to a light yellow color near the endpoint. The endpoint is when the blue color developed by starch TS fades. Perform a blank test in the same manner, and make any necessary correction.

Each ml of 0.1 mol/L sodium thiosulfate = 7.984 mg of Fe_2O_3

Isoamyl Acetate

Isopentyl Acetate

酢酸イソアミル



$C_7H_{14}O_2$ Mol. Wt. 130.18

3-Methylbutyl acetate [123-92-2]

Content Isoamyl Acetate contains not less than 98.0% of isoamyl acetate ($C_7H_{14}O_2$).

Description Isoamyl Acetate is a colorless, transparent liquid having a banana-like odor.

Identification To 1 ml of Isoamyl Acetate, add 5 ml of ethanolic 10% potassium hydroxide TS, and heat in a water bath while shaking. The banana-like odor disappears, and an odor of 3-methyl-1-butanol evolves. After cooling, and add 10 ml of water and 0.5 ml of diluted hydrochloric acid (1 in 4). The solution responds to test (3) for Acetate in the Qualitative Tests.

Purity

(1) Refractive index n_D^{20}: 1.398–1.404.

(2) Specific gravity 0.872–0.878.

(3) Clarity of solution Clear (2.0 ml, 70% (vol) ethanol 4.0 ml).

(4) Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Weigh accurately about 0.5 g of Isoamyl Acetate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 65.09 mg of $C_7H_{14}O_2$

Isoamyl Alcohol

Isopentanol

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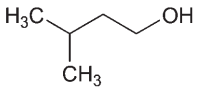


$C_5H_{12}O$ Mol. Wt. 88.15

3-Methylbutan-1-ol [123-51-3]

Content Isoamyl Alcohol contains not less than 98.0% of isoamyl alcohol ($C_5H_{12}O$).

Description Isoamyl Alcohol is a colorless to light yellow, transparent liquid having a characteristic odor.

Identification Determine the absorption spectrum of Isoamyl Alcohol as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

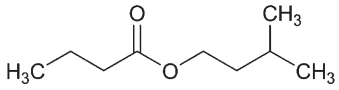
(1) Refractive index n_D^{20}: 1.404–1.410.

(2) Specific gravity d_{25}^{25}: 0.806–0.813.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavor Substance Tests. Use operating conditions (2).

Isoamyl Butyrate

酪酸イソアミル



$C_9H_{18}O_2$ Mol. Wt. 158.24

3-Methylbutyl butanoate [106-27-4]

Content Isoamyl Butyrate contains not less than 98.0% of isoamyl butyrate ($C_9H_{18}O_2$).

Description Isoamyl Butyrate is a colorless to light yellow, transparent liquid having a fruity odor.

Identification To 1 ml of Isoamyl Butyrate, add 5 ml of ethanolic 10% potassium hydroxide TS, and heat in a water bath while shaking. The fruity odor disappears, and an odor of 3-methyl-1-butanol is evolved. Cool, and acidify with diluted sulfuric acid (1 in 20). An odor of butyric acid is evolved.

Purity

(1) Refractive index n_D^{20}: 1.409–1.413.

(2) Specific gravity 0.863–0.867.

(3) Clarity of solution Clear (1.0 ml, 70% (vol) ethanol 5.0 ml).

(4) Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Weigh accurately about 0.8 g of Isoamyl Butyrate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 79.12 mg of $C_9H_{18}O_2$

Isoamyl Formate

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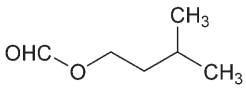

$C_6H_{12}O_2$ Mol. Wt. 116.16

3-Methylbutyl formate [110-45-2]

Content Isoamyl Formate contains not less than 95.0% of isoamyl formate ($C_6H_{12}O_2$).

Description Isoamyl Formate is a colorless, transparent liquid having a characteristic odor.

Identification Determine the absorption spectrum of Isoamyl Formate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Refractive index n_D^{20}: 1.396–1.399.

(2) Specific gravity 0.880–0.886.

(3) Clarity of solution Clear (2.0 ml, 70% (vol) ethanol 4.0 ml).

(4) Acid value Not more than 1.0 (Flavoring Substances Tests).

In the test, titrate to the first light pink color that persists for 10 seconds while cooling in ice water.

Assay Weigh accurately about 0.5 g of Isoamyl Formate, and perform the tests as directed in the Saponification Value and Acid Value Tests, respectively, in the Flavoring Substances Tests. Calculate the content by the formula:

$$\text{Content (\%) of isoamyl formate } (C_6H_{12}O_2) = \frac{\text{Saponification value} - \text{Acid value}}{561.1} \times 116.2$$

Isoamyl Isovalerate

3-Methylbutyl 3-Methylbutyrate

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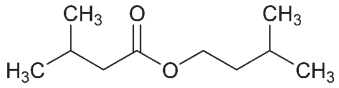


$C_{10}H_{20}O_2$ Mol. Wt. 172.26

3-Methylbutyl 3-methylbutanoate [659-70-1]

Content Isoamyl Isovalerate contains not less than 98.0% of isoamyl isovalerate ($C_{10}H_{20}O_2$).

Description Isoamyl Isovalerate is a colorless to light yellow, transparent liquid having a fruity odor.

Identification To 1 ml of Isoamyl Isovalerate, add 5 ml of ethanolic 10% potassium hydroxide TS, and heat in a water bath while shaking. The fruity odor disappears, and an odor of 3-methyl-1-butanol develops. Acidify with diluted sulfuric acid (1 in 20). An odor of isovaleric acid develops.

Purity

(1) Refractive index n_D^{20}: 1.411–1.414.

(2) Specific gravity 0.855–0.858.

(3) Clarity of solution Clear (1.0 ml, 70% (vol) ethanol 8.0 ml).

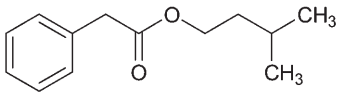
(4) Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Weigh accurately about 1 g of Isoamyl Isovalerate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 86.13 mg of $C_{10}H_{20}O_2$

Isoamyl Phenylacetate

フェニル酢酸イソアミル



$C_{13}H_{18}O_2$ Mol. Wt. 206.28

3-Methylbutyl 2-phenylacetate [102-19-2]

Content Isoamyl Phenylacetate contains not less than 98.0% of isoamyl phenylacetate ($C_{13}H_{18}O_2$).

Description Isoamyl Phenylacetate is a colorless, transparent liquid having a characteristic odor.

Identification Determine the absorption spectrum of Isoamyl Phenylacetate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

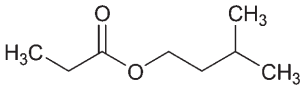
Purity

(1) Refractive index n_D^{20}: 1.485–1.487.

(2) Specific gravity 0.978–0.980.
(3) Clarity of solution Clear (1.0 ml, 80% (vol) ethanol 4.0 ml).
(4) Acid value Not more than 1.0 (Flavoring Substances Tests).
(5) Halogenated compounds Proceed as directed for Halogenated Compounds in the Flavoring Substances Tests.
Assay Weigh accurately about 1.5 g of Isoamyl Phenylacetate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.
Each ml of 0.5 mol/L ethanolic potassium hydroxide = 103.1 mg of $C_{13}H_{18}O_2$

Isoamyl Propionate

プロピオン酸イソアミル



$C_8H_{16}O_2$ Mol. Wt. 144.21
3-Methylbutyl propanoate [105-68-0]
Content Isoamyl Propionate contains not less than 98.0% of isoamyl propionate ($C_8H_{16}O_2$).
Description Isoamyl Propionate is a colorless to light yellow, transparent liquid having a characteristic odor.
Identification To 1 ml of Isoamyl Propionate, add 5 ml of ethanolic 10% potassium hydroxide TS, and heat in a water bath while shaking. The characteristic odor disappears, and an odor of 3-methyl-1-butanol is evolved. Cool, and acidify with diluted sulfuric acid (1 in 20). An odor of propionic acid is evolved.
Purity
(1) Refractive index n_D^{20}: 1.404–1.408.
(2) Specific gravity 0.868–0.872.
(3) Clarity of solution Clear (1.0 ml, 70% (vol) ethanol 4.0 ml).
(4) Acid value Not more than 1.0 (Flavoring Substances Tests).
Assay Weigh accurately about 0.7 g of Isoamyl Propionate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.
Each ml of 0.5 mol/L ethanolic potassium hydroxide = 72.11 mg of $C_8H_{16}O_2$

Isobutanol

Isobutyl Alcohol
2-Methylpropan-1-ol

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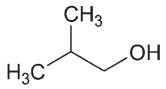
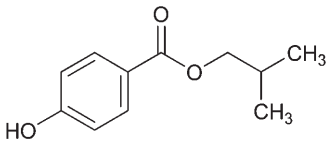


$C_4H_{10}O$ Mol. Wt. 74.12
2-Methylpropan-1-ol [78-83-1]
Content Isobutanol contains not less than 98.0% of isobutanol ($C_4H_{10}O$).
Description Isobutanol is a colorless, transparent liquid having a characteristic odor.
Identification Determine the absorption spectrum of Isobutanol as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.
Purity
(1) Refractive index n_D^{20}: 1.392–1.398.
(2) Specific gravity d_{25}^{25}: 0.799–0.801.
(3) Acid value Not more than 2.0 (Flavoring Substances Tests).
Assay Proceed as directed in Method 1 in the Gas Chromatographic Assay in the Flavor Substances Tests, using operating conditions (2).

Isobutyl *p*-Hydroxybenzoate

パラオキシ安息香酸イソブチル



$C_{11}H_{14}O_3$ Mol. Wt. 194.23
2-Methylpropyl 4-hydroxybenzoate [4247-02-3]
Content Isobutyl *p*-Hydroxybenzoate, when dried, contains not less than 99.0% of isobutyl *p*-hydroxybenzoate ($C_{11}H_{14}O_3$).
Description Isobutyl *p*-Hydroxybenzoate occurs as colorless crystals or as a white crystalline powder. It is odorless.
Identification
(1) Proceed as directed in Identification (1) for Butyl *p*-Hydroxybenzoate.
(2) To 0.05 g of Isobutyl *p*-Hydroxybenzoate, add 2 drops of acetic acid and 5 drops of sulfuric acid, and warm for 5 minutes. An odor of isobutyl acetate is evolved.
Purity
(1) Melting point 75–77°C.

(2) Free acid Not more than 0.55% as *p*-hydroxybenzoic acid.

Proceed as directed in Purity (2) for Butyl *p*-Hydroxybenzoate.

(3) Sulfate Not more than 0.024% as SO_4.

Proceed as directed in Purity (3) for Butyl *p*-Hydroxybenzoate.

(4) Heavy metals Not more than 10 μg/g as Pb.

Proceed as directed in Purity (4) for Butyl *p*-Hydroxybenzoate.

(5) Arsenic Not more than 4.0 μg/g as As_2O_3.

Proceed as directed in Purity (5) for Butyl *p*-Hydroxybenzoate.

Loss on Drying Not more than 0.5% (5 hours).

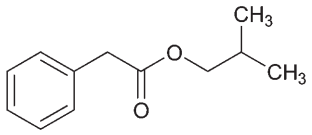
Residue on Ignition Not more than 0.10%.

Assay Proceed as directed in the Assay for Butyl *p*-Hydroxybenzoate.

Each ml of 1 mol/L sodium hydroxide = 194.2 mg of $C_{11}H_{14}O_3$

Isobutyl Phenylacetate

フェニル酢酸イソブチル



$C_{12}H_{16}O_2$ Mol. Wt. 192.25

2-Methylpropyl 2-phenylacetate [102-13-6]

Content Isobutyl Phenylacetate contains not less than 98.0% of isobutyl phenylacetate ($C_{12}H_{16}O_2$).

Description Isobutyl Phenylacetate is a colorless, transparent liquid having a characteristic odor.

Identification Determine the absorption spectrum of Isobutyl Phenylacetate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Refractive index n_D^{20}: 1.486–1.488.

(2) Specific gravity 0.987–0.991.

(3) Clarity of solution Clear (1.0 ml, 70% (vol) ethanol 8.0 ml).

(4) Acid value Not more than 1.0 (Flavoring Substances Tests).

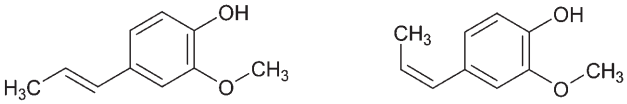
(5) Halogenated compounds Proceed as directed for Halogenated Compounds in the Flavoring Substances Tests.

Assay Weigh accurately about 1.5 g of Isobutyl Phenylacetate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 96.13 mg of $C_{12}H_{16}O_2$

Isoeugenol

イソオイゲノール



$C_{10}H_{12}O_2$ Mol. Wt. 164.20

2-Methoxy-4-(prop-l-en-1-yl)phenol [97-54-1]

Content Isoeugenol contains not less than 99.0% (vol) of isoeugenol ($C_{10}H_{12}O_2$).

Description Isoeugenol is a colorless to light yellow-brown, transparent liquid having a characteristic odor.

Identification Determine the absorption spectrum of Isoeugenol as directed in the liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Refractive index n_D^{20}: 1.572–1.577.

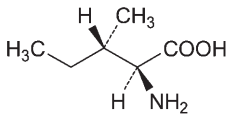
(2) Specific gravity 1.083–1.090.

(3) Clarity of solution Clear (2.0 ml, 70 (vol)% ethanol 4.0 ml).

Assay Proceed as directed for Phenol Content in the Flavoring Substances Tests. Instead of allowing to stand for 30 minutes, heat in a water bath for 30 minutes, and allow to cool to room temperature.

L-Isoleucine

L-イソロイシン



$C_6H_{13}NO_2$ Mol. Wt. 131.17

(*2S*,*3S*)-2-Amino-3-methylpentanoic acid [73-32-5]

Content L-Isoleucine, when calculated on the dried basis, contains 98.0–102.0% of L-isoleucine ($C_6H_{13}NO_2$).

Description L-Isoleucine occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor, and has a slightly bitter taste.

Identification To 5 ml of L-Isoleucine solution (1 in 1,000), add 1 ml of ninhydrin solution (1 in 1,000), and heat for 3 minutes. A purple color develops.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +38.0 to +41.5° (2 g, diluted hydrochloric acid (1 in 2), 50 ml, on the dried basis).

(2) Clarity and color of solution Colorless and almost clear (0.50 g, water 20 ml).

(3) pH 5.5–7.0 (1.0 g, water 100 ml).

(4) Chloride Not more than 0.021% as Cl (0.50 g, 0.01 mol/L hydrochloric acid 0.30 ml).

(5) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 1, warm to dissolve, Control solution Lead Standard Solution 2.0 ml).

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 2, Apparatus B).

Loss on Drying Not more than 0.30% (105°C, 3 hours).

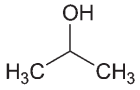
Residue on Ignition Not more than 0.10%.

Assay Weigh accurately about 0.25 g of L-Isoleucine, and proceed as directed in the Assay for DL-Alanine.

Each ml of 0.1 mol/L perchloric acid = 13.12 mg of $C_6H_{13}NO_2$

Isopropanol

イソプロパノール



C_3H_8O Mol. Wt. 60.10

Propan-2-ol [67-63-0]

Content Isopropanol contains not less than 99.7% of isopropanol (C_3H_8O).

Description Isopropanol is a colorless, transparent liquid having a characteristic odor.

Identification Determine the absorption spectrum of isopropanol as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Refractive index n_D^{20}: 1.374–1.380.

(2) Specific gravity 0.784–0.788.

(3) Free acids To 15.0 ml of Isopropanol, add 50 ml of freshly boiled and cooled water and 2 drops of phenolphthalein TS, then 0.20 ml of 0.01 mol/L sodium hydroxide solution. The solution develops red color.

Water Not more than 0.20% (10 g, Direct Titration).

Assay Proceed as directed in Method 1 in the Gas Chromatographic Assay in the Flavor Substance Tests, using operating conditions (2).

Isopropyl Citrate

クエン酸イソプロピル

Mixture of 1-methylethyl esters of 2-hydroxypropane-1,2,3-tricarboxylic acid and glycerol esters of fatty acids

Definition Isopropyl Citrate is a mixture of isopropyl citrate and glycerol esters of fatty acids.

Description Isopropyl Citrate occurs as colorless to white, oily or waxy substances. It is odorless. When it is allowed to stand, crystals may be deposited.

Identification

(1) To 3 g of Isopropyl Citrate, add 50 ml of sodium hydroxide solution (1 in 25), reflux for 1 hour, cool, and neutralize with diluted sulfuric acid (1 in 20). The solution responds to test (2) for Citrate in the Qualitative Tests.

(2) To 2 g of Isopropyl Citrate, add 50 ml of sodium hydroxide solution (1 in 25), reflux for 1 hour, and distill to collect 20 ml of the distillate. Place 8 g of chromium oxide, 15 ml of water, and 2 ml of sulfuric acid into a flask equipped with a reflux condenser. Add gradually 5 ml of the distillate in the flask through the reflux condenser, and reflux for 30 minutes. Cool, distill, and collect 2 ml of the distillate. To the distillate, add 3 ml of water and 10 ml of mercuric sulfate TS, and heat in a water bath for 3 minutes. A white to yellow precipitate is formed within 3 minutes.

Purity

(1) Heavy metals Not more than 30 μg/g as Pb.

Test Solution Weigh 2.0 g of Isopropyl Citrate, transfer into a crucible, and moisten with 2 ml of sulfuric acid. Heat gradually, and incinerate almost all. Cool, add 1 ml of sulfuric acid, heat gradually until fumes of sulfuric acid are no longer evolved. Ignite at 450–550°C until the residue is incinerated. After cooling, add 2 ml of hydrochloric acid and 0.4 ml of nitric acid to the residue, and evaporate to dryness on a water bath. To the residue, add 2 ml of diluted nitric acid (1 in 10) and 30 ml of water, dissolve while heating, and cool. Add 1 drop of phenolphthalein TS, and add ammonia TS dropwise until the color of the solution becomes slightly pink. Add water to make a sample solution of 50 ml. Measure 25 ml of the sample solution, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution To 3.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(2) Lead Not more than 10 μg/g as Pb.

Test Solution Use 10 ml of the sample solution prepared in Purity (1).

Control Solution To 1.0 ml of Lead Standard Solution, add water to make 25 ml.

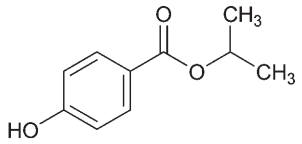
Procedure Proceed as directed in Method 1 in the Lead Limit Test.

(3) Arsenic Not more than 1.3 μg/g as As_2O_3 (1.5 g, Method 3, Apparatus B).

Residue on Ignition Not more than 0.30%.

Isopropyl *p*-Hydroxybenzoate

パラオキシ安息香酸イソプロピル



$C_{10}H_{12}O_3$ Mol. Wt. 180.20
1-Methylethyl 4-hydroxybenzoate [4191-73-5]

Content Isopropyl *p*-Hydroxybenzoate, when dried, contains not less than 99.0% of isopropyl *p*-hydroxybenzoate ($C_{10}H_{12}O_3$).

Description Isopropyl *p*-Hydroxybenzoate occurs as colorless crystals or as a white crystalline powder. It is odorless.

Identification

(1) Proceed as directed in Identification (1) for Butyl *p*-Hydroxybenzoate.

(2) To 0.05 g of Isopropyl *p*-Hydroxybenzoate, add 2 drops of acetic acid and 5 drops of sulfuric acid, and warm for 5 minutes. An odor of Isopropyl Acetate is evolved.

Purity

(1) Melting point 84–86°C.

(2) Free acid Not more than 0.55% as *p*-hydroxybenzoic acid.

Proceed as directed in Identification (2) for Butyl *p*-Hydroxybenzoate.

(3) Sulfate Not more than 0.024% as SO_4.

Proceed as directed in Identification (3) for Butyl *p*-Hydroxybenzoate.

(4) Heavy metals Not more than 10 μg/g as Pb.

Proceed as directed in Identification (4) for Butyl *p*-Hydroxybenzoate.

(5) Arsenic Not more than 4.0 μg/g as As_2O_3.

Proceed as directed in Identification (5) for Butyl *p*-Hydroxybenzoate.

Loss on Drying Not more than 0.50% (5 hours).

Residue on Ignition Not more than 0.10% .

Assay Proceed as directed in the Assay for Butyl *p*-Hydroxybenzoate.

Each ml of 1 mol/L sodium hydroxide = 180.2 mg of $C_{10}H_{12}O_3$

Kansui

かんすい

Definition Kansui contains one or more of the following food additives: "Potassium Carbonate, Anhydrous," "Sodium Carbonate," "Sodium Hydrogen Carbonate," and "Potassium or Sodium Salts of Metaphosphoric Acid, Phosphoric Acids, Polyphosphoric Acid, and Pyrophosphoric Acid."*

There are three types of Kansui: Solid Kansui, Liquid Kansui, and Diluted Powder Kansui, which is diluted with flour.

Solid Kansui

固形かんすい

Description Solid Kansui occurs as colorless to white crystals, powder, or lumps, or as a mixture of these.

Identification

(1) A solution of Solid Kansui (1 in 10) is alkaline.

(2) A solution of Solid Kansui (1 in 10) responds to test (1) for Potassium Salt or to test (1) for Sodium Salt in the Qualitative Tests.

(3) If Solid Kansui contains a carbonate or hydrogen carbonate salt, a solution of it (1 in 10) responds to test (1) for Carbonate in the Qualitative Tests,

(4) If Solid Kansui contains a phosphate salt, a solution of it (1 in 10) responds to test (2) for Phosphate in the Qualitative Tests, when made acidic with the addition of diluted nitric acid (1 in 10).

Purity Weigh 10 g of Solid Kansui, and dissolve it in water to make 200 ml. Refer to this solution as solution A.

(1) Clarity of solution Very slightly turbid.

Test Solution Use 20 ml of solution A.

(2) Alkali hydroxide Measure 40 ml of solution A, add 50 ml of barium chloride solution (3 in 25) and water to make 100 ml, shake vigorously, and filter. Measure 50 ml of the filtrate, and add 3 drops of 0.1 mol/L hydrochloric acid and 3 drops of phenolphthalein TS. No pink color develops.

(3) Chloride Not more than 0.35% as Cl (Solution A 1.0 ml, Control solution 0.01 mol/L hydrochloric acid 0.50 ml).

(4) Silicate Measure 10 ml of solution A, add 1 drop of phenolphthalein TS, then add diluted hydrochloric acid (1 in 4) until the pink color disappears, and heat in a water bath for 15 minutes. Cool, and if the solution is pink in color, add diluted hydrochloric acid (1 in 4) until the pink color disappears. To this solution, add 1 drop of methylene blue TS and 10 ml of ammonium chloride saturated solution, and allow to stand for 2 hours. No colored precipitate or colored turbidity appears.

(5) Heavy metals Not more than 40 μg/g as Pb.

Test Solution Measure 10 ml of solution A, add 3 ml of diluted hydrochloric acid (1 in 4), and evaporate to dryness on a water bath. Dissolve the residue with 2 ml of diluted acetic acid (1 in 20) and 20 ml of water, and add water to make 50 ml.

Control Solution Measure exactly 2 ml of Lead Standard Solution, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(6) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution 10 ml of solution A.

Apparatus Use Apparatus B.

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* "Potassium or Sodium Salts of Metaphosphoric Acid, Phosphoric Acids, Polyphosphoric Acid, and Pyrophosphoric Acid" refers to the following 13 substances: Dipotassium Hydrogen Phosphate, Disodium Dihydrogen Pyrophosphate, Disodium Hydrogen Phosphate, Potassium Dihydrogen Phosphate, Potassium Metaphosphate, Potassium Polyphosphate, Potassium Pyrophosphate, Sodium Dihydrogen Phosphate, Sodium Metaphosphate, Sodium Polyphosphate, Sodium Pyrophosphate, Tripotassium Phosphate, Trisodium Phosphate.

Liquid Kansui

液状かんすい

Description Liquid Kansui is a colorless, clear liquid.
Identification Proceed as directed in Identification (1) through (4) for Solid Kansui.
Purity

(1) Specific gravity 1.20–1.33.

(2) For tests (i), (ii), (iii), (iv), and (v), use solution B prepared as follows: Measure the volume of Liquid Kansui, indicated in Table 1, that corresponds to its specific gravity, and add water to make 200 ml.

(i) Alkali hydroxide Measure 40 ml of solution B, and proceed as directed in Purity (2) for Solid Kansui.

(ii) Chloride Not more than 0.35% as Cl.

Measure 1.0 ml of solution B, and proceed as directed in Purity (3) for Solid Kansui.

(iii) Silicate Measure 10 ml of solution B, and proceed as directed in Purity (4) for Solid Kansui.

(iv) Heavy metals Not more than 40 μg/g as Pb for solid.

Measure 10 ml of solution B, and proceed as directed in Purity (5) for Solid Kansui.

(v) Arsenic Not more than 4.0 μg/g as As_2O_3 for solid.

Measure 10 ml of solution B, and proceed as directed in Purity (6) for Solid Kansui.

Table 1

Specific gravity	Sample volume (ml)	Specific gravity	Sample volume (ml)	Specific gravity	Sample volume (ml)
1.20	39.8	1.25	31.0	1.30	25.4
1.21	37.6	1.26	29.8	1.31	24.4
1.22	35.6	1.27	28.6	1.32	23.6
1.23	34.0	1.28	27.4	1.33	22.8
1.24	32.4	1.29	26.4		

Diluted Powder Kansui

希釈粉末かんすい

Description Diluted Powder Kansui occurs as a homogeneous, white to light yellow powder.
Identification

(1) To 1 g of Diluted Powder Kansui, add 1 drop of iodine TS. A purple color develops.

(2) To 10 g of Diluted Powder Kansui, add 50 ml of water, shake well, filter, and proceed as directed in Identification (1) through (4) for Solid Kansui, using the filtrate.

Purity

(1) Specific gravity Weigh 60 g of Diluted Powder Kansui, add water to make 200 ml, shake well, and filter. The specific gravity of the filtrate is 1.12–1.17.

(2) Insoluble substances Not more than 2.0%.

Weigh 0.50 g of Diluted Powder Kansui, add 100 ml of sodium hydroxide solution (1 in 100), boil for 15 minutes, and allow to stand for 30 minutes. No precipitate is observed. If a precipitate is observed, filter through a filter paper for quantitative analysis (5C), wash with water until the washings are no longer alkaline, and ignite the residue together with the filter paper at about 550°C to constant weight. Weigh the residue.

(3) For tests (i), (ii), and (iii), use solution C prepared as follows: Measure the volume of the filtrate obtained in Purity (1), indicated in Table 2, that corresponds to the specific gravity determined in Purity (1), and add water to make 100 ml.

(i) Alkali hydroxide Measure 40 ml of solution C, and proceed as directed in Purity (2) for Solid Kansui.

(ii) Chloride Not more than 0.35% as Cl for water-soluble solid.

Measure 1.0 ml of solution C, and proceed as directed in Purity (3) for Solid Kansui.

(iii) Silicate Measure 10 ml of solution C, and proceed as directed in Purity (4) for Solid Kansui.

Table 2

Specific gravity	Filtrate volume (ml)	Specific gravity	Filtrate volume (ml)	Specific gravity	Filtrate volume (ml)
1.12	34.3	1.14	29.2	1.16	25.4
1.13	31.7	1.15	27.2	1.17	23.7

(4) Heavy metals Not more than 30 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 3.0 ml).

(5) Arsenic Not more than 2.5 μg/g as As_2O_3 (2.0 g, Method 3, Apparatus B).

Standard Color Use 5 ml of Arsenic Standard Solution for its preparation.

Kaolin

カオリン

Definition Kaolin is a refined product of natural hydrated aluminum silicate.
Description Kaolin occurs as a white or whitish powder.
Identification

(1) Mix 0.2 g of Kaolin with 1.5 g of a 1:1 mixture of anhydrous sodium carbonate/anhydrous potassium carbonate, transfer to a platinum or nickel crucible, and heat until completely fused. After cooling, add 5 ml of water, allow to stand for about 3 minutes, and heat gently the bottom of the crucible to remove the fused mixture like a lump. Transfer the fused lump together with water into a beaker, and add hydrochloric acid in small portions until effervescence ceases. Add another 10 ml of hydrochloric acid, and evaporate to dryness on a water bath. Add 200 ml of water, boil, and filter. Transfer the gelatinous residue to a platinum dish, and add 5 ml of hydrofluoric acid. The residue dissolves. Then heat the solution. It almost completely evaporates.

(2) The filtrate obtained in Identification (1) responds to all tests for Aluminum Salt in the Qualitative Tests.

(3) To 8 g of Kaolin, add 5 ml of water, and mix well. The resulting mixture has a plasticity.

Purity

(1) pH 6.0–8.0.

Weigh 10.0 g of Kaolin, add 100 ml of water, and heat on a water bath for 2 hours while shaking occasionally and replenishing the lost water. After cooling, filter with suction, using a filter holder equipped with a 47-mm diameter membrane filter (0.45 μm in pore diameter). If the filtrate is turbid, repeat the filtration with suction through the same filter. Wash the container and the residue on the filter with water, combine the filtrate and the washings, and add water to make 100 ml. Refer to this solution as solution A. Measure the pH of solution A.

(2) Water-soluble substances Not more than 0.30 %.

Measure 50 ml of solution A prepared in Purity (1), evaporate to dryness, and dry the residue at 105°C for 2 hours. Weigh the residue.

(3) Sulfuric acid-soluble substances Not more than 2.0%.

Weigh 1.0 g of Kaolin, add 20 ml of diluted sulfuric acid (1 in 15), shake for 15 minutes, and filter. Wash the container and the residue on the filter paper with a small amount of water, combine the filtrate and the washings, and add water to make 20 ml. Measure 10 ml of this solution, evaporate to dryness, and ignite at 550°C to constant weight. Weigh the residue.

(4) Heavy metals Not more than 10 μg/g as Pb.

Test Solution Weigh 4.0 g of Kaolin, add 70 ml of water, and add 10 ml of hydrochloric acid and 5 ml of nitric acid, heat on a water bath for 15 minutes while shaking, cool, and filter. Wash the residue with water, combine the filtrate and the washings, and add water to make 100 ml. Measure 50 ml of this solution, evaporate to dryness on a water bath, dissolve the residue in 2 ml of diluted acetic acid (1 in 20) and 20 ml of water, filter if necessary, and add water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(5) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.50 g of Kaolin, add 2.5 ml of water and 0.5 ml of sulfuric acid, and heat on a hot plate until white fumes are evolved. Cool, and add water to make 5 ml.

Apparatus Use Apparatus B.

(6) Foreign matter Weigh 5 g of Kaolin, add 300 ml of water, shake, and allow to stand for 30 seconds. Discard most of the solution containing fine particles by decantation, and press the portion remaining on the bottom of the container using a glass rod with a flat tip. There is no sound of sand.

Loss on Ignition Not more than 15.0% (550°C, constant weight).

Karaya Gum

カラヤガム

[9000-36-6]

Definition Karaya Gum is obtained from the exudate of the karaya tree *Sterculia urens* Roxburgh or the silk cotton tree *Cochlospermum gossypium* de Candolle and consists mainly of polysaccharides.

Description Karaya Gum occurs as a light gray to light red-brown powder or as light yellow to light red-brown lumps having an acetic acid odor.

Identification

(1) To 1 g of a powder of Karaya Gum, add 50 ml of water, and mix it. A viscous liquid is produced, and it is acidic.

(2) Suspend 0.4 g of a powder of Karaya Gum in 6 ml of ethanol, and add 4 ml of water while stirring. The powder swells.

Purity

(1) Hydrochloric acid-insoluble substances Not more than 3.0 %.

Weigh accurately about 5 g of Karaya Gum, and transfer into a Erlenmeyer flask containing 100 ml of diluted hydrochloric acid (10 in 100) to dissolve. Cover with a watch dish, heat gradually until the gum dissolves, and boil. While warming, filter with suction through a glass filter (1G3), previously dried at 105°C for 1 hour and weighed. Wash the residue well with warm water. Dry the residue with the glass filter at 105°C for 1 hour, and weigh.

(2) Heavy metals Not more than 40 μg/g as Pb (0.50 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(3) Lead Not more than 10 μg/g as Pb (1.0 g, Method 1).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(5) Starch and dextrin Add 0.2 g of Karaya Gum to 10 ml of water, and boil. Allow to cool, and add 2 drops of Iodine TS. No dark blue or red purple color develops.

Loss on Drying Not more than 20.0% (105°C, 5 hours).

Ash Not more than 8.0%.

Acid-insoluble Ash Not more than 1.0%.

Microbial Limits Proceed as directed in the Microbial Limit Tests. The total bacterial count is not more than 10,000/g, and *Escherichia coli* is negative.

Lac Color

ラック色素

Definition Lac Color is obtained from the secretion of lac scale insects, *Laccifer* spp., and consists mainly of laccaic acids.

Color Value The color value of Lac Color ($E^{10\%}_{1cm}$) is not less than 1,000 and is in the range of 95–115% of the labeled value.

Description Lac Color occurs as a red to dark red powder or granules having a slight characteristic odor.

Identification

(1) Weigh the equivalent of 0.05 g of Lac Color with a Color Value 1,000, and dissolve it in 500 ml of 0.1 mol/L sodium hydroxide solution. A purplish red color develops.

(2) To 10 ml of the solution prepared in Identification (1), add 20 ml of 0.1 mol/L hydrochloric acid. The solution turns orange and exhibits an absorption maximum at a wavelength of 485–495 nm.

(3) Weigh the equivalent of 0.1 g of Lac Color with a Color Value 1,000, and dissolve it in 10 ml of ethanol. Centrifuge this solution, and use the supernatant as the test

solution. Analyze a 2-µl portion of the test solution by paper chromatography using a 4:2:1 mixture of 1-butanol/water/acetic acid as the developing solvent. No control solution is used. Use No. 2 filter paper for chromatography. Stop the development when the solvent front has ascended to a point about 10 cm above the original line, and air-dry. A yellowish red to red spot is observed at an R_f value of about 0.4. An additional spot may be observed at an R_f value of about 0.2. When sprayed with ammonia solution, these spots turn dark red-purple.

Purity

(1) Heavy metals Not more than 40 µg/g as Pb (0.50 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 8.0 µg/g (1.25 g , Method 1).

(3) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50g, Method 3, Apparatus B).

Color Value Test Weigh accurately an appropriate amount of Lac Color so that its absorbance is in the range of 0.3–0.7. Dissolve it in 20 ml of anhydrous sodium carbonate solution (1 in 200), and add water to make exactly 100 ml. Measure exactly 5 ml of this solution, and add 0.1 mol/L hydrochloric acid to make exactly 50 ml. Use the last solution as the test solution. Centrifuge the solution if necessary, and use the supernatant for the test. Perform the test as directed in the Color Value Test according to the following operating conditions.

Operating Conditions

Reference solution: 0.1 mol/L hydrochloric acid.

Determination wavelength: Maximum absorption wavelength of 485–495 nm.

Lactic Acid

乳酸

Definition Lactic Acid is a mixture of lactic acid and condensation polymers of lactic acid.

Content Lactic Acid contains the equivalent of not less than 40.0% of lactic acid ($C_3H_6O_3$ = 90.08), and the equivalent of 95–105% of the labeled content.

Description Lactic Acid occurs as a white to light yellow solid or as a colorless to light yellow, clear liquid. It is odorless or has little or no unpleasant odor. It has an acid taste.

Identification

(1) A solution of Lactic Acid (1 in 10) is acidic.

(2) Lactic Acid responds to the test for Lactate in the Qualitative Tests.

Purity Dissolve Lactic Acid in water, and heat in a water bath if necessary to make a solution with the concentration of 40.0%. Refer to the obtained solution as solution A. Perform tests (1) through (4) specified below using solution A.

(1) Clarity of solution Concentrate solution A to make a solution with the concentration of 80%. Weigh 10 g of solution A, previously evaporated to the concentration of 80%, add 12 ml of diethyl ether, and mix. The resulting solution is clear, or passes the following test:

Filter the solution through a glass filter (G3), wash the residue three times with 10 ml of diethyl ether each time, then once with 10 ml of acetone, dry the residue together with the filter under reduced pressure at 50°C for 14 hours. The residue weighs not more than 0.07 g. (Diethyl ether-insoluble substances: Not more than 0.7% for 80% lactic acid)

(2) Citric acid, oxalic acid, tartaric acid, and phosphoric acid Weigh 2.0 g of solution A, add 8 ml of water and 40 ml of calcium hydroxide TS, and boil for 2 minutes. No turbidity appears.

(3) Sulfate Not more than 0.010% as SO_4 for 80% lactic acid (solution A 2.0 g, Control Solution 0.005 mol/L sulfuric acid 0.20 ml).

(4) Cyanide Weigh 2.0 g of solution A, and add water to make 100 ml. Measure 10 ml of this solution, transfer into a Nessler tube, add 1 drop of phenolphthalein TS, and add sodium hydroxide solution (1 in 10) until the solution is pink. Add another 1.5 ml of sodium hydroxide solution (1 in 10) and water to make 20 ml, and heat in a water bath for 10 minutes. Cool, neutralize with diluted acetic acid (1 in 20), and after the pink color of the solution disappears, add another drop. Add 10 ml of phosphate buffer (pH 6.8) and 0.25 ml of chloramine T TS, stopper tightly, shake gently, and allow to stand for 3–5 minutes. Add 15 ml of pyridine–pyrazolone TS and water to make 50 ml, and allow to stand at about 25°C for 30 minutes. The solution does not turn blue.

(5) Heavy metals Not more than 10 µg/g as Pb for 80% lactic acid.

Test Solution Weigh 4.0 g of solution A, add 1 drop of phenolphthalein TS, and add ammonia TS dropwise until the solution is slightly pink. Add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(6) Iron Not more than 10 µg/g as Fe for 80% lactic acid (solution A 2.0 g, Method 1, Control Solution Iron Standard Solution 1.0 ml).

(7) Arsenic Not more than 4.0 µg/g as As_2O_3 for 80% lactic acid.

Test Solution Weigh 2.0 g of solution A, and add water to make 10 ml. Use 5 ml of this solution as the test solution.

Apparatus Use Apparatus B.

(8) Volatile fatty acids Weigh 5.0 g of solution A, and heat on a water bath. This solution emits no butyric acid-like odor.

(9) Methanol Not more than 0.20% (v/w) as CH_3OH for 80% lactic acid.

Tests Solution Weigh 10 g of solution A, add 8 ml of water and 5 g of calcium carbonate, and distill the solution. To about 5 ml of the initial distillate, add water to make 100 ml.

Control Solution To 1.0 ml of methanol, add water to 100 ml. Then add water to 1.0 ml of the resulting solution to make 100 ml.

Procedure Measure 1.0 ml of the test solution, add 0.1 ml of phosphoric acid (1 in 20) and 0.2 ml of potassium permanganate solution (1 in 300), allow to stand for 10 minutes, add 0.4 ml of anhydrous sodium sulfite solution (1 in 5) and 3 ml of sulfuric acid, and then add 0.2 ml of chromotropic acid TS. The resulting solution is not darker in color than the control solution treated in the same manner as the test solution.

(10) Readily carbonizable substances Weigh 5.0 g of solution A, and adjust to 15°C. Place gradually this solution on the surface of 5-ml sulfuric acid pre-adjusted to 15°C, and

keep at 15°C. No circular band is formed on the boundary surface within 15 minutes. Or, a circular band formed on the boundary surface within 15 minutes is not dark gray.

Residue on Ignition Not more than 0.10%.

Assay Weigh accurately an amount equivalent to about 1.2 g of lactic acid, add exactly 20 ml of 1 mol/L sodium hydroxide, and add water to make 100 ml. Heat on a water bath for 20 minutes, and while hot, titrate the excess alkali with 0.5 mol/L sulfuric acid (indicator: 1–2 drops of phenolphthalein TS). Perform a blank test in the same manner.

Each ml of 1 mol/L sodium hydroxide = 90.08 mg of $C_3H_6O_3$

Lanolin

ラノリン

Definition Lanolin is obtained from the waxy substance of sheep wool and consists mainly of esters of higher alcohols and α-hydroxy acids.

Description Lanolin occurs as a slightly yellow to pale yellow-brown viscous substance in paste form. It has a slight characteristic odor.

Identification Pour carefully 1 ml of a solution of Lanolin in cyclohexane (1 in 50) on the surface of 2 ml of sulfuric acid. The boundary surface of the two liquids turns red-brown. The sulfuric acid phase has a green fluorescence.

Purity

(1) Melting point 37–44°C (Melting Point Determination, Procedure for Class 2 Substances).

(2) Acid value Not more than 1.0.

Test Solution Weigh accurately about 5 g of Lanolin, and dissolve it in 80 ml of a 1:1 mixture of ethanol/xylene.

Procedure Proceed as directed in the Acid Value Test in the Fats and Related Substances Tests. Titration should be done while warm.

(3) Iodine value 18–36.

Test Solution Weigh accurately about 0.8 g of Lanolin in a 500-ml stoppered flask, and dissolve it in 10 ml of cyclohexane.

Procedure Proceed as directed in the Iodine Value Test in the Fats and Related Substances Tests.

(4) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50g, Method 3, Apparatus B).

Residue on Ignition Not more than 0.10%.

Lecithin

レシチン

Definition Lecithin is obtained from oil seeds or animal materials. It consists mainly of phospholipids.

Description Lecithin occurs as a white to brown powder or granules or as light yellow to dark brown lumps or viscous fluid. It has a slight, characteristic odor.

Identification

(1) Proceed as directed in Identification (1) for Enzymatically Decomposed Lecithin.

(2) To 0.5 g of Lecithin, add 5 ml of diluted hydrochloric acid (1 in 2), heat in a water bath for 2 hours, and filter. Use this solution as the test solution. Analyze 10 μl of the test solution by paper chromatography, using choline chloride solution (1 in 200) as the control solution and a 4:2:1 mixture of butanol/water/acetic acid as the developing solvent. For the filter paper, use a No. 2 filter paper for chromatography. Stop the development when the developing solvent ascends to a point about 25 cm above the base line, air-dry, spray with Dragendorff TS to let color develop, and observe in daylight. A red-orange spot corresponding to the spot obtained from the control solution is observed.

Purity

(1) Acid value Not more than 40.

Test Solution Weigh accurately about 2 g of Lecithin, dissolve it in 50 ml of petroleum ether, and add 50 ml of ethanol.

Procedure Proceed as directed in the Acid Value Test in the Fats and Related Substances Tests, using this solution as the test solution.

(2) Toluene-insoluble substances Not more than 0.30%.

Weigh accurately about 10 g of Lecithin, dissolve it in 100 ml of toluene, and filter the insoluble substances through a crucible-type glass filter (1G4). Wash several times with 25 ml of toluene, dry together with the glass filter at 105°C for 1 hour, and allow to cool in a desiccator. Weigh accurately the glass filter containing the residue.

(3) Acetone-soluble substances Not more than 40%.

Weigh accurately about 2 g of Lecithin in a scaled, 50-ml centrifuge tube with a ground-glass stopper, dissolve by adding 3 ml of petroleum ether, add 15 ml of acetone, and stir well, and allow to stand in ice water for 15 minutes. Then proceed as directed in Purity (2) for Enzymatically Decomposed Lecithin.

(4) Peroxide value Not more than 10.

Weigh accurately about 5 g of Lecithin, transfer into a 250-ml Erlenmeyer flask with a ground-glass stopper, add 35 ml of a 2:1 mixture of chloroform/acetic acid, and dissolve to a transparent state while shaking gently. Then proceed as directed in Purity (3) for Enzymatically Decomposed Lecithin.

(5) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

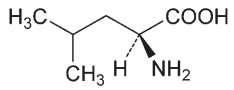
(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Loss on Drying Not more than 2.0%.

Proceed as directed in the Loss on Drying Test for Enzymatically Decomposed Lecithin.

L-Leucine

L-ロイシン



$C_6H_{13}NO_2$ Mol. Wt. 131.17
(2*S*)-2-Amino-4-methylpentanoic acid [61-90-5]

Content L-Leucine, when calculated on the dried basis, contains 98.0–102.0% of L-leucine ($C_6H_{13}NO_2$).

Description L-Leucine occurs as white crystals or crystalline powder. It is odorless or has a very slight characteristic odor. It has a very slight bitter taste.

Identification

(1) To 5 ml of a solution of L-Leucine (1 in 1,000), add 1 ml of ninhydrin solution (1 in 50), and heat for 3 minutes in a water bath. A bluish purple color develops.

(2) Dissolve 0.3 g of L-Leucine in 10 ml of water while warming, and add 10 drops of diluted hydrochloric acid (1 in 4) and 2 ml of sodium nitrite solution (1 in 10). A colorless gas emitted while bubbles are formed.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +14.5 to +16.5°.

Weigh accurately about 4 g of L-Leucine, and dissolve it in 6 mol/L hydrochloric acid to make exactly 100 ml. Measure the angular rotation of this solution, and calculate on the dried basis.

(2) Clarity and color of solution Colorless and clear (1.0 g, water 50 ml).

(3) pH 5.5–6.5 (1.0 g, water 100 ml).

(4) Chloride Not more than 0.1% as Cl (0.070 g, Control solution 0.01 mol/L hydrochloric acid 0.20 ml).

(5) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.5 g, Method 3, Apparatus B).

Loss on Drying Not more than 0.30% (105°C, 3 hours).

Residue on Ignition Not more than 0.10%.

Assay Weigh accurately about 0.3 g of L-Leucine, and proceed as directed in the Assay for L-Asparagine.

Each ml of 0.1 mol/L perchloric acid =13.12 mg of $C_6H_{13}NO_2$

Licorice Extract

カンゾウ抽出物

Definition Licorice Extract is obtained from the roots or rhizomes of *Glycyrrhiza uralensis* Fischer, *Glycyrrhiza inflata* Batalin, or *Glycyrrhiza glabra* Linné, or their allied plants. It consists mainly of glycyrrhizic acid. There are two types of products: Licorice Extract, Crude and Licorice Extract, Purified.

Licorice Extract, Crude

粗製物

Content Crude Licorice Extract, when calculated on the dried basis, contains not less than 5.0% and less than 50.0% of glycyrrhizic acid ($C_{42}H_{62}O_{16}$ = 822.93).

Description Crude Licorice Extract occurs as a yellow to blackish brown powder, flakes, granules, lumps, paste, or liquid.

Identification Prepare a test solution by dissolving 0.01–0.10 g of Crude Licorice Extract in 10 ml of 50% ethanol. Prepare a control solution by dissolving 5 mg of glycyrrhizic acid for thin-layer chromatography in 10 ml of 50% ethanol. Analyze a 2-μl portion each of both solutions by thin-layer chromatography using a 7:2:1 mixture of 1-butanol/water/acetic acid as the developing solvent. Use a thin-layer plate coated with fluorescent silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the original line, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm) in a dark place. One of the spots from the test solution corresponds in color tone and R_f value to the dark purple spot of glycyrrhizic acid from the control solution.

Purity

(1) Insoluble substances Dissolve 5.0 g of Crude Licorice Extract, previously dried, in 100 ml of 50% ethanol, filter through a filter paper whose weight is known, wash the residue on the filter paper with 50% ethanol, and dry the residue with the filter paper at 105°C for 5 hours. The residue weighs not more than 1.25 g.

(2) pH 2.5–7.0 (1.0 g of a solid sample or previously dried paste or liquid sample, 50% ethanol 100 ml).

(3) Heavy metals Not more than 10μg/g as Pb (2.0 g of a solid sample or previously dried paste or liquid sample, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 2.0 μg/g as As_2O_3 (1.0 g of a solid sample or previously dried paste or liquid sample, Method 3, Apparatus B).

Loss on Drying

Solid sample: Not more than 8.0% (105°C, 2 hours).
Paste or liquid sample: Not more than 60.0% (105°C, 5 hours).

Residue on Ignition Not more than 15.0% (dry the sample before use if it is paste or liquid).

Assay

Test Solution Weigh accurately 0.04–0.4 g of Crude Licorice Extract, and dissolve it in 50% ethanol to make exactly 100 ml.

Standard Solution Weigh accurately about 0.02 g of Glycyrrhizic Acid Reference Standard—the water content should be measured previously—and dissolve it in 50% ethanol to make exactly 100 ml.

Procedure Analyze 20 μl portions of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Measure the peak areas (A_T and A_S) of glycyrrhizic acid for the test solution and the standard solution, and calculate the content by the formula:

$$\text{Content (\%) of glycyrrhzic acid } (C_{42}H_{62}O_{16}) = \frac{\left(\begin{array}{c}\text{Anhydrous basis weight (g) of}\\ \text{Glycyrrhizic Acid Reference Standard}\end{array}\right)}{\text{Dry basis weight (g) of the sample}} \times \frac{A_T}{A_S} \times 100$$

Operating Conditions

Detector: Ultraviolet spectrophotometer (determination wavelength: 254 nm).

Column: A stainless steel tube of 4–6 mm internal diameter and 15–30 cm length.

Column packing material: 5- to 10-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 40°C.

Mobile phase: A 3:2 mixture of diluted acetic acid (1 in 50)/acetonitrile.

Flow rate: Adjust so that the retention time of glycyrrhizic acid is about 10 minutes.

Column selection: Use a column capable of eluting glycyrrhizic acid and propyl *p*-hydroxybenzoate in that order and completely separating their peaks when 20 μl of the solution (prepared by dissolving 5 mg of Glycyrrhizic Acid Reference Standard and 1 mg of propyl *p*-hydroxybenzoate in 20 ml of 50% ethanol) is chromatographed according to the above operating conditions.

Licorice Extract, Purified

精製物

Content Purified Licorice Extract, when calculated on the dried basis, contains 50.0–80.0% of glycyrrhizic acid ($C_{42}H_{62}O_{16}$ = 822.93).

Description Purified Licorice Extract occurs as white to yellow crystals or powder.

Identification Weigh 5–10 mg of Purified Licorice Extract, proceed as directed in Identification for Crude Licorice Extract.

Purity

(1) pH 2.5–5.0 (1.0 g, 50% ethanol 100 ml).

(2) Heavy metals Not more than 10μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(3) Arsenic Not more than 2.0 μg/g as As_2O_3 (1.0 g, Method 3, Apparatus B).

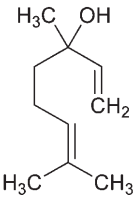
Loss on Drying Not more than 8.0% (105°C, 2 hours).

Residue on Ignition Not more than 15.0%.

Assay Weigh accurately 0.02–0.04 g of Purified Licorice Extract, and proceed as directed in the Assay for Crude Licorice Extract.

Linalool

リナロオール



$C_{10}H_{18}O$ Mol. Wt. 154.25

3,7-Dimethylocta-1,6-dien-3-ol [78-70-6]

Content Linalool contains not less than 92.0% of linalool ($C_{10}H_{18}O$).

Description Linalool is a colorless, transparent liquid having a characteristic odor.

Identification Determine the absorption spectrum of Linalool as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum of Linalool. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Refractive index n_D^{20}: 1.461–1.465.

(2) Specific gravity 0.860–0.876.

(3) Clarity of solution Clear (2.0 ml, 70% (vol) ethanol 4.0 ml).

(4) Acid Value Not more than 1.0 (Flavoring Substances Tests).

(5) Ester value Not more than 2.0 (5.0 g, Flavoring Substances Tests).

(6) Halogenated compounds Proceed as directed in the Flavoring Substances Tests.

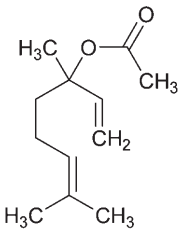
Assay Measure exactly 10 ml of Linalool, transfer into a flask, allow to stand in ice water for 10 minutes, add 20 ml of dimethylaniline, and shake well. Add 10 ml of acetyl chloride for linalool assay and 5 ml of acetic anhydride, equip the flask with a ground-glass air condenser, shake well, allow to stand in ice water for 5 minutes, and allow to stand for 30 minutes at room temperature. Heat in a water bath at 50°C for 4 hours, cool, transfer the contents to a separating funnel, and wash three times with 75 ml of ice water each time. Wash the oily layer with 25 ml of diluted sulfuric acid (1 in 20) each time as well. Repeat this procedure until no turbidity is observed when the washings are made alkaline with sodium hydroxide solution (1 in 25). Wash the layer repeatedly with 10 ml of anhydrous sodium carbonate solution (1 in 8) each time until the washings are alkaline. Then wash repeatedly with 25 ml of sodium chloride solution (1 in 10) until the washings are neutral, and transfer the oil layer into a dried flask. Add 2 g of anhydrous sodium sulfate, shake well, allow to stand for 30 minutes, and filter through a dry filter paper. Weigh accurately about 1 g of the filtrate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests. Perform a blank test in the same manner, and calculate the content by the formula:

$$\text{Content (\%) of linalool } (C_{10}H_{18}O) = \frac{(a-b)\times 77.12}{[S-(a-b)\times 0.02102]\times 1{,}000}\times 100$$

a = volume (ml) of 0.5 mol/L hydrochloric acid consumed in the blank test,
b = volume (ml) of 0.5 mol/L hydrochloric acid consumed in the test,
S = weight (g) of the filtrate.

Linalyl Acetate

酢酸リナリル



$C_{12}H_{20}O_2$ Mol. Wt. 196.29
3,7-Dimethylocta-1,6-dien-3-yl acetate [115-95-7]

Content Linalyl Acetate contains not less than 90.0% of linalyl acetate ($C_{12}H_{20}O_2$).

Description Linalyl Acetate is a colorless to light yellow, transparent liquid having a characteristic odor.

Identification Determine the absorption spectrum of Linalyl Acetate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum of Linalyl Acetate. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Refractive index n_D^{20}: 1.449–1.457.

(2) Specific gravity 0.902–0.917.

(3) Clarity of solution Clear (1.0 ml, 70% (vol) ethanol 5.0 ml).

(4) Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Weigh accurately about 1 g of Linalyl Acetate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 98.14 mg of $C_{12}H_{20}O_2$

Liquid Paraffin

流動パラフィン

Definition Liquid Paraffin is a mixture of hydrocarbons obtained from petroleum.

Description Liquid Paraffin is a colorless, clear, and viscous liquid having almost no fluorescence. It is odorless and tasteless.

Identification Determine the absorption spectrum of Liquid Paraffin as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Free acid and free alkali Measure 10 ml of Liquid Paraffin, add about 10 ml of boiling water and 1 drop of phenolphthalein TS, and shake vigorously. No pink color develops. To the resulting solution, add 0.20 ml of 0.02 mol/L sodium hydroxide, and shake. A pink color develops.

(2) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(3) Sulfur compounds Measure 4.0 ml of Liquid Paraffin, add 2 ml of absolute ethanol, add 2 drops of a transparent solution of sodium hydroxide solution (1 in 5) saturated with lead monoxide, warm at 70°C for 10 minutes with occasional shaking, and allow to cool. The color of the solution does not change to dark brown.

(4) Polycyclic aromatic hydrocarbons Place 25 ml of Liquid Paraffin into a 25-ml measuring cylinder, and transfer into a 100-ml separating funnel. Place 25 ml of hexane for ultraviolet absorption spectrum measurement into the same measuring cylinder, transfer into the separating funnel, and shake well. Add 5 ml of dimethylsulfoxide for ultraviolet spectrum measurement, shake vigorously for 2 minutes, and allow to stand for 15 minutes. Transfer the lower layer into a 50-ml separating funnel, add 2 ml of hexane for ultraviolet absorption spectrum measurement, shake vigorously for 2 minutes, and allow to stand for 2 minutes. Transfer the lower layer into a 10-ml centrifuge tube with a stopper, and centrifuge at 2,500–3,000 rpm for about 10 minutes. Transfer the supernatant into a cell with a tight stopper, and immediately measure the absorbance at a wavelength of 260–350 nm against the reference solution prepared as follows: To 25 ml of hexane for ultraviolet absorption spectrum measurement, add 5 ml of dimethylsulfoxide for ultraviolet spectrum measurement, and proceed in the same manner as test solution. The absorbance is not more than 0.10.

(5) Readily carbonizable substances Measure 5 ml of Liquid Paraffin, transfer into a Nessler tube, add 5 ml of 94.5–94.9% sulfuric acid, heat in a water bath for 2 minutes, and immediately shake up and down vigorously for 5 seconds. Repeat this procedure four times. The color of liquid paraffin layer does not change. In addition, the color of the sulfuric acid layer is not deeper than that of the solution prepared as follows: Mix 3.0 ml of Ferric Chloride Standard Stock Solution, 1.5 ml of Cobaltous Chloride Standard Stock Solution, and 0.5 ml of Cupric Sulfate Standard Stock Solution together in a Nessler tube.

Luohanguo Extract

ラカンカ抽出物

Definition Luohanguo Extract is obtained from the fruits of the luohanguo plant *Siraitia grosvenorii* C. Jeffrey ex A.

M. Lu & Zhi Y. Zhang (*Momordica grosvenori* Swingle) and consists mainly of mogrosides.

Content Luohanguo Extract, when dried, contains not less than 20% of mogroside V ($C_{60}H_{102}O_{29}$ = 1,287.43).

Description Luohanguo Extract occurs as a light yellow to light brown power having a sweet taste.

Identification

(1) To 5–10 mg of Luohanguo Extract, previously dried, add 2 ml of acetic anhydride, warm for 2 minutes, and slowly add 0.5 ml of sulfuric acid. The boundary surface turns red-brown.

(2) Prepare a test suspension containing 0.05–0.1 g of Luohanguo Extract in 1–3 ml of 70% (vol) methanol. Separately, prepare a control solution by dissolving 5–10 mg of mogroside V for assay in 1–3 ml of 70% (vol) methanol. Analyze 2 µl portions of the test suspension and the control solution by thin-layer chromatography using a 15:15:4 mixture of methanol/butyl acetate/water as the developing solvent. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the original line. Air-dry the plate, spray uniformly with diluted sulfuric acid (1 in 10), and heat at 105°C for 10 minutes. One of the spots from the test solution corresponds in color tone and R_f value to the dark purple spot of mogroside V from the control solution.

Purity

(1) Heavy metals Not more than 10 µg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 1.0 ml).

(2) Arsenic Not more than 1.0 µg/g as As_2O_3 (2.0 g, Method 1, Apparatus B).

Loss on Drying Not more than 6.0% (105°C, 2 hours).

Residue on Ignition Not more than 2.0%.

Assay

Test Solution Weigh accurately about 0.2 g of Luohanguo Extract, previously dried, suspend in 70% (vol) methanol to make exactly 100 ml, and filter through a membrane filter (0.45 µm pore diameter).

Standard Solution Weigh accurately about 5 mg of mogroside V for assay, previously dried, and dissolve it in 70% (vol) methanol to make exactly 10 ml.

Procedure Analyze 20 µl portions of the test solution and the standard solution by liquid chromatography according to the operating conditions given below. Measure the peak areas (A_T and A_S) of mogroside V for the test solution and the standard solution. Calculate the content by the formula:

$$\text{Content (\%) of mogroside V } (C_{60}H_{102}O_{29}) = \frac{\text{Weight (g) of mogroside for assay}}{\text{Weight (g) of the sample}} \times \frac{A_T}{A_S} \times 10 \times 100$$

Operating Conditions

Detector: Ultraviolet spectrophotometer (determination wavelength 203 nm).

Column: A stainless steel tube of 4–6 mm internal diameter and 25–30 cm length.

Column packing material: 5-µm of aminated polyvinyl alcohol gel for liquid chromatography.

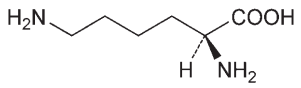
Column temperature: 40°C.

Mobile phase: A 74:26 mixture of acetonitrile/water.

Flow rate: Adjust so that the retention time of mogroside V is 15–20 minutes.

L-Lysine

L-リシン



$C_6H_{14}N_2O_2$ Mol. Wt. 146.19

(2*S*)-2,6-Diaminohexanoic acid [56-87-1]

Content L-Lysine, when calculated on the anhydrous basis, contains 97.0–103.0% of L-lysine ($C_6H_{14}N_2O_2$).

Description L-Lysine occurs as white crystals or crystalline powder. It has a characteristic odor, and has a characteristic taste.

Identification

(1) To 5 ml of a solution of L-Lysine (1 in 1,000), add 1 ml of ninhydrin solution (1 in 50), and heat for 3 minutes in a water bath. A reddish purple color develops.

(2) A solution of L-Lysine is alkaline.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +23.3 to +29.3°.

Weigh accurately about 2 g of L-Lysine, and dissolve it in 6 mol/L hydrochloric acid to make exactly 100 ml. Measure the angular rotation of this solution, and calculate on the anhydrous basis.

(2) Clarity and color of solution Colorless and almost clear (1.0 g, water 40 ml).

(3) Chloride Not more than 0.1% as Cl (0.070 g, Control solution 0.01 mol/L hydrochloric acid 0.20 ml).

(4) Heavy metals Not more than 20 µg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(5) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Water Content Not more than 8.0% (0.20 g, Back Titration).

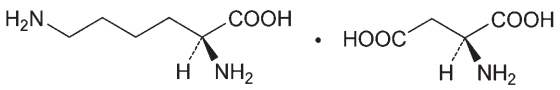
Residue on Ignition Not more than 0.20%.

Assay Weigh accurately about 0.2 g of L-Lysine, and proceed as directed in the Assay in L-Asparagine.

Each ml of 0.1 mol/L perchloric acid = 7.310 mg of $C_6H_{14}N_2O_2$

L-Lysine L-Aspartate

L-リシン L-アスパラギン酸塩



$C_{10}H_{21}N_3O_6$ Mol. Wt. 279.29

(2*S*)-2,6-Diaminohexanoic acid
mono[(2*S*)-2-aminobutanedioate]

Content L-Lysine L-Aspartate, when calculated on the dried basis, contains 98.0–102.0% of L-lysine L-aspartate ($C_{10}H_{21}N_3O_6$).

Description L-Lysine L-Aspartate occurs as a white powder. It is odorless or has a slight odor, and a characteristic taste.

Identification

(1) To 5 ml of a solution of L-Lysine L-Aspartate (1 in 1,000), add 1 ml of ninhydrin solution (1 in 1,000), and heat for 3 minutes. A purple color develops.

(2) Use a solution of L-Lysine L-Aspartate (1 in 500) as the test solution. Prepare a control solution by dissolving together 0.1 g of sodium L-aspartate and 0.1 g of L-lysine monohydrochloride in water to make exactly 100 ml. Analyze 5 µl each of the test solution and the control solution by paper chromatography using a 5:2:1 mixture of butanol/water/acetic acid as the developing solvent. Use a No. 2 filter paper for chromatography. Stop the development when the developing solvent has ascended to a point about 30 cm above the original line. Air-dry the filter paper, then dry at 100°C for 20 minutes, and spray with a solution of ninhydrin in acetone (1 in 50). Heat at 100°C for 5 minutes to allow a color to develop. Examine in daylight. Two major spots from the test solution correspond to the spots from the control solution.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +24.0 to +26.5° (4.0 g, diluted hydrochloric acid (1 in 2), 50 ml, on the dried basis).

(2) Clarity and color of solution Colorless and almost clear (1.0 g, water 20 ml).

(3) pH 5.0–7.0 (1.0 g, water 20 ml).

(4) Chloride Not more than 0.041% as Cl (0.30 g, Control solution 0.01 mol/L hydrochloric acid 0.35 ml).

(5) Heavy metals Not more than 20 µg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(6) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Drying Not more than 0.5% (reduced pressure, 5 hours).

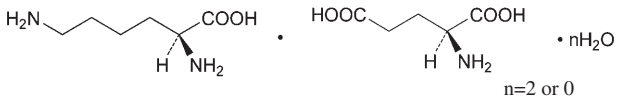
Residue on Ignition Not more than 0.30%.

Assay Proceed as directed in the Assay for DL-Alanine.

Each ml of 0.1 mol/L perchloric acid = 9.310 mg of $C_{10}H_{21}N_3O_6$

L-Lysine L-Glutamate

L-リシン L-グルタミン酸塩



$C_{11}H_{23}N_3O_6 \cdot nH_2O$ (n=2 or 0) Mol. Wt. dihydrate 329.35
anhydrous 293.32

(2*S*)-2,6-Diaminohexanoic acid
mono[(2*S*)-2-aminopentanedioate] dihydrate
(2*S*)-2,6-Diaminohexanoic acid
mono[(2*S*)-2-aminopentanedioate]

Content L-Lysine L-Glutamate, when calculated on the dried basis, contains 98.0–102.0% of L-lysine L-glutamate ($C_{11}H_{23}N_3O_6$).

Description L-Lysine L-Glutamate occurs as a white powder. It is odorless or has a slight odor, and a characteristic taste.

Identification

(1) To 5 ml of a solution of L-Lysine L-Glutamate (1 in 1,000), add 1 ml of ninhydrin solution (1 in 1,000), and heat for 3 minutes. A purple color develops.

(2) Proceed as directed in Identification (2) for L-Lysine L-Aspartate. Prepare a control solution by dissolving 0.1 g of sodium L-glutamate and 0.1 g of L-lysine monohydrochloride in water to make 100 ml.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +27.5 to +29.5° (4.0 g, diluted hydrochloric acid (1 in 2) 50 ml, on the dried basis).

(2) Clarity and color of solution Colorless and almost clear (1.0 g, water 20 ml).

(3) pH 6.0–7.5 (1.0 g, water 20 ml).

(4) Chloride Not more than 0.041% as Cl (0.30 g, Control solution 0.01 mol/L hydrochloric acid 0.35 ml).

(5) Heavy metals Not more than 20 µg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(6) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Drying Not more than 11.4% (105°C, 5 hours).

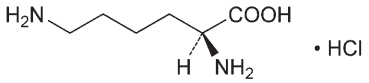
Residue on Ignition Not more than 0.30%.

Assay Proceed as directed in the Assay in DL-Alanine.

Each ml of 0.1 mol/L perchloric acid = 9.777 mg of $C_{11}H_{23}N_3O_6$

L-Lysine Monohydrochloride

L-リシン塩酸塩



$C_6H_{14}N_2O_2$·HCl Mol. Wt. 182.65
(2*S*)-2,6-Diaminohexanoic acid monohydrochloride
[657-27-2]

Content L-Lysine Monohydrochloride, when dried, contains not less than 98.0% of L-lysine monohydrochloride ($C_6H_{14}N_2O_2$·HCl).

Description L-Lysine Monohydrochloride occurs as a white powder. It is odorless or has a slight, characteristic odor, and has a slight, characteristic taste.

Identification

(1) To 5 ml of a solution of L-Lysine Monohydrochloride (1 in 1,000), add 1 ml of ninhydrin solution (1 in 1,000), and heat for 3 minutes. A purple color develops.

(2) L-Lysine Monohydrochloride responds to all tests for Chloride in the Qualitative Tests.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +19.0 to +21.5° (Dried sample 4 g, diluted hydrochloric acid (1 in 2), 50 ml).

(2) Clarity and color of solution Colorless and clear (1.0 g, water 10 ml).

(3) pH 5.0–6.0 (1.0 g, water 20 ml).

(4) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 4, Control solution Lead Standard Solution 2.0 ml).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Drying Not more than 1.0% (105°C, 3 hours).

Residue on Ignition Not more than 0.30%.

Assay Proceed as directed in the Assay for L-Histidine Monohydrochloride.

Each ml of 0.1 mol/L perchloric acid = 9.132 mg of $C_6H_{14}N_2O_2$·HCl

L-Lysine Solution

L-リシン液

Content L-Lysine Solution contains not more than 80% of L-lysine ($C_6H_{14}N_2O_2$ = 146.19) and 95.0–110.0% of the labeled content.

Description L-Lysine Solution is a yellow liquid. It has a characteristic odor and has a characteristic taste.

Identification

(1) To 5 ml of diluted L-Lysine Solution (1 in 200), add 1 ml of ninhydrin solution (1 in 50), and heat for 3 minutes in a water bath. A reddish purple color develops.

(2) To 5 g of L-Lysine Solution, add 50 ml of diluted hydrochloric acid (1 in 2), and mix. It shows dextrorotatory.

Purity

(1) Heavy metals Not more than 20 μg/g of L-lysine ($C_6H_{14}N_2O_2$) as Pb.

Test Solution Weigh an amount of L-Lysine Solution equivalent to 1.0 g of L-lysine ($C_6H_{14}N_2O_2$), add about 30 ml of water, and stir. Add a drop of Phenolphthalein TS, and neutralize with diluted hydrochloric acid (1 in 4). Add 2 ml of acetic acid (1 in 20), and add water to make 50 ml.

Control Solution To 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20), and add water to make 50 ml.

(2) Arsenic Not more than 4.0 μg/g of L-lysine ($C_6H_{14}N_2O_2$) as As_2O_3.

Test Solution Weigh an amount of L-Lysine Solution equivalent to 0.50 g of L- lysine ($C_6H_{14}N_2O_2$), add 5 ml of water, and dissolve while heating if necessary.

Apparatus Use Apparatus B.

Residue on Ignition Not more than 0.20% on the basis of L-lysine ($C_6H_{14}N_2O_2$).

Assay Weigh accurately an amount of L-Lysine Solution equivalent to about 0.2 g of L-lysine ($C_6H_{14}N_2O_2$), and proceed as directed in the Assay for L-Asparagine.

Each ml of 0.1 mol/L perchloric acid = 7.310 mg of $C_6H_{14}N_2O_2$

Lysozyme

リゾチーム

Definition Lysozyme is an enzyme that dissolves the cell wall substances of bacteria. It is obtained from hen egg white by resin purification process after treatment with an alkaline aqueous solution and a saline solution or by column purification or re-crystallization after resin or salting treatment.

Enzyme Activity Lysozyme has enzyme activity equivalent to not less than 0.9 mg (potency) per mg, when dried.

Description Lysozyme occurs as an odorless white powder.

Identification A solution (1 in 10,000) of Lysozyme in acetate buffer (pH 5.4) exhibits an absorption maximum at a wavelength of 279–281 nm.

Purity

(1) Clarity of solution Adjust 5 ml of a solution of Lysozyme (1 in 100) to pH 3.0 by adding dilute hydrochloric acid if necessary. It has a transmittance of not less than 80.0% at 660 nm.

(2) pH Not less than 5.0 (3.0 g, water 200 ml).

(3) Chloride Not more than 4.5% as Cl.

Weigh accurately about 0.5 g of Lysozyme, and dissolve it in 50 ml of water. To this solution, add 0.1 ml of 10% potassium chromate solution, and titrate with 0.1 mol/L silver nitrate solution. The endpoint is when the solution turns light red-brown. Each ml of 0.1 mol/L silver nitrate = 3.545 mg of Cl

(4) Lead Not more than 5.0 μg/g as Pb (2.0 g, Method 1).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50g, Method 3, Apparatus B).

Loss on Drying Not more than 6.0% (1.0 g, silica gel, reduced pressure, 2 hours).

Enzyme Activity Determination

(i) *Test Solution* Weigh accurately an amount of Lysozyme equivalent to about 50 mg potency, previously dried, and add phosphate buffer (pH 6.2) to make exactly 100 ml. Measure exactly 2 ml of this solution, and add phosphate buffer (pH 6.2) to make exactly 100 ml of solution. Then measure exactly 2 ml of the second solution, and add phosphate buffer (pH 6.2) to make exactly 50 ml.

(ii) *Standard Solution* Dry about 0.1 g of Lysozyme Reference Standard in a vacuum desiccator for about 2 hours. Weigh accurately an amount of the dried Standard equivalent to about 50 mg potency, and add phosphate buffer (pH 6.2) to make exactly 100 ml. Measure exactly 2 ml of this solution, and add phosphate buffer (pH 6.2) to make exactly 100 ml. Then measure exactly 2 ml of the second solution, and add phosphate buffer (pH 6.2) to make exactly 50 ml.

(iii) *Procedure* Place exactly 3 ml of the substrate solution for lysozyme, into each of three test tubes, and warm the tubes at 35°C for 3 minutes. Warm the standard solution, test solution, and phosphate buffer (pH 6.2) at 35°C for 3 minutes. To the test tubes, separately add exactly 3 ml each of the warmed three solutions, and allow to react at 35°C for 10 ± 0.1 minutes. Immediately measure the absorbances at a wavelength of 640 nm, using water as the reference. Express the absorbances of the standard solution, sample solution, and phosphate buffer as A_S, A_T, and A_0, respectively. Repeat the whole procedure twice, obtain the average of the three absorbances, and calculate the enzyme activity by the formula:

$$\text{Enzyme activity of dried Lysozyme (mg potency/mg)} = \frac{\left(\begin{array}{c}\text{Weight (mg potency) of}\\ \text{dried Lysozyme Reference Standard}\end{array}\right)}{\text{Weight (mg) of the dried sample}} \times \frac{A_0 - A_T}{A_0 - A_S}$$

Macrophomopsis Gum

マクロホモプシスガム

Definition Macrophomopsis Gum is obtained from the culture fluid of *Macrophomopsis* (*Fisicoccum*) and consists mainly of polysaccharides. It may contain sucrose, glucose, lactose, dextrin, or maltose.

Description Macrophomopsis Gum occurs as a light yellow to light brown powder having a slight characteristic odor.

Identification

(1) To 100 ml of hot water, gradually add 0.5 g of Macrophomopsis Gum while stirring, and cool to room temperature. A viscous liquid is produced.

(2) To 100 ml of hot water, gradually add 0.1 g of Macrophomopsis Gum while stirring, and dissolve by homogenizing at 8,000 rpm for 15 minutes. Use this solution as the test solution. After cooling, measure 5 ml of the test solution into a test tube, add 1 ml of 2-propanol, mix well, and heat for 10 minutes in a water bath. Stir well, and allow to stand at room temperature for 2 hours. A gel is formed.

Purity

(1) Heavy metals Not more than 20 µg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 5.0 µg/g as Pb (2.0 g, Method 1).

(3) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50g, Method 3, Apparatus B).

(4) Total nitrogen Not more than 1.0% (on the dried basis).

Weigh accurately about 0.3 g of Macrophomopsis Gum, and proceed as directed in the Semi-micro Kjeldahl Method under Nitrogen Determination.

(5) 2-Propanol Not more than 0.50%.

(i) Apparatus Use the apparatus illustrated in Purity (9) for Processed Eucheuma Algae.

(ii) Method Prepare a test solution and an internal standard solution as directed in Purity (9) for Processed Eucheuma Algae.

Standard Solution Weigh accurately about 0.5 g of 2-propanol, and add water to make exactly 50 ml. Measure exactly 5 ml of this solution, and add water to make 50 ml. To exactly 10 ml of the second solution, add exactly 4 ml of the internal standard solution and water to make exactly 100 ml.

Procedure Analyze 2.0 µl portions of the test solution and the standard solution by gas chromatography using the operating conditions given below. Determine the peak area ratios of 2-propanol to *tert*-butanol for the test solution and the standard solution, and express as Q_T and Q_S, respectively. Calculate the amount of 2-propanol by the formula:

$$\text{Amount (\%) of 2 - propanol} = \frac{\text{Weight (g) of 2 - propanol}}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 2$$

Operating Conditions

Detector: Hydrogen flame ionization detector.

Column: A glass tube of 3 mm internal diameter and 2 m length.

Column packing material: 180- to 250-µm styrene-divinylbenzene porous polymer for gas chromatography.

Column temperature: A constant temperature at about 120°C.

Inlet temperature: A constant temperature at about 200°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust so that the retention time of 2-propanol is 10 minutes.

Loss on Drying Not more than 15.0% (105°C, 2.5 hours).

Ash Not more than 10.0% (on the dried basis).

Microbial Limits Proceed as directed in the Microbial Limit Tests. The total bacterial count is not more than 10,000/g. *Escherichia coli* is negative for a test solution prepared using 1 g of the sample.

Magnesium Carbonate

炭酸マグネシウム

Content Magnesium Carbonate contains the equivalent of 40.0–44.0% of magnesium oxide (MgO = 40.30).

Description Magnesium Carbonate occurs as a white, bulky powder or brittle lumps.

Identification To 0.2 g of Magnesium Carbonate, add gradually 3 ml of diluted hydrochloric acid (1 in 4). It dissolves with effervescence. Add ammonia TS to make the solution alkaline. The solution responds to the test for Magnesium Salt in the Qualitative Tests.

Purity

(1) Clarity of solution Very slightly turbid.

Test Solution Weigh 1.0 g of Magnesium Carbonate, dissolve it in 10 ml of diluted hydrochloric acid (2 in 3), and add 10 ml of water.

(2) Water-soluble substances Not more than 1.0%.

Weigh 2.0 g of Magnesium Carbonate, add 100 ml of freshly boiled and cooled water, and boil for 5 minutes while stirring. Cool and filter it. Combine the filtrate and the washings, add water to make 100 ml. Measure 50 ml of this solution, evaporate to dryness in a water bath, and dry the residue at 105°C for 1 hour. Weigh the residue.

(3) Heavy metals Not more than 30 μg/g as Pb.

Test Solution Weigh 1.0 g of Magnesium Carbonate, dissolve it in 10 ml of diluted hydrochloric acid (1 in 4), and evaporate to dryness in a water bath. Dissolve the residue in about 40 ml of water, filter if necessary, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution To 3.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(4) Calcium oxide Not more than 0.60% as CaO.

Weigh exactly 0.600g of Magnesium Carbonate, dissolve by adding 35 ml of water and 6 ml of diluted hydrochloric acid (1 in 4), and add 250 ml of water and 5 ml of tartaric acid solution (1 in 5). Add 10 ml of triethanolamine solution (3 in 10) and 10 ml of potassium hydroxide solution (1 in 2), and allow to stand for 5 minutes. Titrate with 0.01 mol/L EDTA (indicator: 0.1 g of NN indicator), and calculate the content of calcium oxide. The endpoint is when the color of the solution changes from red-purple to blue. Perform a blank test in the same manner, and make any necessary correction.

Each ml of 0.01 mol/L EDTA = 0.5608 mg of CaO

(5) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.50 g of Magnesium Carbonate, and moisten with 1.5 ml of water, and add 3.5 ml of diluted hydrochloric acid (1 in 4) to dissolve it.

Apparatus Use Apparatus B.

Assay Weigh accurately about 0.4g of Magnesium Carbonate, dissolve by adding 10 ml of water and 3.5 ml of diluted hydrochloric acid (1 in 4), and add water to make exactly 500 ml. Measure exactly 25 ml of this solution, add 50 ml of water and 5 ml of ammonia–ammonium chloride buffer (pH 10.7), and titrate with 0.01 mol/L EDTA (indicator: 0.04 g of eriochrome black T–sodium chloride indicator). Perform a blank test in the same manner, make any necessary correction, and calculate the volume (a ml) of the EDTA solution consumed. Refer to the volume of 0.01 mol/L EDTA consumed by the titration in Purity (4) as b ml, and calculate the content by the formula:

$$\text{Content (\%) of magnesium oxide (MgO)} = \frac{(a - 0.033b) \times 0.8061}{\text{Weight (g) of the sample}}$$

Magnesium Chloride

塩化マグネシウム

$MgCl_2 \cdot 6H_2O$ Mol. Wt. 203.30

Magnesium chloride hexahydrate [7791-18-6]

Content Magnesium Chloride contains not less than 95.0% of magnesium chloride ($MgCl_2 \cdot 6H_2O$).

Description Magnesium Chloride occurs as colorless to white crystals, powder, fragments, granules, or lumps.

Identification Magnesium Chloride responds to all tests for Magnesium Salt and for Chloride in the Qualitative Tests.

Purity

(1) Clarity of solution Slightly turbid (1.0 g, water 10 ml).

(2) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(3) Zinc Not more than 70 μg/g as Zn.

Weigh 4.0 g of Magnesium Chloride, dissolve it in water to make 40 ml, and use it as the sample solution. Measure 30 ml of the sample solution, add 5 drops of acetic acid and 2 ml of potassium ferrocyanide solution (1 in 20), shake, and allow to stand for 10 minutes. The turbidity of this solution does not exceed that of the solution prepared as follows: Measure 14 ml of Zinc Standard Solution, and add 10 ml of the sample solution and water to make 30 ml. Add 5 drops of acetic acid and 2 ml of potassium ferrocyanide solution (1 in 20), shake, and allow to stand for 10 minutes.

(4) Calcium Weigh 0.50 g of Magnesium Chloride, and dissolve it in water to make 50 ml. Measure 5 ml of the solution, add 1 ml of ammonium oxalate solution (1 in 25), and allow to stand for 5 minutes. The solution is very slightly turbid.

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Assay Weigh accurately about 0.3 g of Magnesium Chloride, and dissolve it in water to make exactly 100 ml. Measure exactly 20 ml of this solution, add 50 ml of water and 5 ml of ammonia–ammonium chloride buffer (pH 10.7), and titrate with 0.01 mol/L EDTA (indicator: 2 drops of eriochrome black T TS) until the red color of the solution changes to blue. Calculate the content by the formula:

$$\text{Content (\%) of magnesium chloride } (MgCl_2 \cdot 6H_2O) = \frac{\left(\begin{array}{c}\text{Volume (ml) of}\\ \text{0.01mol/L EDTA consumed}\end{array}\right) \times 1.017}{\text{Weight (g) of the sample}}$$

Magnesium Oxide

酸化マグネシウム

MgO Mol. Wt. 40.30

Magnesium oxide [1309-48-4]

Content Magnesium Oxide, when ignited, contains not less than 96.0% of magnesium oxide (MgO).

Description Magnesium Oxide occurs as a white or whitish powder or granules.

Identification Dissolve 1 g of Magnesium Oxide in 25 ml of diluted hydrochloric acid (1 in 4). The resulting solution responds to the test for Magnesium Salt in the Qualitative Tests.

Purity

(1) Water-soluble substances Not more than 2.0%.

Weigh 2.0 g of Magnesium Oxide, add 100 ml of water, heat in a water bath for 5 minutes, and immediately filter. Cool, measure 25 ml of the filtrate, evaporate to dryness in a water bath, and dry at 105°C for 1 hour. Weigh the residue.

(2) Hydrochloric acid-insoluble substances Not more than 1.0%.

Weigh 2.0 g of Magnesium Oxide, and add 75 ml of water. Add, dropwise, hydrochloric acid to the suspension while shaking until the insoluble substances no longer reduce, and boil for 5 minutes. Cool, filter through a filter paper for quantitative analysis (5C), wash the residue on the filter paper thoroughly with water until the washings do not give positive tests for Chloride, and ignite together with the filter paper. Weigh the residue.

(3) Free alkali Measure 50 ml of the filtrate obtained in test (1), add 2 drops of methyl red TS, and add 2.0 ml of 0.05 mol/L sulfuric acid. A red color develops.

(4) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of Magnesium Oxide, and dissolve it in 25 ml of diluted hydrochloric acid (1 in 4). Evaporate to dryness in a water bath, and near the end of evaporation, stir the residue well to make a fine powder. Dissolve with 20 ml of water, evaporate to dryness in the same manner, and dissolve again with 20 ml of water. Filter if necessary. Add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(5) Calcium oxide Not more than 1.5%.

Measure exactly 50 ml of solution A prepared in the Assay below, and add water to make 300 ml. Add 0.6 ml of tartaric acid (1 in 5), then 10 ml of triethanolamine (3 in 10) and 10 ml of potassium hydroxide solution (1 in 2). Allow to stand for 5 minutes, titrate with 0.01 mol/L EDTA using a microburet (indicator: about 0.1 g of NN indicator), and express the volume consumed as b (ml). The endpoint is observed when the red-purple color of the solution completely disappears and the solution becomes blue. Calculate the content by the formula:

$$\text{Content of calcium oxide (CaO) (\%)} = \frac{\text{b (ml)} \times 0.5608}{\text{Weight (g) of the sample}}$$

(6) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.50 g of Magnesium Oxide, and dissolve it in 5 ml of diluted hydrochloric acid (1 in 4).

Apparatus Use Apparatus B.

Loss on Ignition Not more than 10.0% (1,000°C, 30 minutes).

Assay Weigh accurately about 0.5 g of Magnesium Oxide, previously ignited, moisten with 5 ml of water, add 10 ml of hydrochloric acid and 10 ml of perchloric acid, cover with a watch glass, and heat gradually. After thick white fumes are evolved, heat for another 10 minutes. After cooling, add about 50 ml of hot water and 5 ml of diluted hydrochloric acid (1 in 2), heat slightly, and immediately filter through a filter paper for quantitative analysis (5C), and add water to the filtrate to make exactly 500 ml. Refer to this solution as solution A. Measure exactly 10 ml of solution A, add water to make 100 ml, add 5 ml of ammonia–ammonium chloride buffer (pH 10.7) and 2 drops of eriochrome black T TS, and immediately titrate with 0.01 mol/L EDTA until the red color of the solution changes to blue. Determine the consumed volume (a ml). From the consumed volume (b ml) obtained in Purity (5), calculate the content by the formula:

$$\text{Content (\%) of magnesium oxide (MgO)} = \frac{(a - 0.2b) \times 2.015}{\text{Weight (g) of the sample}}$$

Magnesium Stearate

ステアリン酸マグネシウム

Definition Magnesium Stearate is a mixture of magnesium salts consisting principally of stearic acid and palmitic acid.

Content Magnesium Stearate, when calculated on the dried basis, contains 4.0%–5.0% of magnesium (Mg = 24.31).

Description Magnesium Stearate occurs as a white, light, bulky powder. It has no odor or a faint, characteristic odor.

Identification

(1) Place 5.0 g of Magnesium Stearate in a round-bottom flask, and add 50 ml of peroxide-free diethyl ether, 20 ml of dilute nitric acid, and 20 ml of water. Heat under a reflux condenser until the sample dissolves completely. Transfer the contents of the flask into a separating funnel, shake, and allow to stand. Transfer the aqueous layer into a flask. Extract twice from the diethyl ether layer with 4 ml of water each time, and combine these extracts with the aqueous layer. Wash the extract with 15 ml of peroxide-free diethyl ether, and add water to make exactly 50 ml. Shake this solution and use as the test solution. This solution responds to the test for magnesium salts described in the Qualitative Tests.

(2) Prepare a test solution and standard solutions as directed in Purity (5). Analyze equal portions of the solutions by gas chromatography using the conditions given in Purity (5). The chromatogram from the test solution shows peaks at the retention times of methyl stearate and methyl palmitate for the corresponding standard solutions.

Purity

(1) Acid or alkali To 1.0 g of Magnesium Stearate, add 20 ml of freshly boiled and cooled water, heat on a water bath for 1 minute with shaking, and filter after cooling. To 10 ml of the filtrate, add 0.05 ml of bromothymol blue TS, then exactly 0.05 ml of 0.1 mol/L hydrochloric acid or 0.1 mol/L sodium hydroxide. The color of the solution changes.

(2) Chloride Not more than 0.10% as Cl.

Perform the test on 10.0 ml of the test solution obtained in Identification (1). Prepare a control solution, using 1.40 ml of 0.02 mol/L hydrochloric acid.

(3) Sulfate Not more than 1.0% as SO_4.

Perform the test on 10.0 ml of the test solution obtained in Identification (1). Prepare a control solution, using 10.2 ml

of 0.01 mol/L sulfuric acid.

(4) Heavy metals Not more than 20 μg/g.

Test Solution Heat 1.0 g of Magnesium Stearate gently at first, then incinerate at about 500 ± 25ºC. After cooling, add 2 ml of hydrochloric acid, and evaporate on a water bath to dryness. To the residue, add 20 ml of water and 2 ml of dilute acetic acid, and heat for 2 minutes. After cooling, filter the mixture through a filter paper. Wash the filter paper with 15 ml of water, combine the washings with the filtrate, and add water to make 50 ml.

Control Solution Evaporate 2 ml of hydrochloric acid on a water bath to dryness, and add 2 ml of dilute acetic acid, 2.0 ml of Standard Lead Solution, and water to make 50 ml.

(5) Relative content ratio of stearic acid and palmitic acid

Test Solution Weigh 0.10 g of Magnesium Stearate, and transfer into a small conical flask fitted with a reflux condenser. Add 5.0 ml of boron trifluoride–methanol TS, shake, and heat for about 10 minutes to dissolve. Add 4.0 ml of heptane through the condenser, and heat for about 10 minutes. After cooling, add 20 ml of saturated sodium chloride solution, shake, and allow the layers to separate. Transfer the heptane layer into another flask through about 0.1 g of anhydrous sodium sulfate, previously rinsed with heptane. Transfer 1.0 ml of this solution into a 10-ml volumetric flask, add heptane to volume, and mix.

Standard Solutions Weigh 0.050 g each of stearic acid and palmitic acid in separate small conical flasks fitted with a reflux condenser. Add 5.0 ml of boron trifluoride–methanol TS to each, and shake. Prepare methyl stearate standard solution and methyl palmitate standard solution, respectively, in the same manner as for the test solution.

Procedure Analyze 1 μl portions of the test solution and the standard solutions by gas chromatography using the conditions given below. Determine the peak areas (A_A and A_B) of methyl stearate and methyl palmitate for the test solution, respectively. Also, determine the total peak area (A_T) of all the peaks of fatty acid esters (all peaks detected) for the test solution. The main solvent peak is excluded from measurement. The chromatography should be continued for about 1.5 times the retention time of methyl stearate, and solvent peaks should be excluded from the measurement. Determine the percentages of stearic acid and the sum of stearic acid and palmitic acid in the fatty acid fraction of magnesium stearate using the following formula:

$$\text{Ratio (\%) of stearic acid} = \frac{A_A}{A_T} \times 100$$

$$\text{Ratio (\%) of the sum of stearic acid and palmitic acid} = \frac{A_A + A_B}{A_T} \times 100$$

The area of the methyl stearate peak and the total area of the methyl stearate peak and methyl palmitate peak are not less than 40% and not less than 90%, respectively, of the total area of all the peaks of fatty acid esters obtained.

Operating Conditions

Detector: Hydrogen flame-ionization detector.

Column: A silicate glass capillary tube (about 0.32 mm internal diameter and about 30 m length) coated with a 0.5-μm thick layer of polyethylene glycol 15000-diepoxide for gas chromatography.

Column temperature: Maintain the temperature at 70ºC for 2 minutes, raise to 240ºC at a rate of 5ºC/minute, and then maintain at 240ºC for 5 minutes.

Injection port temperature: A constant temperature of about 220ºC.

Injection method: Splitless.

Carrier gas: Helium.

Flow rate: Adjust so that the retention time of methyl stearate is about 32 minutes.

Loss on Drying Not more than 6.0 % (105ºC, 2 hours).

Assay Weigh accurately about 0.5 g of Magnesium Stearate, and add 50 ml of an equal-volume mixture of absolute ethanol and 1-butanol, 5 ml of ammonia solution, and 3 ml of ammonium chloride buffer solution (pH10). To this, add exactly 30.0 ml of 0.1 mol/L EDTA solution, and shake, and heat at 45–50ºC until the solution is clear. After cooling, titrate with 0.1 mol/L zinc sulfate. The endpoint is when the solution turns from blue to red-purple. Use 1 to 2 drops of eriochrome black T TS as the indicator. Perform a blank test and make any necessary correction.

Each ml of 0.1 mol/L EDTA = 2.431 mg of Mg

Magnesium Sulfate

硫酸マグネシウム

$MgSO_4 \cdot nH_2O$ (n=7 or 3) Mol. Wt. heptahydrate 246.48
trihydrate 174.41

Magnesium sulfate heptahydrate [10034-99-8]
Magnesium sulfate trihydrate

Definition Magnesium Sulfate occurs in crystalline form (heptahydrate) called Magnesium Sulfate (crystal), and in dried form (trihydrate) called Magnesium Sulfate (dried).

Content Magnesium Sulfate, when ignited, contains not less than 99.0% of magnesium sulfate ($MgSO_4$ = 120.37).

Description Magnesium Sulfate (crystal) occurs as colorless prisms or needles having a salty and bitter taste. Magnesium Sulfate (dried) occurs as a white powder having a salty and bitter taste.

Identification Magnesium Sulfate responds to all tests for Magnesium Salt and for Sulfate in the Qualitative Tests.

Purity

(1) Clarity and color of solution
Crystal: Colorless, almost clear (1.0 g, water 10 ml).
Dried: Colorless, very slightly turbid (1.0 g, water 10 ml).

(2) Chloride Not more than 0.014% as Cl (1.0 g, Control solution 0.01 mol/L hydrochloric acid 0.40 ml).

(3) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Ignition

Crystal: 40.0–52.0% (100°C for 2 hours, then 300–400°C for 4 hours).

Dried: 25.0–35.0% (300–400°C, 4 hours).

Assay Weigh accurately about 0.6 g of Magnesium Sulfate, previously ignited, and dissolve it in 2 ml of diluted hydrochloric acid (1 in 4) and water to make exactly 100 ml. Measure exactly 25 ml of this solution, add 50 ml of water and 5 ml of ammonia–ammonium chloride buffer (pH 10.7), and

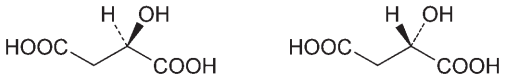
titrate with 0.05 mol/L EDTA (indicator: 5 drops of eriochrome black T TS) until the color of the solution changes from the red-purple to blue. Perform a blank test, and make any necessary correction.

Each ml of 0.05 mol/L EDTA = 6.018 mg of $MgSO_4$

DL-Malic Acid

***dl*-Malic Acid**
Malic Acid

DL-リンゴ酸



$C_4H_6O_5$ Mol. Wt. 134.09
(2*RS*)-2-Hydroxybutanedioic acid [6915-15-7]

Content DL-Malic Acid contains not less than 99.0% of DL-malic acid ($C_4H_6O_5$).

Description DL-Malic Acid occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor, and a characteristic acid taste.

Identification

(1) Place 1 ml of a solution of DL-Malic Acid (1 in 20) into a porcelain dish, neutralize with ammonia TS, add 0.010 g of sulfanilic acid, and heat on a water bath for a few minutes. Add 5 ml of sodium nitrite solution (1 in 5), warm slightly, and make the solution alkaline with sodium hydroxide solution (1 in 25). A red color develops.

(2) Place 1 ml of DL-Malic Acid solution (1 in 20) into a test tube, add 2–3 mg of resorcinol and 1 ml of sulfuric acid, shake, heat at 120–130°C for 5 minutes, cool, and add water to make 5 ml. Make the solution alkaline by adding sodium hydroxide solution (3 in 10) dropwise while cooling, and add water to make 10 ml. A light blue fluorescence is observed under ultraviolet light.

Purity

(1) Melting point 127–132°C.

(2) Clarity of solution Clear (1.0 g, water 20 ml).

(3) Chloride Not more than 0.004% as Cl (1.0 g, Control solution 0.01 mol/L hydrochloric acid 0.10 ml).

(4) Heavy metals Not more than 20 µg/g as Pb.

Test Solution Weigh 1.0 g of DL-Malic Acid, dissolve it in 40 ml of water, add 1 drop of phenolphthalein TS, and then add ammonia TS dropwise until the solution is slightly pink. Add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml (not more than 20 µg/g as Pb).

(5) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

(6) Readily oxidizable substances Weigh 0.10 g of DL-Malic Acid, dissolve it in 25 ml of water and 25 ml of diluted sulfuric acid (1 in 20), keep at 20°C, and add 1.0 ml of 0.02 mol/L potassium permanganate. The pink color of the solution does not disappear within 3 minutes.

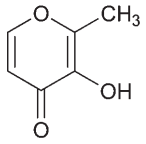
Residue on Ignition Not more than 0.05% (5 g).

Assay Weigh accurately about 1.5 g of DL-Malic Acid, and dissolve it in water to make exactly 250 ml. Measure exactly 25 ml of this solution, and titrate with 0.1 mol/L sodium hydroxide (indicator: 2 drops of phenolphthalein TS).

Each ml of 0.1 mol/L sodium hydroxide = 6.704 mg of $C_4H_6O_5$

Maltol

マルトール



$C_6H_6O_3$ Mol. Wt. 126.11
3-Hydroxy-2-methyl-4*H*-pyran-4-one [118-71-8]

Content Maltol contains not less than 99.0% of maltol ($C_6H_6O_3$).

Description Maltol occurs as white or slightly yellowish needles or crystalline powder having a sweet odor.

Identification Determine the absorption spectrum of Maltol as directed in the Paste Method under Infrared Spectrophotometry, and compare with the Reference Spectrum of Maltol. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Melting point 160–163°C.

(2) Clarity of solution Clear (0.10 g, 70% (vol) ethanol 5.0 ml).

(3) Heavy metals Not more than 10 µg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 4, Apparatus B).

Loss on Drying Not more than 0.5% (4 hours).

Residue on Ignition Not more than 0.05%.

Assay

Test Solution Weigh accurately about 0.2 g of Maltol, and dissolve it in 0.1 mol/L hydrochloric acid to make exactly 500 ml. Measure exactly 5 ml of this solution, and add 0.1 mol/L hydrochloric acid to make exactly 200 ml.

Standard Solution Weigh accurately about 0.2 g of maltol for assay, and dissolve it in 0.1 mol/L hydrochloric acid to make exactly 500 ml. Measure exactly 5 ml of this solution, and add 0.1 mol/L hydrochloric acid to make exactly 200 ml.

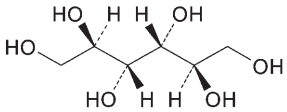
Procedure Measure the absorbances (A_T and A_S) of the test solution and the standard solution at a wavelength of 274 nm, using 0.1 mol/L hydrochloric acid as the reference. Calculate the content by the formula:

$$\text{Content (\%) of maltol } (C_6H_6O_3) = \frac{\text{Weight(g) of maltol for assay}}{\text{Weight (g) of the sample}} \times \frac{A_T}{A_S} \times 100$$

D-Mannitol

Mannitol
D-Mannite

D-マンニトール



$C_6H_{14}O_6$ Mol. Wt. 182.17

D-Mannitol [69-65-8]

Content D-Mannitol, when dried, contains 96.0–101.0% of D-mannitol ($C_6H_{14}O_6$).

Description D-Mannitol occurs as white crystals or powder. It is odorless and has a cool, sweet taste.

Identification

(1) Transfer 3 ml of a solution of D-Mannitol (1 in 5) to a test tube containing 1 ml of iron(III) chloride solution (1 in 10), and add 1.5 ml of sodium hydroxide solution (1 in 25). A yellow precipitate is formed. Shake vigorously. The precipitate dissolves, and the liquid is yellow and transparent. Even when sodium hydroxide solution (1 in 25) is added, no precipitate is formed.

(2) To 0.5 g of D-Mannitol, add 3 ml of acetic anhydride and 1 ml of pyridine, and dissolve completely by heating in a water bath with occasional shaking. Continue heating for 5 minutes, and cool. To this solution, add 20 ml of water, mix well, and allow to stand for 5 minutes. Collect the deposited crystals by filtration, wash with water, and recrystallize from diethyl ether. The melting point of crystals is 120–125°C.

Purity

(1) Melting point 165–169°C.

(2) Free acid Weigh 5 g of D-Mannitol, dissolve it in 50 ml of freshly boiled and cooled water, add 1 drop of phenolphthalein TS and 0.5 ml of 0.01 mol/L sodium hydroxide solution, and shake. The color of the solution changes to pink. The pink color persists for not less than 30 seconds.

(3) Heavy metals Not more than 10 µg/g as Pb (2.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(4) Nickel Weigh 0.5 g of D-Mannitol, dissolve it in 5 ml of water, add 3 drops of a solution of dimethylglyoxime in ethanol (1 in 100) and 3 drops of ammonia TS, and allow to stand for 5 minutes. The color of the solution does not change to pink.

(5) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

(6) Saccharide Weigh 0.5 g of D-Mannitol, add 10 ml of water and 2 ml of diluted hydrochloric acid (1 in 4), boil for 2 minutes, and cool. Add 5 ml of anhydrous sodium carbonate solution (1 in 8), allow to stand for 5 minutes, add 2 ml of Fehling's TS, and boil for 1 minute. An orange-yellow to red precipitate is not immediately formed.

Loss on Drying Not more than 0.30% (105°C, 4 hours).

Residue on Ignition Not more than 0.02% (5 g).

Assay

Test Solution and Standard Solution Weigh accurately about 1 g each of D-Mannitol and D-mannitol for assay, previously dried, separately dissolve them in water to make two solutions of exactly 50 ml each. Use these solutions as the test solution and the standard solution, respectively.

Procedure Analyze 10 µl portions of these solutions by liquid chromatography using the conditions given below. Measure the peak areas (A_T and A_S) of D-mannitol for the test solution and the standard solution, and determine the content by the formula:

$$\text{Content (\%) of D-mannitol } (C_6H_{14}O_6) = \frac{\text{Weight (g) of D-mannitol for assay}}{\text{Weight (g) of the sample}} \times \frac{A_T}{A_S} \times 100$$

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube of 4–8 mm internal diameter and 20–50 cm length.

Column packing material: 5- to 12-µm strongly acidic cation-exchange resin for liquid chromatography.

Column temperature: A constant temperature of 40–85°C.

Mobile phase: Water.

Flow rate: A constant rate of 0.5–1.0 ml/minute.

Marigold Color

マリーゴールド色素

Definition Marigold Color is obtained from the flowers of the marigold plant, *Tagetes patula* Linné, *Tagetes erecta* Linné, or their interspecific hybrids. It consists mainly of xanthophylls.

Color Value The Color Value ($E_{1cm}^{10\%}$) of Marigold Color is not less than 2,500 and is in the range of 95–115% of the labeled value.

Description Marigold Color is a dark brown solid or liquid having a characteristic odor.

Identification

(1) Weigh the equivalent of 0.1 g of Marigold Color with a Color Value 2,500, and dissolve it in 100 ml of a 1:1 mixture of ethanol/hexane. A deep yellow color develops.

(2) A solution of Marigold Color in a 1:1 mixture of ethanol/hexane exhibits absorption maxima at wavelengths of 469–475 nm and 441–447 nm. It may, in addition, exhibit an absorption maximum at a wavelength of 420–426 nm.

(3) Weigh the equivalent of 0.1 g of Marigold Color with a Color Value 2,500, and dissolve it in 10 ml of a 1:1 mixture of ethanol/hexane. Use the solution obtained as the test solution. Analyze a 5-µl portion of the test solution by thin-layer chromatography using a 15:4:1 mixture of toluene/ethyl acetate/ethanol as the developing solvent. No control solution is used. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the original line, and air-dry. One yellow spot is observed at each or either of R_fs of approximately 0.8 (fatty acid esters of lutein) and approximately 0.35 (lutein). The color disappears when

the spot is sprayed with 5% sodium nitrite solution followed by with 0.5 mol/L sulfuric acid.

Purity

(1) Heavy metals Not more than 40 μg/g as Pb (0.50 g, Method 2, Control solution Lead standard solution 2.0 ml).

(2) Lead Not more than 10 μg/g as Pb (1.0g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Color Value Test Proceed as directed in the Color Value Test.

Operating Conditions

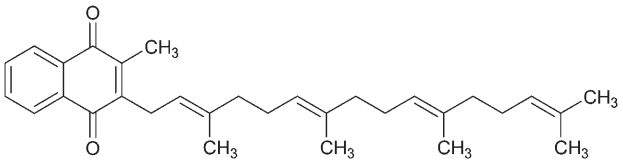
Solvent: A 1:1 mixture of Ethanol and hexane.

Wavelength: Maximum absorption wavelength of 441–447 nm.

Menaquinone (Extract)

Vitamin K_2 (Extract)

メナキノン（抽出物）



$C_{31}H_{40}O_2$ Mol. Wt. 444.65

2-Methyl-3-[(2*E*,6*E*,10*E*)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenyl]naphthalene-1,4-dione [863-61-6]

Definition Menaquinone (Extract) is obtained from the culture fluid of *Arthrobacter nicotianae* and consists mainly of menaquinone-4.

Content Menaquinone (Extract), when calculated on the anhydrous basis, contains 98.0–102.0% of menaquinone-4 ($C_{31}H_{40}O_2$).

Description Menaquinone (Extract) occurs as yellow crystals, crystalline powder, wax-like lumps, or oil-like liquid.

Identification Determine the absorption spectrum of Menaquinone (Extract), previously kept in a vacuum desiccator containing phosphorous(V) oxide at 40°C for 24 hours, as directed in the Potassium Bromide Disk Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Arsenic Not more than 2.0 μg/g as As_2O_3 (1.0 g, Method 3, Apparatus B).

(3) Menadion To 0.20 g of Menaquinone (Extract), add 5 ml of diluted absolute ethanol (1 in 2), shake well, and filter. To 0.5 ml of the filtrate, add 1 drop of a solution (1 in 20) of 3-methyl-1-phenyl-5-pyrazoron in absolute ethanol and 1 drop of aqueous ammonia, and allow to stand for 2 hours. A blue-purple color does not develop.

Water Content Not more than 0.50% (0.50 g, Direct Titration).

Residue on Ignition Not more than 0.10%.

Assay Throughout this assay, all procedures should be protected from direct light and the apparatus used should be light-resistant. Before the assay, determine the water content of menaquinone-4 in the same manner as Menaquinone (Extract).

Test Solution and Standard Solution Weigh accurately about 0.1 g each of Menaquinone (Extract) and menaquinone-4 for assay, dissolve separately in 50 ml of 2-propanol, and add absolute ethanol to make 2 solutions of exactly 100 ml each. Measure exactly 10 ml each of these solutions, and add absolute ethanol to each to make exactly 100 ml. To exactly 2 ml each of the resulting solutions, add 4 ml of a solution of phytonadion in 2-propanol (1 in 20,000). Use these solutions as the test solution and the standard solution, respectively.

Procedure Analyze 20 μl portions of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Determine the peak area ratios (Q_T and Q_S) of menaquinone-4 to phytonadion for the test solution and the standard solution, and calculate the content of menaquinone-4 by the formula:

$$\text{Content (\%) of menaquinone-4 } (C_{31}H_{40}O_2) = \frac{\left(\begin{array}{c}\text{Ahydrous basis weight (g) of}\\ \text{menaquinone-4 for assay}\end{array}\right)}{\text{Anhydrous basis weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 100$$

Operating Conditions

Detector: Ultraviolet spectrophotometer (determination wavelength: 270 nm).

Column: A stainless steel tube of about 5 mm internal diameter and about 15 cm length.

Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: A constant temperature at about 40°C.

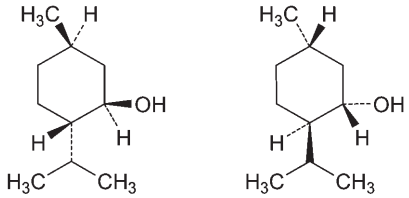
Mobile phase: Methanol.

Flow rate: Adjust so that the retention time of menaquinone-4 is about 7 minutes.

dl-Menthol

_dl_-Peppermint Camphor

dl-メントール



$C_{10}H_{20}O$ Mol. Wt. 156.27
(1*RS*,2*SR*,5*RS*)-5-Methyl-2-(1-methylethyl)cyclohexan-1-ol
[89-78-1]

Content *dl*-Menthol contains not less than 98.0% of *dl*-menthol ($C_{10}H_{20}O$).

Description *dl*-Menthol occurs as colorless prisms or needles or as a white crystalline powder having a peppermint-like odor.

Identification

(1) Triturate *dl*-Menthol with an equal quantity of camphor or thymol. The mixture is liquefied.

(2) To 1 g of *dl*-Menthol, add 20 ml of sulfuric acid, and shake. Yellowish red turbidity appears. Allow to stand for 24 hours. A transparent, oily layer having no odor of menthol separates.

Purity

(1) Congealing point 27–28°C.

(2) Specific rotation $[\alpha]_D^{20}$: − 2.0 to +2.0° (2.5 g, ethanol, 25 ml).

(3) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 4, Apparatus B).

(5) Thymol Weigh 0.20 g of *dl*-Menthol, and add to a cool mixture of 2 ml of acetic acid, 6 drops of sulfuric acid, and 2 drops of nitric acid. No color develops.

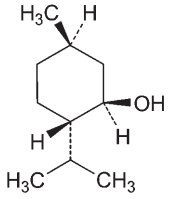
Assay Weigh accurately about 1 g of *dl*-Menthol, and proceed as directed in Method 2 in the Alcohol Content Test in the Flavoring Substances Tests.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 78.13 mg of $C_{10}H_{20}O$

l-Menthol

Peppermint Camphor

l-メントール



$C_{10}H_{20}O$ Mol. Wt. 156.27
(1*R*,2*S*,5*R*)-5-Methyl-2-(1-methylethyl)cyclohexan-1-ol
[2216-51-5]

Content *l*-Menthol contains not less than 98.0% of *l*-menthol ($C_{10}H_{20}O$).

Description *l*-Menthol occurs as colorless prisms or needles or as a white crystalline powder having a peppermint-like odor and a cool taste.

Identification

(1) A solution of *l*-Menthol in ethanol (1 in 10) is levorotatory.

(2) Proceed as directed in Identification (1) and (2) for *dl*-Menthol.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: − 45.0 to −51.0° (2.5 g, ethanol, 25 ml).

(2) Melting point 42–44°C.

(3) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 4, Apparatus B).

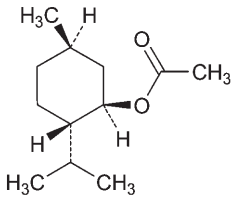
(5) Thymol Proceed as directed in Purity (5) for *dl*-Menthol.

Assay Weigh accurately about 1 g of *l*-Menthol, and proceed as directed in Method 2 in the Alcohol Content Test in the Flavoring Substances Tests.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 78.13 mg of $C_{10}H_{20}O$

l-Menthyl Acetate

酢酸*l*-メンチル



$C_{12}H_{22}O_2$ Mol. Wt. 198.30
(1*R*,2*S*,5*R*)-5-Methyl-2-(1-methylethyl)cyclohexyl acetate [2623-23-6]

Content *l*-Menthyl Acetate contains not less than 98.0% of *l*-menthyl acetate ($C_{12}H_{22}O_2$).

Description *l*-Menthyl Acetate is a colorless or slightly yellowish, transparent liquid having a cool odor.

Identification To 1 ml of *l*-Menthyl Acetate, add 5 ml of ethanolic 10% potassium hydroxide TS, and heat under a reflux condenser in a water bath for 1 hour. The cool odor disappears, and an odor of menthol is evolved. Cool, and add 2 ml of water and 2 ml of diluted hydrochloric acid (1 in 4). The solution responds to test (3) for Acetate in Qualitative Tests.

Purity

(1) Refractive index n_D^{20}: 1.445–1.448.

(2) Angular rotation α_D^{20}: –70 to –75°.

(3) Specific gravity 0.924–0.928.

(4) Clarity of solution Clear (1.0 ml, 70% (vol) ethanol 7.0 ml).

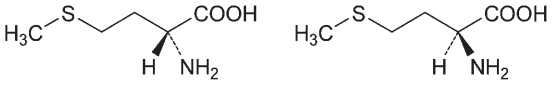
(5) Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Weigh accurately about 1.5 g of *l*-Methyl Acetate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests. In the test, boil the mixture for 2 hours before titrating.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 99.15 mg of $C_{12}H_{22}O_2$.

DL-Methionine

DL-メチオニン



$C_5H_{11}NO_2S$ Mol. Wt. 149.21
(2*RS*)-2-Amino-4-(methylsulfanyl)butanoic acid [59-51-8]

Content DL-Methionine, when dried, contains 98.5–101.0% of DL-methionine ($C_5H_{11}NO_2S$).

Description DL-Methionine occurs as white plates or crystalline powder having a characteristic odor and a slightly sweet taste.

Identification

(1) Determine the absorption spectrum of DL-Methionine, previously dried as directed in the Potassium Bromide Disk Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) A solution of DL-Methionine (1 in 100) exhibits no optical rotation.

Purity

(1) Clarity and color of solution Colorless and clear (0.50 g, water 20 ml).

(2) pH 5.6–6.1 (1.0 g, water 100 ml).

(3) Chloride Not more than 0.021% as Cl.

Test Solution Weigh 0.5 g of DL-Methionine, dissolve by adding 6 ml of diluted nitric acid (1 in 10) and water, and make 40 ml.

Control Solution To 0.30 ml of 0.01 mol/L hydrochloric acid, add 6 ml of diluted nitric acid (1 in 10) and water to make 40 ml.

Procedure In the test, use 10 ml of silver nitrate solution (1 in 50).

(4) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 1, Dissolve while warming, Control solution Lead Standard Solution 2.0 ml).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3.

Proceed as directed in Purity (4) for L-Cysteine Monohydrochloride.

Loss on Drying Not more than 0.5% (105°C, 3 hours).

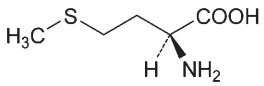
Residue on Ignition Not more than 0.10% .

Assay Weigh accurately about 0.3 g of DL-Methionine, and proceed as directed in the Assay for DL-Alanine.

Each ml of 0.1 mol/L perchloric acid = 14.92 mg of $C_5H_{11}NO_2S$

L-Methionine

L-メチオニン



$C_5H_{11}NO_2S$ Mol. Wt. 149.21
(2*S*)-2-Amino-4-(methylsulfanyl)butanoic acid [63-68-3]

Content L-Methionine, when dried, contains 98.5–101.0% of L-methionine ($C_5H_{11}NO_2S$).

Description L-Methionine occurs as white plates or crystalline powder having a characteristic odor and a slight bitter taste.

Identification

(1) To 5 ml of a solution of L-Methionine (1 in 1,000), add 1 ml of ninhydrin solution (1 in 1,000), and heat for 3 minutes. A purple color develops.

(2) To 0.025 g of L-Methionine, add 1 ml of anhydrous cupric sulfate-saturated sulfuric acid. A yellow color develops.

(3) To 2 ml of a solution of L-Methionine (1 in 100), add 2 ml of sodium hydroxide solution (1 in 25), shake, add 0.3 ml

of sodium nitroprusside solution (1 in 20), and shake again. Allow to stand for 1–2 minutes, and add 4 ml of diluted hydrochloric acid (1 in 10). A red-purple color develops.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +21.0 to +25.0° (1.0 g, hydrochloric acid (1 in 2), 50 ml, on the dried basis).

(2) Clarity and color of solution Colorless and clear (0.50 g, water 20 ml).

(3) pH 5.6–6.1 (1.0 g, water 100 ml).

(4) Chloride Not more than 0.021% as Cl.

Proceed as directed in Purity (3) for DL-Methionine.

(5) Heavy metals Not more than 20 μg/g as Pb.

Proceed as directed in Purity (4) for DL-Methionine.

(6) Arsenic Not more than 4.0 μg/g as As_2O_3.

Proceed as directed in Purity (4) for L-Cysteine Monohydrochloride.

Loss on Drying Not more than 0.5% (105°C, 3 hours).

Residue on Ignition Not more than 0.10% .

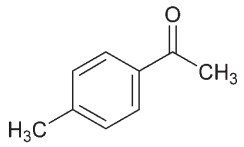
Assay Weigh accurately about 0.3 g of L-Methionine, and proceed as directed in the Assay for DL-Alanine.

Each ml of 0.1 mol/L iodine = 14.92 mg of $C_5H_{11}NO_2S$

p-Methylacetophenone

4-Methyl Acetophenone

パラメチルアセトフェノン



$C_9H_{10}O$ Mol. Wt. 134.18

1-(4-Methylphenyl)ethanone [122-00-9]

Content *p*-Methylacetophenone contains not less than 98.0% of *p*-methylacetophenone ($C_9H_{10}O$).

Description *p*-Methylacetophenone is a colorless or slightly yellowish, transparent liquid having a characteristic odor.

Identification Determine the absorption spectrum of *p*-Methylacetophenone as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Refractive index n_D^{20}: 1.532–1.535.

(2) Specific gravity 1.005–1.008.

(3) Clarity of solution Clear (1.0 ml, 70% (vol) ethanol 3.0 ml).

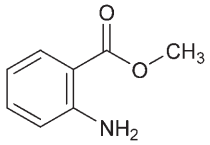
(4) Halogenated compounds Proceed as directed in the Halogenated Compounds Test in the Flavoring Substances Tests.

Assay Weigh accurately about 1 g of *p*-Methylacetophenone, and proceed as directed in Method 2 in the Aldehyde and Ketone Content Test in the Flavoring Substances Tests. In the test, boil the mixture for 1 hour before titrating.

Each ml of 0.5 mol/L hydrochloric acid = 67.09 mg of $C_9H_{10}O$

Methyl Anthranilate

アントラニル酸メチル



$C_8H_9NO_2$ Mol. Wt. 151.16

Methyl 2-aminobenzoate [134-20-3]

Content Methyl Anthranilate contains not less than 98.0% of methyl anthranilate ($C_8H_9NO_2$).

Description Methyl Anthranilate occurs as colorless to light yellow lumps or as a colorless to light yellow liquid. It has a grape-like odor. The liquid fluoresces a blue-purple color.

Identification

(1) Dissolve 0.1 g of Methyl Anthranilate in 10 ml of diluted hydrochloric acid (1 in 40). Add 1 ml of sodium nitrite solution (1 in 10) freshly prepared and 2 ml of the solution prepared by dissolving 0.1 g of β-naphthol in 5 ml of sodium hydroxide solution (1 in 25). An orange-red precipitate is formed.

(2) To 1 g of Methyl Anthranilate, add 5 ml of ethanolic 10% potassium hydroxide TS, and heat in a water bath for 5 minutes. Add 5 ml of water while hot, cool, and add 4 ml of diluted hydrochloric acid (1 in 4). A white to grayish white precipitate is formed.

Purity

(1) Congealing point Not less than 22°C.

(2) Refractive index n_D^{20}: 1.580–1.585.

(3) Clarity of solution Clear.

Measure 1.0 ml of Methyl Anthranilate, melted by warming at 30°C, and dissolve it in 5.0 ml of 60% (vol) ethanol.

(4) Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Weigh accurately about 0.5 g of Methyl Anthranilate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 75.58 mg of $C_8H_9NO_2$

Methyl Cellulose

メチルセルロース

Methyl ether of cellulose [9004-67-5]

Content Methyl Cellulose, when dried, contains 25.0–33.0% of methoxy group (-OCH_3 = 31.03).

Description Methyl Cellulose occurs as a white to whitish powder or fibrous substance. It is odorless.

Identification Add 1.0 g of Methyl Cellulose to 100 ml of water at about 70°C, stir well, cool while shaking, and allow to stand in a cold place until it becomes a homogeneous paste. Use the resulting solution as the test solution.

(1) Heat about 10 ml of the test solution in a water bath. White turbidity or precipitate is formed. Cool it. The white turbidity or precipitate dissolves and becomes a homogeneous pasty again.

(2) On the top of about 2 ml of the test sample, superimpose 1 ml of anthrone TS gently along the tube wall. The junction between the two solutions turns blue to green.

Purity

(1) Viscosity When the viscosity is declared, perform the following test. The viscosity is 80–120% of the declared amount when the declared amount is not more than 100 mm²/s, and 70–140% when it exceeds 100 mm²/s.

Weigh accurately an amount of Methyl Cellulose equivalent to 2 g, calculated on the dried basis, add 50 ml of water of 85°C, and stir for 10 minutes using a stirrer. Add 40 ml of water, dissolve the sample in ice water while stirring for 40 minutes, and add water to make exactly 100 ml. Remove the effervescence by centrifuging if necessary, and measure the viscosity at 20±0.15°C.

(2) Chloride Not more than 0.57% as Cl.

Sample Solution Weigh 0.5 g of Methyl Cellulose, transfer into a beaker, add 30 ml of hot water, stir well, and filter while hot with a warmed funnel. Wash the beaker and the residue on filter paper three times, using 15 ml of hot water each time, combine the filtrate and the washings, and add water to make 100 ml. Refer to this solution as solution A. Use 5 ml of solution A as the sample solution.

Control Solution Use 0.40 ml of 0.01 mol/L hydrochloric acid.

(3) Sulfate Not more than 0.096% as SO_4.

Sample Solution Use exactly 40 ml of solution A obtained in Purity (2).

Control Solution Use 0.40 ml of 0.01 mol/L hydrochloric acid.

(4) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.5 g, Method 3, Apparatus B).

Loss on Drying Not more than 8.0% (105°C, 1 hour)

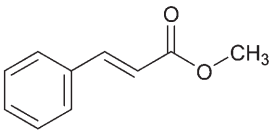
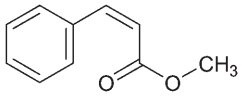
Residue on Ignition Not more than 1.5% (calculated on the dried basis).

Assay Weigh accurately about 0.025 g of Methyl Cellulose, previously dried, and proceed as directed under Methoxy Determination.

$$\text{Content (\%) of methoxy group (-OCH}_3\text{)} = \frac{\left(\text{Volume (ml) of 0.01 mol/L sodium thiosulfate consumed}\right) \times 0.0517}{\text{Weight (g) of the sample} \times 1{,}000} \times 100$$

Methyl Cinnamate

ケイ皮酸メチル

$C_{10}H_{10}O_2$ Mol. Wt. 162.19

Methyl 3-phenylprop-2-enoate [103-26-4]

Content Methyl Cinnamate contains not less than 98.0% of methyl cinnamate ($C_{10}H_{10}O_2$).

Description Methyl Cinnamate occurs as a white solid having a *matsutake* mushroom-like odor.

Identification To 1 g of Methyl Cinnamate, add 10 ml of ethanolic 10% potassium hydroxide TS, and heat in a water bath. Methyl Cinnamate dissolves, a white precipitate is formed, and the characteristic odor disappears. The precipitate dissolves on the addition of 10 ml of water while it is warm. When the solution is acidified with diluted sulfuric acid (1 in 20), a white crystalline precipitate is formed.

Purity

(1) Congealing point Not less than 33.8°C.

(2) Clarity of solution Almost clear.

Test Solution Weigh 1.0 g of Methyl Cinnamate, add 3.0 ml of 70% (vol) ethanol, and dissolve by warming at 40°C.

(3) Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Weigh accurately about 0.9 g of Methyl Cinnamate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests. In this test, add 5 ml of water before heating.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 81.09 mg of $C_{10}H_{10}O_2$

Methyl Hesperidin

Soluble Vitamin P

メチルヘスペリジン

Content Methyl Hesperidin, when dried, contains 97.5–103.0% of methyl hesperidin.

Description Methyl Hesperidin occurs as a yellow to orange-yellow powder. It is odorless or has a slight odor.

Identification

(1) To 0.01 g of Methyl Hesperidin, add 2 ml of sulfuric acid. A red color develops. Add 1–2 drops of hydrogen peroxide TS. A dark red color develops.

(2) To 0.1 g of Methyl Hesperidin, add 5 ml of ethanol and 1 ml of sodium hydroxide solution (1 in 25), boil for 3 minutes, cool, and filter. The color of the filtrate is yellow to orange-yellow. To the filtrate, add 1 ml of hydrochloric acid and about 0.010 g of magnesium dust, and allow to stand. A pink color develops.

(3) To 0.1 g of Methyl Hesperidin, add 10 ml of diluted hydrochloric acid (1 in 3), boil for 5 minutes, cool, and filter. Neutralize the filtrate with sodium hydroxide solution (1 in 5), add 2 ml of Fehling's TS, and heat. A red precipitate is formed.

Purity

(1) Clarity of solution Almost clear (1.0 g, water 10 ml).

(2) Sulfate Not more than 0.019% as SO_4 (1.0 g, Control solution 0.005 mol/L sulfuric acid 0.40 ml).

(3) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

Loss on Drying Not more than 3.0% (reduced pressure, 24 hours).

Residue on Ignition Not more than 0.5%.

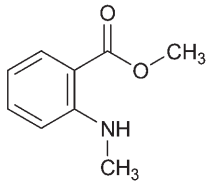
Assay Weigh accurately about 0.3 g of Methyl Hesperidin, previously dried, and dissolve it in water to make exactly 1,000 ml. Measure exactly 10 ml of this solution, and add water to make exactly 100 ml. Measure the absorbance (A) of the resulting solution at a wavelength of 300 nm, and calculate the content of methyl hesperidin by the formula:

$$\text{Content (\%) of methyl hesperidin} = \frac{A \times 0.754}{\text{Weight (g) of the sample}} \times 100$$

Methyl *N*-Methylanthranilate

Dimethyl Anthranilate

N-メチルアントラニル酸メチル



$C_9H_{11}NO_2$ Mol. Wt. 165.19

Methyl 2-(methylamino)benzoate [85-91-6]

Content Methyl *N*-Methylanthranilate contains 98.0–101.0% of methyl *N*-methylanthranilate ($C_9H_{11}NO_2$).

Description Methyl *N*-Methylanthranilate occurs as colorless to light yellow, transparent crystalline lumps or liquid. It has a grape-like odor. A liquid product generates a blue-purple fluorescence.

Identification To 1 ml of Methyl *N*-Methylanthranilate, add 5 ml of ethanolic 10% potassium hydroxide TS, and heat under a reflux condenser for 1 hour. The grape-like odor disappears. Cool, and acidify with diluted hydrochloric acid (1 in 4). Crystals are deposited. Collect the crystals by filtration, and recrystallize from 50% (vol) ethanol. The melting point of the crystals is 164–174°C.

Purity

(1) Congealing point Not less than 11°C.

(2) Refractive index n_D^{20}: 1.578–1.581.

(3) Specific gravity 1.129–1.135.

(4) Clarity of solution Clear (1.0 ml, 70% (vol) ethanol 10 ml).

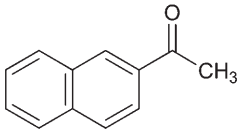
(5) Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Weigh accurately about 1 g of Methyl *N*-Methylanthranilate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 82.60 mg of $C_9H_{11}NO_2$

Methyl β-Naphthyl Ketone

メチルβ-ナフチルケトン



$C_{12}H_{10}O$ Mol. Wt. 170.21

1-(Naphthalen-2-yl)ethanone [93-08-3]

Content Methyl β-Naphthyl Ketone contains not less than 99.0% of methyl β-naphthyl ketone ($C_{12}H_{10}O$).

Description Methyl β-Naphthyl Ketone occurs as white to light yellow crystals or crystalline powder having a characteristic odor.

Identification Determine the absorption spectrum of Methyl β-Naphthyl Ketone as directed in the Potassium Bromide Disk Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Melting point 52–54°C.

(2) Clarity of solution Clear.

Weigh 0.10 g of Methyl β-Naphthyl Ketone, add 10 ml of 70% (vol) ethanol, and dissolve by warming to 30°C.

(3) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 4, Apparatus B).

(5) Halogenated compounds Proceed as directed in the Halogenated Compounds Test in the Flavoring Substances Tests.

Loss on Drying Not more than 0.5% (4 hours).

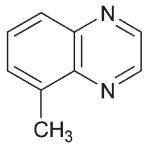
Residue on Ignition Not more than 0.05%.

Assay Weigh accurately about 1g of Methyl β-Naphthyl Ketone, and proceed as directed in Method 2 in the Aldehyde and Ketone Content Test in the Flavoring Substances Tests. In the test, boil the mixture for 1 hour before titrating.

Each ml of 0.5 mol/L hydrochloric acid = 85.10 mg of $C_{12}H_{10}O$

5-Methylquinoxaline

5-メチルキノキサリン



$C_9H_8N_2$ Mol. Wt. 144.17

5-Methylquinoxaline [13708-12-8]

Content 5-Methylquinoxaline contains not less than 98.0% of 5-methylquinoxaline ($C_9H_8N_2$).

Description 5-Methylquinoxaline occurs as a colorless to orange-color liquid or crystalline lumps having a characteristic odor.

Identification Determine the infrared absorption spectrum of 5-Methylquinoxaline as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit absorptions having about the same intensity at the same wavenumbers.

Purity

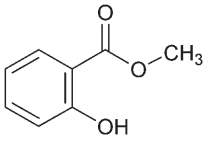
(1) Refractive index n_D^{20}: 1.615–1.625.

(2) Specific gravity d_{25}^{25}: 1.102–1.128.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay under the Flavor Substance Tests. Use operating conditions (1).

Methyl Salicylate

サリチル酸メチル



$C_8H_8O_3$ Mol. Wt. 152.15

Methyl 2-hydroxybenzoate [119-36-8]

Content Methyl Salicylate contains not less than 98.0% of methyl salicylate ($C_8H_8O_3$).

Description Methyl Salicylate is a colorless to light yellow, transparent liquid having a cool odor.

Identification Determine the absorption spectrum of Methyl Salicylate as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Refractive index n_D^{20}: 1.535–1.538.

(2) Specific gravity 1.183–1.189.

(3) Clarity of solution Clear (1.0 ml, 70% (vol) ethanol 8.0 ml).

(4) Acid value Not more than 0.5 (Flavoring Substances Tests).

Use Phenol Red TS as the indicator.

Assay Weigh accurately about 0.9 g of Methyl Salicylate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests, using Phenol Red TS as the indicator.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 76.07 mg of $C_8H_8O_3$

Microcrystalline Cellulose

微結晶セルロース

Definition Microcrystalline Cellulose is obtained from pulp and consists mainly of crystalline cellulose. There are two forms of products: the desiccated form and the hydrated form.

Description A desiccated product occurs as a white or almost white, odorless, free- flowable crystalline powder. A hydrated product occurs as white or almost white, odorless, moist, cotton-like lumps.

Identification

(1) *Sample Preparation* In the case of a desiccated product: Confirm the percentage of residue by sieving 20 g of Microcrystalline Cellulose through a 38-μm sieve for 5 minutes, using a vacuum suction-type sieving machine. If not less than 5% of residue remains on the sieve, add 270 ml of water to 30 g of Microcrystalline Cellulose, and if less than 5% of residue remains, add 255 ml of water to 45 g of Microcrystalline Cellulose. Then lightly stir the mixture with a spatula.

In the case of a hydrated product: To an amount equivalent to 30 g of the sample on the dried basis, add water to make a 300-g mixture, and lightly stir with a spatula.

Procedure Mix the corresponding mixture in a high-speed (18,000 rpm) blender for 5 minutes, transfer 100 ml of the mixture to a 100-ml measuring cylinder, allow to stand for 3 hours. A white opaque, bubble-free dispersion is obtained. No separation is observed.

(2) Proceed as directed in the Potassium Bromide Disk Method under Infrared Spectrometry. Compare the spectrum obtained with the Reference Spectrum of Microcrystalline Cellulose. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) pH 5.0–7.5.

To an amount equivalent to 5.0 g of Microcrystalline Cellulose on the dried basis, add 40 ml of freshly boiled and cooled water, shake for 20 minutes, and centrifuge. Use the supernatant for pH determination.

(2) Water-soluble substances Not more than 0.26%.

Weigh accurately an amount equivalent to 5.0 g of Microcrystalline Cellulose on the dried basis, add water to make 85 g, shake for 10 minutes, and filter by suction through a filter paper (5C). Transfer the filtrate to a beaker, previously dried and weighed, evaporate to dryness, taking care not to scorch. Dry at 105°C for 1 hour, cool in a desiccator, and weigh accurately. Separately, perform a blank test for cor-

rection.

(3) Heavy metals Not more than 10 μg/g as Pb (2.0 g on the dried basis, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g on the dried basis, Method 3, Apparatus B)

(5) Starch To 20 ml of the liquid obtained under the Identification (1), add a few drops of iodine TS, and mix. No bluish purple or blue color develops.

Loss on Drying

Desiccated sample Not more than 7.0% (105°C, 3 hours).

Hydrated sample 40.0–70.0% (4 g, 105°C, 3 hours).

Residue on ignition Not more than 0.05% (2 g on the dried basis).

Microcrystalline Wax

マイクロクリスタリンワックス

Definition Microcrystalline Wax is a mixture of solid hydrocarbons obtained from petroleum vacuum distillation residues or heavy distillates. It consists mainly of branched and linear saturated hydrocarbons.

Description Microcrystalline Wax is a colorless or white to yellow, partially translucent solid at room temperature. It has a slight characteristic odor.

Identification Determine the absorption spectrum of Microcrystalline Wax as directed in the Thin Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Melting point 70–95°C (Melting Point Determination, procedure for Class 2 Substances).

(2) Lead Not more than 3.0 μg/g as Pb (3.3 g, Method 1).

(3) Arsenic Not more than 2.0 μg/g as As_2O_3 (1.0 g, Method 3, Apparatus B).

(4) Polycyclic aromatic hydrocarbons

Proceed as directed in Purity (5) for Paraffin Wax in the Monographs.

Residue on Ignition Not more than 0.10%.

Microfibrillated Cellulose

微小繊維状セルロース

Definition Microfibrillated Cellulose is obtained by microfibrillating pulp or cotton and consists mainly of cellulose.

Description Microfibrillated Cellulose is a white, wet-cotton-like substance.

Identification

(1) Dry Microfibrillated Cellulose to produce a thin film-form sample, and cut into small pieces or break into flakes. Determine the absorption spectrum of the prepared sample as directed in the Potassium Bromide Disk Method under Infrared Spectrophotometry. The sample disk should be prepared so that the transmittances in the main absorption bands are in a range of 30–80%. Compare the obtained spectrum with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) Weigh an amount of Microfibrillated Cellulose equivalent to 5.0 g on the dried basis, and add water to make 100 g. Mix it at 10,000–12,000 rpm for 3 minutes, using a homogenizer with a rotor blade of 35 mm in diameter and a cup of 150 ml in volume (59 mm in upper internal diameter, 44 mm in lower internal diameter, and 75 mm in depth). The mixture is in a white opaque dispersed-form and remains the same even after 3 hours without separating.

(3) Weigh an amount of Microfibrillated Cellulose equivalent to 1.0 g on a dry basis, and add water to make 100 g. Homogenize for 3 minutes as specified in Identification (1). Place the white turbid liquid obtained on a 20-cm×25-μm standard sieve with a receiver, and gently vibrate horizontally for 10 seconds. Evaporate the liquid passing through the sieve to dryness. The weight of the residue is not more than 0.30 g.

Purity

(1) pH 5.0–8.0 for suspension (2.0 g, water 100 ml).

(2) Lead Not more than 2.0 μg/g as Pb (sample amount: equivalent of 5.0 g on the dried basis, Method 1).

(3) Arsenic Not more than 2.0 μg/g as As_2O_3 (sample amount: equivalent of 1.0 g on the dried basis, Method 3, Apparatus B).

(4) Water-soluble substance Not more than 0.50%.

Weigh an amount of Microfibrillated Cellulose equivalent to 4.0 g on the dried basis, add 200 ml of water, and disperse using a high-speed disperser (composed of 4 blades about 13 mm in length and about 16 mm in maximum width) at 5,000 rpm for 5 minutes. Filter the dispersed liquid by suction through a 5-C filter paper. Evaporate 50 ml of the filtrate to dryness on a water bath, dry the residue at 120°C for 1 hour, and allow to stand in a desiccator. Weigh the residue accurately.

Loss on Drying 60.0–92% (5 g, 120°C, 5hours).

Ash Not more than 0.50 % (sample amount: equivalent of 2.0 g on the dried basis).

Microbial Limits Proceed as directed in the Microbial Limit Tests. The total bacterial count is not more than 5,000/g, and *Escherichia coli* is negative.

Milt Protein

しらこたん白抽出物

Definition Milt Protein is obtained from the testes of the ainame *Hexagrammos otakii* Jordan et Starks, the humpback salmon *Oncorhynchus gorbuscha* (Walbaum), the chum salmon *Oncorhynchus keta* (Walbaum), the sockeye salmon *Oncorhynchus nerka* (Walbaum), the skipjack tuna *Katsuwonus pelamis* (Linnaeus), or the Pacific herring *Clupea pallasii pallasii* Valenciennes, and consists mainly of basic proteins.

Content Milt Protein, when calculated on the dried basis, contains the equivalent of not less than 50% of protamine.

Description Milt Protein occurs as a white to light yellow powder having a slight characteristic odor.

Identification

(1) Dissolve 1 mg of Milt Protein in 2 ml of water, and add 5 drops of a solution of 0.1 g of α-naphthol in 100 ml of diluted ethanol (7 in 10) and 5 drops of sodium hypochlorite TS. When the solution is made alkaline with sodium hydroxide solution (1 in 20), a bright red color develops.

(2) Dissolve 5 mg of Milt Protein in 1 ml of water while warming. To this solution, add 1 drop of sodium hydroxide solution (1 in 10) and 2 drops of cupric sulfate solution (1 in 7). A blue-purple color develops.

Purity

(1) Clarity and color of solution Colorless to light yellow and turbid (0.5 g, water 50 ml, 5 minutes of stirring).

(2) Lead Not more than 5.0 μg/g (2.0 g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50g, Method 3, Apparatus B).

Loss on Drying Not more than 7.0% (100°C, 3 hours).

Ash Not more than 15.0%.

Assay Determine the amount of nitrogen in about 0.1–0.15 g of Milt Protein, accurately weighed, as directed in the Kjeldehl Method under Nitrogen Determination. Calculate the content of protamine by the formula given below.

Each ml of 0.05 mol/L sulfuric acid = 1.401 mg of N

$$\text{Content (\%) of protamine} = \frac{\text{Amount (mg) of nitrogen} \times 3.19}{\text{Dry basis weight (g) of the sample} \times 1{,}000} \times 100$$

Mixed Tocopherols

ミックストコフェロール

Definition Mixed Tocopherols are obtained from vegetable fats or oils and consist mainly of *d*-α-tocopherol, *d*-β-tocopherol, *d*-γ-tocopherol, and *d*-δ-tocopherol. They may contain edible fats or oils.

Contents Mixed Tocopherols contain not less than 34% of total tocopherols.

Description Mixed Tocopherols are slightly yellow to reddish brown, clear viscous liquids having a characteristic odor.

Identification Proceed as directed under Identification for *d*-α-Tocopherol.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: not less than +20°C.

Proceed as directed in Purity (1) for *d*-α-Tocopherol.

(2) Acid value Not more than 5.0.

Proceed as directed in Purity (2) for Tocotrienol.

(3) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(5) Antioxidation value Not less than 40.

Test Solution Weigh accurately an amount of the sample equivalent to about 0.030 g of total tocopherols, transfer to a 200-ml brown volumetric flask, dissolve it in absolute ethanol to make 200 ml. Place 2 ml of this solution in a 25-ml brown volumetric flask, add 1 ml of a solution of iron(III) chloride in absolute ethanol (1 in 500), immediately add 1 ml of a solution of α,α′-dipyridyl in absolute ethanol (1 in 200), shake mildly, and add absolute ethanol to make exactly 25 ml.

Control Solution Place 2 ml of absolute ethanol in a 25-ml brown volumetric flask, and then proceed as directed for the test solution.

Procedure Exactly 10 minutes after the addition of the solution of iron(III) chloride in absolute ethanol, measure the absorbances (A and A′) of the test solution and the control solution at 520 nm against absolute ethanol. Calculate the antioxidation value by the formula:

$$\text{Antioxidation value} = \frac{A - A'}{\text{Weight (g) of the sample}} \times 2.82 \times 2$$

Assay Proceed as directed in the Assay for *d*-α-Tocopherol.

Monascus Color

ベニコウジ色素

Definition Monascus Color is obtained from the culture fluid of *Monascus pilosus* or *Monascus purpureus* and consists mainly of ankaflavins and monascorubrins.

Color Value The Color Value ($E_{1cm}^{10\%}$) of Monascus Color is not less than 50 and is in the range of 90–110% of the labeled value.

Description Monascus Color is a dark red powder, paste, or liquid having a slightly characteristic odor.

Identification

(1) Weigh the equivalent of 1 g of Monascus Color with a Color Value 50, and dissolve it in 100 ml of a 1:1 mixture of water/ethanol. A red-orange to dark red color develops.

(2) To 1 ml of the solution obtained in Identification (1), add 1 ml of ammonia solution and 1 ml of acetone, and heat at 45–55°C for 1 minute. A yellow-orange color develops. Allow it to stand for 10 minutes. A yellow-green fluorescence is emitted.

(3) To 0.1 ml of the solution obtained in Identification (1), add 3 ml of nitric acid, and shake immediately. A yellow color develops.

(4) A solution of Monascus Color in a 1:1 mixture of water/ethanol exhibits an absorption maximum at a wavelength of 480–520 nm.

Purity

(1) Heavy metals Not more than 40 μg/g as Pb (0.50 g, Method 2, Control solution Lead standard solution 2.0 ml).

(2) Lead Not more than 10 μg /g as Pb (1.0g, Method 1).

(3) Arsenic Not more than 4.0 μg /g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(4) Citrinine Not more than 0.2 μg/g (on the basis of a Color Value 50).

Test Solution As a packing material, use styrene-divinylbenzene resin for adsorption or acrylic ester resin for adsorption. Rinse the resin with methanol to replace water, pack a

glass column with a 1 cm internal diameter to the height of 10 cm. Check the adsorption resin to confirm that citrinine runs off in the first 20 ml. Weigh accurately the equivalent of about 1 g of Monascus Color with a Color Value 50, and place on the surface of resin-layer in the glass column. Allow a 7:3 mixture of methanol/water to flow through the column at a flow rate of 2–3 ml/minute, and collect the first 20-ml effluent. Filter the effluent through a membrane filter with a pore diameter of not more than 0.5 µm.

Standard Solutions Weigh exactly 0.0100 g of citrinine, and dissolve it in methanol to make exactly 100 ml. Dissolve exactly 1 ml of this solution in a 7:3 mixture of methanol/water to make exactly 100 ml. Then transfer exactly 1.0 ml, 5.0 ml, and 10.0 ml of the second solution into separate 100-ml volumetric flasks, and dilute each with a 7:3 mixture of methanol/water to volume.

Procedure Analyze 5 µl potions of the test solution and the standard solutions by liquid chromatography using the conditions below. The procedure should be promptly carried out. Measure the peak areas of citrinine for the standard solutions, and prepare a calibration curve. To determine citrinine in the test solution, correct the peak area as the peak overlapping on the tailing because the peak of citrinine is interfered with tailing of another peak.

Operating Conditions

Detector: Fluorescence spectrophotometer (excitation wavelength 330 nm; fluorescence wavelength 500 nm).

Column: A stainless steel tube of 3.9–4.6 mm internal diameter and 25–30 cm length.

Column packing material: 5-µm octadecylsilanized silica gel.

Column temperature: Room temperature.

Mobile phase: A 100:100:0.1 mixture of water/acetonitril/trifluoroacetic acid.

Flow rate: 1 ml/minute.

Color Value Test Proceed as directed in the Color Value Test.

Operating Conditions

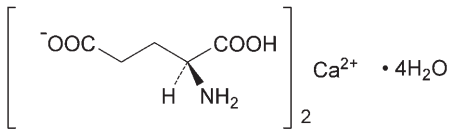
Solvent: A 1:1 mixture of water/ethanol.

Wavelength: Maximum absorption wavelength of 480–520 nm.

Monocalcium Di-L-Glutamate

Calcium Diglutamate

L-グルタミン酸カルシウム



$C_{10}H_{16}N_2CaO_8 \cdot 4H_2O$ Mol.Wt. 404.38

Monocalcium bis[monohydrogen (2*S*)-2-aminopentanedioate] tetrahydrate [69704-19-4]

Content Monocalcium Di-L-Glutamate, when calculated on the anhydrous basis, contains 98.0–102.0% of monocalcium L-glutamate ($C_{10}H_{16}N_2CaO_8$ = 332.32).

Description Monocalcium Di-L-Glutamate occurs as colorless to white prismatic crystals or as white crystals having a characteristic taste.

Identification

(1) To 5 ml of a solution of Monocalcium Di-L-Glutamate (1 in 1,000), add 1 ml of ninhydrin solution (1 in 1,000) and heat for 3 minutes. A purple color develops.

(2) Monocalcium Di-L-Glutamate responds to all tests for Calcium Salt in the Qualitative Tests.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +27.4 to +29.2° (10 g, diluted hydrochloric acid (1 in 4), 100 ml, on the anhydrous basis).

(2) Clarity and color of solution Colorless and almost clear (1.0 g, water 10 ml).

(3) pH 6.7–7.3 (1.0 g, water 10 ml).

(4) Chloride Not more than 0.10% as Cl (0.07 g, Control solution 0.01 mol/L hydrochloric acid 0.20 ml).

(5) Heavy metals Not more than 10 µg/g as Pb (2.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(6) Arsenic Not more than 2.5 µg/g as As_2O_3 (0.80 g, Method 1, Apparatus B).

Water Content Not more than 19% (0.3 g, Direct Titration).

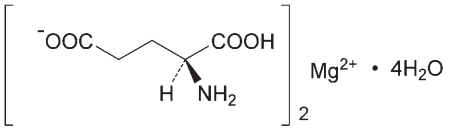
Assay Weigh accurately about 0.2 g of Monocalcium Di-L-Glutamate, dissolve it in 50 ml of water, add about 2 ml of ammonia–ammonium chloride buffer solution (pH10.7), and titrate with 0.02 mol/L EDTA (indicator: 3 drops of Eriochrome Black T TS) until the color of the solution changes from red to blue. Perform a blank test in the same manner, make any necessary correction, and calculate on the anhydrous basis.

Each ml of 0.02 mol/L EDTA = 6.646 mg of $C_{10}H_{16}N_2CaO_8$

Monomagnesium Di-L-Glutamate

Magnesium Diglutamate

L-グルタミン酸マグネシウム



$C_{10}H_{16}N_2MgO_8 \cdot 4H_2O$ Mol. Wt. 388.61

Monomagnesium bis[monohydrogen (2*S*)-2-aminopentanedioate] tetrahydrate [129160-51-6]

Content Monomagnesium Di-L-Glutamate, when calculated on the anhydrous basis, contains 95.0–105.0% of monomagnesium di-L-glutamate ($C_{10}H_{16}N_2MgO_8$ = 316.55).

Description Monomagnesium Di-L-Glutamate occurs as colorless to white prismatic crystals or as white crystals having a characteristic taste.

Identification

(1) To 5 ml of a solution of Monomagnesium Di-L-Glutamate (1 in 1,000), add 1 ml of ninhydrin solution (1 in 1,000)

and heat for 3 minutes. A purple color develops.

(2) Monomagnesium Di-L-Glutamate responds to the test for Magnesium Salt in the Qualitative Tests.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +28.8 to +30.7° (10 g, diluted hydrochloric acid (1 in 4), 100 ml, on the anhydrous basis).

(2) Clarity and color of solution Colorless and almost clear (1.0 g, water 10 ml).

(3) pH 6.5–7.5 (1.0 g, water 10 ml).

(4) Chloride Not more than 0.10% as Cl (0.07 g, Control solution 0.01 mol/L hydrochloric acid 0.20 ml).

(5) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(6) Arsenic Not more than 2.5 μg/g as As_2O_3 (0.80 g, Method 1, Apparatus B).

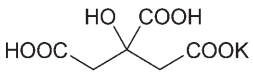
Water Content Not more than 24% (0.2 g, Direct Titration).

Assay Weigh accurately about 0.2 g of Monomagnesium Di-L-Glutamate, dissolve it in 50 ml of water, add about 2 ml of ammonia–ammonium chloride buffer solution (pH10.7), and titrate with 0.02 mol/L EDTA (indicator: 3 drops of Eriochrome Black T TS) until the color of the solution changes from red to blue. Perform a blank test in the same manner, make any necessary correction, and calculate on the anhydrous basis.

Each ml of 0.02 mol/L EDTA = 6.331 mg of $C_{10}H_{16}N_2MgO_8$

Monopotassium Citrate

クエン酸一カリウム



$C_6H_7KO_7$ Mol. Wt. 230.21

Monopotassium dihydrogen 2-hydroxypropane-1,2,3-tricarboxylate [866-83-1]

Content Monopotassium Citrate, when calculated on the dried basis, contains 99.0–101.0% of monopotassium citrate ($C_6H_7KO_7$).

Description Monopotassium Citrate occurs as colorless crystals or crystalline powder. It is odorless.

Identification Monopotassium Citrate responds to all tests for Potassium Salt and to test (2) for Citrate in the Qualitative Tests.

Purity

(1) Clarity and color of solution Colorless and almost clear (1.0 g, water 20 ml).

(2) pH 3.0–4.2 (1.0 g, water 20 ml).

(3) Sulfate Not more than 0.024% as SO_4 (1.0 g, Control solution 0.005 mol/L sulfuric acid 0.50 ml).

(4) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Drying Not more than 0.5% (105°C, 3 hours).

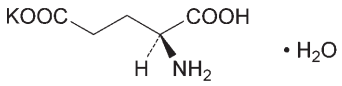
Assay Weigh accurately about 0.4 g of Monopotassium Citrate, add 30 ml of acetic acid for nonaqueous titration, and dissolve by warming. Cool, and titrate with 0.1 mol/L perchloric acid. Usually, a potentiometer is used to confirm the endpoint. When crystal violet–acetic acid TS (1 ml) is used as the indicator, the endpoint is when the color of the solution changes from purple through blue to green. Perform a blank test in the same manner to make any necessary correction, and calculate on the dried basis.

Each ml of 0.1 mol/L perchloric acid = 23.022 mg of $C_6H_7KO_7$

Monopotassium L-Glutamate

Monopotassium Glutamate

L-グルタミン酸カリウム



$C_5H_8NKO_4 \cdot H_2O$ Mol. Wt. 203.23

Monopotassium monohydrogen (2*S*)-2-aminopentanedioate monohydrate [6382-01-0]

Content Monopotassium L-Glutamate, when calculated on the dried basis, contains 99.0–101.0% of monopotassium L-glutamate ($C_5H_8NKO_4 \cdot H_2O$).

Description Monopotassium L-Glutamate occurs as colorless to white prismatic crystals or as a white crystalline powder. It is hygroscopic and has a characteristic taste.

Identification

(1) To 5 ml of a solution of Monopotassium L-Glutamate (1 in 1,000), add 1 ml of ninhydrin solution (1 in 1,000) and heat for 3 minutes. A purple color develops.

(2) Monopotassium L-Glutamate responds to all tests for Potassium Salt in the Qualitative Tests.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +22.5 to +24.0° (10 g, diluted hydrochloric acid (1 in 4), 100 ml, on the dried basis).

(2) Clarity and color of solution Colorless and clear (1.0 g, water 10 ml).

(3) pH 6.7–7.3 (1.0 g, water 10 ml).

(4) Chloride Not more than 0.10% as Cl (0.07 g, Control solution 0.01 mol/L hydrochloric acid 0.20 ml).

(5) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(6) Arsenic Not more than 2.5 μg/g as As_2O_3 (0.80 g, Method 1, Apparatus B).

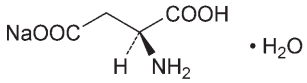
Loss on Drying Not more than 0.5% (80°C, 5 hours).

Assay Weigh accurately about 0.15 g of Monopotassium L-Glutamate, dissolve it in 3 ml of formic acid, add 50 ml of acetic acid for nonaqueous titration, and titrate with 0.1 mol/L perchloric acid. The endpoint is usually confirmed by using a potentiometer. When crystal violet–acetic acid TS (1 ml) is used as the indicator, the endpoint is when the color of the solution changes from brown to green. Perform a blank test in the same manner, make any necessary correction, and calculate on the dried basis.

Each ml of 0.1 mol/L perchloric acid = 10.16 mg of $C_5H_8NKO_4 \cdot H_2O$

Monosodium L-Aspartate

L-アスパラギン酸ナトリウム



$C_4H_6NNaO_4 \cdot H_2O$ Mol. Wt. 173.10
Monosodium (2*S*)-2-aminobutanedioate monohydrate
[3792-50-5]

Content Monosodium L-Aspartate, when calculated on the dried basis, contains not less than 98.0% of monosodium L-aspartate ($C_4H_6NNaO_4 \cdot H_2O$).

Description Monosodium L-Aspartate occurs as colorless to white prisms or as a white crystalline powder having a characteristic taste.

Identification

(1) To 5 ml of a solution of Monosodium L-Aspartate (1 in 1,000), add 1 ml of ninhydrin solution (1 in 1,000), and heat for 3 minutes. A purple color develops.

(2) Monosodium L-Aspartate responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +18.0 to +21.0° (4 g, diluted hydrochloric acid (1 in 2), 50 ml, on the dried basis).

(2) Clarity and color of solution Colorless and clear (1.0 g, water 10 ml).

(3) pH 6.0–7.5 (1.0 g, water 20 ml).

(4) Chloride Not more than 0.041% as Cl (0.30 g, Control solution 0.01 mol/L hydrochloric acid 0.35 ml).

(5) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.5 g, Method 1, Apparatus B).

Loss on Drying Not more than 0.30% (reduced pressure, 5 hours).

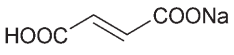
Assay Weigh accurately about 0.1g of Monosodium L-Aspartate, add 3 ml of formic acid and 100 ml of acetic acid, and titrate with 0.1 mol/L perchloric acid. Proceed as directed in the Assay for Asparagine.

Each ml of 0.1 mol/L perchloric acid = 8.655 mg of $C_4H_6NNaO_4 \cdot H_2O$

Monosodium Fumarate

Sodium Fumarate

フマル酸一ナトリウム



$C_4H_3NaO_4$ Mol. Wt. 138.05
Monosodium monohydrogen (2*E*)-but-2-enedioate
[5873-57-4]

Content Monosodium Fumarate, when dried, contains 98.0–102.0% of monosodium fumarate ($C_4H_3NaO_4$).

Description Monosodium Fumarate occurs as a white crystalline powder. It is odorless and has a characteristic acid taste.

Identification

(1) Proceed as directed in Identification (3) and (4) for Fumaric Acid.

(2) Monosodium Fumarate responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Clarity and color of solution Colorless and clear.

Test Solution Weigh 0.50 g of Monosodium Fumarate, add 10 ml of water, warm to 40°C, and dissolve by shaking for 10 minutes.

(2) pH 3.0–4.0 (1.0 g, water 30 ml).

(3) Sulfate Not more than 0.010% as SO_4.

Proceed as directed in Purity (2) for Fumaric Acid.

(4) Heavy metals Not more than 20 μg/g as Pb.

Weigh 1.0 g of Monosodium Fumarate, and proceed as directed in Purity (3) for Fumaric Acid.

(5) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.5 g of Monosodium Fumarate, add 10 ml of water, dissolve by warming, and cool.

Apparatus Use Apparatus B. Use 10 ml of acidic stannous chloride TS and 3 g of arsenic-free zinc.

Loss on Drying Not more than 0.5% (120°C, 4 hours).

Residue on Ignition 50.5–52.5% (dried sample).

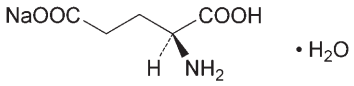
Assay Weigh accurately about 0.3 g of Monosodium Fumarate, previously dried, dissolve it in 30 ml of water, and titrate with 0.1 mol/L sodium hydroxide (indicator: 2 drops of phenolphthalein TS).

Each ml of 0.1 mol/L sodium hydroxide = 13.81 mg of $C_4H_3NaO_4$

Monosodium L-Glutamate

Monosodium Glutamate
Soda Glutamate

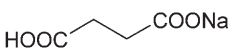
L-グルタミン酸ナトリウム



$C_5H_8NNaO_4 \cdot H_2O$ Mol. Wt. 187.13
Monosodium monohydrogen (2*S*)-2-aminopentanedioate monohydrate [6106-04-3]
Content Monosodium L-Glutamate, when calculated on the dried basis, contains not less than 99.0% of monosodium L-glutamate ($C_5H_8NNaO_4 \cdot H_2O$).
Description Monosodium L-Glutamate occurs as colorless to white prisms or as a white crystalline powder having a characteristic taste.
Identification
(1) To 5 ml of a solution of Monosodium L-Glutamate (1 in 1,000), add 1 ml of ninhydrin solution (1 in 1,000), and heat for 3 minutes. A purple color develops.
(2) Monosodium L-Glutamate responds to all tests for Sodium Salt in the Qualitative Tests.
Purity
(1) Specific rotation $[\alpha]_D^{20}$: +24.8 to +25.3° (10 g, diluted hydrochloric acid (1 in 5), 100 ml, on the dried basis).
(2) Clarity and color of solution Colorless and clear (1.0 g, water 10 ml).
(3) pH 6.7–7.2 (1.0 g, water 20 ml).
(4) Chloride Not more than 0.041% as Cl (0.30 g, Control solution 0.01 mol/L sulfuric acid 0.35 ml).
(5) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).
(6) Arsenic Not more than 2.5 μg/g as As_2O_3 (0.80 g, Method 1, Apparatus B).
Loss on Drying Not more than 0.5% (97–99°C, 5 hours).
Assay Weigh accurately about 0.15 g of Monosodium L-Glutamate, and proceed as directed in the Assay for DL-Alanine.
Each ml of 0.1 mol/L perchloric acid = 9.356 mg of $C_5H_8NNaO_4 \cdot H_2O$

Monosodium Succinate

コハク酸一ナトリウム



$C_4H_5NaO_4$ Mol. Wt. 140.07
Monosodium monohydrogen butanedioate [2922-54-5]
Content Monosodium Succinate contains 98.0–102.0% of monosodium succinate ($C_4H_5NaO_4$).
Description Monosodium Succinate occurs as colorless to white crystals or as a white crystalline powder. It is odorless and has a characteristic taste.
Identification Monosodium Succinate responds to all tests for Sodium Salt and for Succinate in the Qualitative Tests.
Purity
(1) pH 4.3–5.3 (1.0 g, water 10 ml).
(2) Sulfate Not more than 0.019% as SO_4 (1.0 g, Control solution 0.005 mol/L sulfuric acid 0.40 ml).
(3) Heavy metals Not more than 20 μg/g as Pb.
Test Solution Weigh 1.0 g of Monosodium Succinate, dissolve it in 20 ml of water, add 1 drop of phenolphthalein TS, and add ammonia TS dropwise until the color of the solution changes to a slightly pink color. Add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.
Control Solution To 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.
(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).
(5) Readily oxidizable substances Weigh 2.0 g of Monosodium Succinate, dissolve it in 25 ml of water and 25 ml of diluted sulfuric acid (1 in 20), and add 4.0 ml of 0.02 mol/L potassium permanganate. The pink color of the solution does not disappear within 3 minutes.
Residue on Ignition 49.5–51.5%.
Assay Weigh accurately about 0.3 g of Monosodium Succinate, dissolve it in 30 ml of water, and titrate with 0.1 mol/L sodium hydroxide (indicator: 2 drops of phenolphthalein TS).
Each ml of 0.1 mol/L sodium hydroxide = 14.01 mg of $C_4H_5NaO_4$

Morpholine Salts of Fatty Acids

モルホリン脂肪酸塩

Description Morpholine Salts of Fatty Acids occur as light yellow to yellow-brown waxy or oily substances.
Identification
(1) To 2 g of the sample, add 10 ml of diluted hydrochloric acid (3 in 5), heat in a water bath for 10 minutes with occasional shaking, and allow to cool. Remove the oily or solid portions deposited, and make the resulting solution alkaline with sodium hydroxide solution (1 in 25). Dissolve this solution in methanol (1 in 3), and use as the test solution. Separately, prepare a solution of morpholine in methanol (1 in 200), and use as the standard solution.
Analyze 1.0 μl portions of the test solution and the standard solution by gas chromatography using the conditions below. The retention time of the main peak from the test solution corresponds to that of the peak of morpholine from the standard solution.
Operating Conditions
Detector: Flame-inonization detector.
Column: A silicate glass capillary tube (0.25 mm internal diameter and 30 m length) coated with a 0.25-μm thick layer of a mixture of 5% diphenyl/95% dimethylpolysiloxane.

Column temperature: Maintain the temperature at 50ºC for 1 minute, and raise at a rate of 10ºC/minute to 250ºC and then at a rate of 5ºC/minute to 325ºC.

Carrier gas: Nitrogen.

Flow rate: A constant rate of about 1.2 ml/minute.

(2) Dissolve 1 g of the sample in 2 ml of ethanol while heating, add 5 ml of diluted sulfuric acid (1 in 20), heat in a water bath for 30 minutes, and cool. Oil drops or white to yellow-white solids are deposited.

Collect the oil drops or solids by separation, add 5 ml of diethyl ether, and shake. The oil drops or solids dissolve.

Purity

(1) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Arsenic Not more than 4.0 μg/g as As_2O_3.

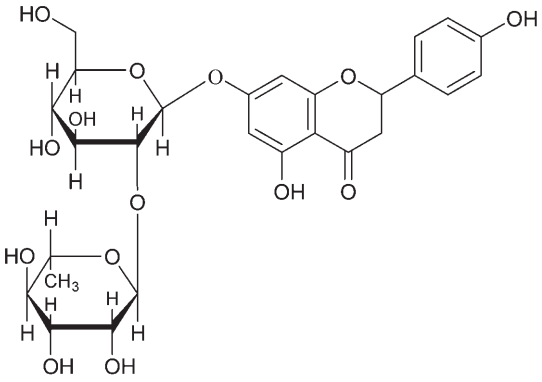
Test Solution Weigh 0.50 g of the sample, add 5 ml of diluted sulfuric acid (1 in 20), heat in a water bath for 30 minutes, and cool. Remove the deposited fatty acid by extraction with diethyl ether, heat the resulting solution on a water bath, and remove the diethyl ether.

Apparatus Use Apparatus B.

Residue on Ignition Not more than 1.0%.

Naringin

ナリンジン



$C_{27}H_{32}O_{14}$ Mol. Wt. 580.53

5-Hydroxy-2-(4-hydroxyphenyl)-4-oxochroman-7-yl α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside

[10236-47-2]

Definition Naringin is obtained from the peels, juice, or seeds of the grapefruit *Citrus paradisi* Macfadyen by extraction and separation with water, ethanol, or methanol. It consists of naringin ($C_{27}H_{32}O_{14}$).

Content Naringin, when dried, contains 90–110% of naringin ($C_{27}H_{32}O_{14}$ = 580.53).

Description Naringin occurs as white to pale yellow crystals.

Identification

(1) Dissolve 5 mg of Naringin in 10 ml of 50% (vol) ethanol, and add 1 to 2 drops of iron(III) chloride solution (1 in 500). A brown color develops.

(2) Dissolve 5 mg of Naringin in 5 ml of sodium hydroxide TS. A yellow to orange color develops.

(3) Dissolve 0.010 g of Naringin in 500 ml of water. The solution has a bitter taste, and exhibits an absorption maximum at a wavelength of 280–285 nm.

Purity

(1) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 5.0 μg/g as Pb (1.0 g, Method 1).

(3) Arsenic Not more than 2.0 μg/g as As_2O_3 (1.0 g, Method 3, Apparatus B).

(4) Methanol Not more than 50 μg/g.

(i) Apparatus Use the apparatus specified in Purify (4) for Enju Extract in the Monographs.

(ii) Method

Test Solution Weigh accurately about 5 g of Naringin into eggplant-shaped flask A, add 100 ml of water, a few boiling chips, and 3–4 drops of silicon resin, and stir well. Place exactly 2 ml of the internal standard solution in volumetric flask E, and assemble the apparatus. Moisten the joint parts with water. Distill at a rate of 2 to 3 ml/minute while adjusting so that bubbles do not enter into delivery tube C, and collect about 45 ml of the distillate. To the distillate, add water to make exactly 50 ml. Use *tert*-butanol solution (1 in 1,000) as the internal standard solution.

Standard Solution Weigh accurately about 0.5 g of methanol, and add water to make exactly 100 ml. Measure exactly 5 ml of this solution, and add water to make exactly 100 ml. Next, measure exactly 2 ml of the second solution and 4 ml of the internal standard solution into a 100-ml volumetric flask, and add water exactly to volume.

Procedure Analyze 2.0 μl portions of the test solution and the standard solution by gas chromatography using the operating conditions given below. Determine the peak area ratios (Q_T and Q_S) of methanol to *tert*-butanol for the test solution and the standard solution, and calculate the methanol content by the formula:

$$\text{Content of methanol (μg/g)} = \frac{\text{Weight (g) of methanol}}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 500$$

Operating Conditions

Detector: Flame-ionization detector.

Column: A glass tube of 3 mm internal diameter and 2 m length.

Column packing material: 180- to 250-μm styrene-divinylbenzene porous polymer for gas chromatography.

Column temperature: A constant temperature at about 120°C.

Injection port: A constant temperature at about 200°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust so that the retention time of methanol is about 2 minutes.

Loss on Drying Not more than 10 % (105°C, 3 hours).

Assay Weigh accurately about 0.2 g of Naringin, previously dried at 105°C for 3 hours, and dissolve it in 50% (vol) ethanol to make exactly 100 ml. Filter this solution through a membrane filter (0.45-μm pore size), measure exactly 1 ml of the filtrate, and add water to make exactly 100 ml. Measure the absorbance (A) of this solution at a wavelength of 280 nm, using water as the control. Calculate the content by the formula:

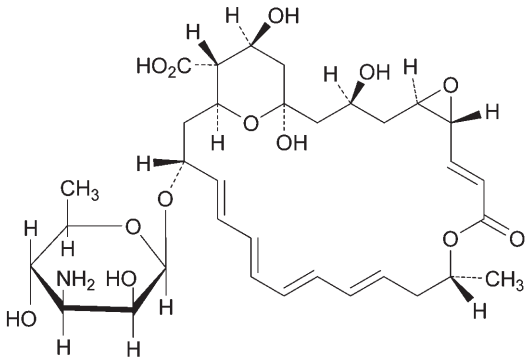
Content (%) of naringin ($C_{27}H_{32}O_{14}$)

$$= \frac{A}{28.0} \times \frac{10}{\text{weight (g) of the sample}} \times 100$$

Natamycin

Pimaricin

ナタマイシン



$C_{33}H_{47}NO_{13}$ Mol. Wt. 665.73

(1*R**,3*S**,5*R**,7*R**,8*E*,12*R**,14*E*,16*E*,18*E*,20*E*,22*R**,24*S**,25*R**,26*S**)-22-(3-Amino-3,6-dideoxy-β-D-mannopyranosyloxy)-1,3,26-trihydroxy-12-methyl-10-oxo-6,11,28-trioxatricyclo[22.3.1.0^{5,7}]octacosa-8,14,16,18,20-pentaene-25-carboxylic acid [7681-93-8]

Content Natamycin, when calculated on the anhydrous basis, contains not less than 95.0% of natamycin ($C_{33}H_{47}NO_{13}$).

Description Natamycin occurs as a white to creamy-white crystalline powder.

Identification

(1) To 1 mg of Natamycin, add 1 ml of hydrochloric acid, and shake. A blue color develops.

(2) Dissolve 5 mg of Natamycin in 1,000 ml of a solution of acetic acid in methanol (1 in 1,000). The solution exhibits absorption maximum at wavelengths of approximately 290, 303, and 318 nm.

(3) Determine the infrared absorption spectrum of Natamycin as directed in the Potassium Bromide Disk Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +250 to +295° (1 g, acetic acid, 100 ml, on the anhydrous basis).

(2) pH 5.0–7.5 (1% suspension).

(3) Lead Not more than 2.0 μg/g (5.0 g, Method 1).

Water Content 6.0–9.0% (0.03 g, coulometric titration).

Residue on Ignition Not more than 0.5%.

Assay Throughout this assay, all procedures should be protected from direct light and the apparatus used should be light-resistant.

Test Solution and Standard Solution Weigh accurately about 0.02 g each of Natamycin and Natamycin Reference Standard (the water content should be previously determined in the same manner as for the sample). Add 5 ml of tetrahydrofuran to each, sonicate for 10 minutes, and add 60 ml of methanol to dissolve. Then add 25 ml of water, and allow to cool to room temperature. Add water to make two solutions of exactly 100 ml each. Use them as the test solution and the standard solution, respectively.

Procedure Analyze 20 μl portions of both solutions by liquid chromatography using the operation conditions given below. Measure the peak areas (A_T and A_S) of natamycin for the test solution and the standards solution, and calculate on the anhydrous basis, and then calculate the natamycin content in the sample by the formula:

Content (%) of natamycin ($C_{33}H_{47}NO_{13}$)

$$= \frac{\left(\begin{array}{c}\text{Anhydrous basis weight (mg) of}\\ \text{Natamycin Reference Standard}\end{array}\right)}{\text{Anhydrous basis weight (mg) of the sample}} \times \frac{A_T}{A_S} \times 100$$

Operating Conditions

Detector: Ultraviolet spectrophotometer (determination wavelength: 303 nm).

Column: A stainless steel of 4.6 mm internal diameter and 25 cm length.

Column packing material:
5- to 10-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: Room temperature.

Mobile phase: Use the mobile phase prepared by dissolving 3.0 g of ammonium acetate and 1.0 g of ammonium chloride in 760 ml of water, and adding 5.0 ml of tetrahydrofuran and 240 ml of acetonitrile.

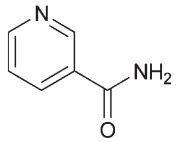
Flow rate: 2 ml/minute.

Storage standard Store in a light-resistant bottle in a cold place.

Nicotinamide

Niacinamide

ニコチン酸アミド



$C_6H_6N_2O$ Mol. Wt. 122.12

Pyridine-3-carboxamide [98-92-0]

Content Nicotinamide, when calculated on the dried basis, contains 98.5–101.0% of nicotinamide ($C_6H_6N_2O$).

Description Nicotinamide occurs as a white crystalline powder. It is odorless and has a bitter taste.

Identification

(1) Proceed as directed in Identification (1) for Nicotinic Acid.

(2) To 0.02 g of Nicotinamide, add 5 ml of sodium hydroxide solution (1 in 25), and boil gently. An odor of ammonia is evolved.

Purity

(1) Melting point 128–131°C.

(2) pH 6.0–7.5.

Weigh 1.0 g of Nicotinamide, and add water to make 20 ml.

(3) Heavy metals Not more than 30 μg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 3.0 ml).

(4) Readily carbonizable substances Perform the test with 0.20 g of Nicotinamide, using Matching Fluid A.

Loss on Drying Not more than 0.5% (4 hours).

Residue on Ignition Not more than 0.10%.

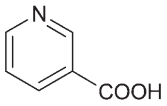
Assay Weigh accurately about 0.2 g of Nicotinamide, dissolve it in 30 ml of acetic acid, and titrate with 0.1 mol/L perchloric acid (indicator: 1 ml of crystal violet–acetic acid TS) until the color of the solution changes from purple through blue to green. Perform a blank test in the same manner, make any necessary correction, and calculate on the dried basis.

Each ml of 0.1 mol/L perchloric acid = 12.21 mg of $C_6H_6N_2O$

Nicotinic Acid

Niacin

ニコチン酸



$C_6H_5NO_2$ Mol. Wt. 123.11

Pyridine-3-carboxylic acid [59-67-6]

Content Nicotinic Acid, when calculated on the dried basis, contains 99.5–101.0% of nicotinic acid ($C_6H_5NO_2$).

Description Nicotinic Acid occurs as white crystals or crystalline powder. It is odorless and has a slightly acid taste.

Identification

(1) To 5 mg of Nicotinic Acid, add 10 mg of 2,4-dinitrochlorobenzene, mix, and fuse by heating for several seconds. Cool, and add 4 ml of ethanolic potassium hydroxide TS. A dark purple color develops.

(2) To 20 ml of a solution of Nicotinic Acid (1 in 400), add sodium hydroxide solution (1 in 250) to neutralize it, and add 3 ml of cupric sulfate solution (1 in 8). A blue precipitate is gradually formed.

Purity

(1) Melting point 234–238°C.

(2) Chloride Not more than 0.021% as Cl (0.50 g, Control solution 0.01 mol/L hydrochloric acid 0.30 ml).

(3) Sulfate Not more than 0.019% as SO_4 (0.50 g, Control solution 0.005 mol/L sulfuric acid 0.20 ml).

(4) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

Loss on Drying Not more than 1.0% (105°C, 1 hour).

Residue on Ignition Not more than 0.10%.

Assay Weigh accurately about 0.3 g of Nicotinic Acid, dissolve it in 50 ml of water, and titrate with 0.1 mol/L sodium hydroxide (indicator: 5 drops of phenolphthalein TS). Calculate on the dried basis.

Each ml of 0.1 mol/L sodium hydroxide = 12.31 mg of $C_6H_5NO_2$

Nitrous Oxide

亜酸化窒素

N_2O Mol. Wt. 44.01

Nitrous oxide [10024-97-2]

Definition Nitrous Oxide is a gas consisting mainly of nitrous oxide (N_2O). It is filled in a hermetic, pressure-resistant metal container other than a cartridge-type container.

Content Nitrous Oxide contains not less than 97.0% (vol) of nitrous oxide (N_2O).

Description Nitrous Oxide is a colorless gas at room temperature under atmospheric pressure. It has no odor.

Identification

(1) A wood-chip ember bursts into flame when exposed to Nitrous Oxide.

(2) Analyze 1 ml portions of Nitrous Oxide (the sample) and nitrous oxide (N_2O) by gas chromatography using the operating conditions specified for the Assay. The retention time of the main peak of the sample corresponds to that of nitrous oxide (NO_2).

Purity The amounts of Nitrous Oxide indicated in Purity are the volumes at 20°C at an atmospheric pressure of 101.3 kPa. Collect appropriate amounts of the sample, taking into account the temperature and pressure at the time of testing.

(1) Chloride To 2.5 ml of 0.1 mol/L silver nitrate TS, add water to make 50 ml. When 10 L of Nitrous Oxide is passed through this solution and allowed to stand for 5 minutes, a white turbidity appears. The turbidity is not higher than that of the solution prepared as follows: To 2.5 ml of 0.1 mol/L silver nitrate TS, add 1 ml of Chloride Ion Standard Stock Solution, 0.15 ml of diluted nitric acid, and water to make 50 ml, and allow to stand for 5 minutes.

(2) Arsenic hydride and hydrogen phosphide Place 5 ml of silver diethyldithiocarbamate–quinoline TS into a Nessler tube. Into the Nessler tube, insert a gas introduction tube connected to a glass tube filled with absorbent cotton moistened with lead acetate TS. Keep the end of the gas introduction tube 2 mm above the bottom of the Nessler tube. Introduce 10 L of Nitrous Oxide into the Nessler tube over 10 minutes. The color of the silver diethyldithiocarbamate–quinoline TS does not change.

(3) Carbon monoxide Measure 5 ml of Nitrous Oxide into a gas-measuring tube or syringe for gas chromatography, analyze it by gas chromatography using the operating conditions given below. No peak is observed at the retention time of carbon monoxide.

Operating Conditions

Detector: Thermal conductivity detector. When 5 ml of hydrogen or helium containing 0.1% (vol) carbon

monoxide is charged, the peak height obtained is about 10 cm or more.
Column: A glass tube of about 3 mm internal diameter and about 3 m length.
Column packing material: 300- to 500-μm zeolite for gas chromatography.
Column temperature: A constant temperature at about 50°C.
Carrier gas: Hydrogen or helium.
Flow rate: Adjust so that the peak of carbon monoxide appears about 20 minutes after injection.

(4) Nitrogen monoxide and nitrogen dioxide Not more than 2 μl/L as total volume.

Use a detector tube type gas measuring instrument connected with a nitrogen oxide detector tube.

Assay The collection of Nitrous Oxide should follow the requirement given in Purity.

Measure 1.0 ml of Nitrous Oxide into a gas-measuring tube or syringe for gas chromatography, analyze by gas chromatography using the operating conditions given below, and measure the peak area (A_T) of air. Separately, introduce 3.0 ml of nitrogen into a gas mixer, and add the carrier gas to make exactly 100 ml. Mix them well to prepare a standard mixed gas. Using 1.0 ml of the mixed gas, measure the peak area (A_S) of nitrogen in the same manner as the sample.

$$\text{Content (\% (vol)) of nitrous oxide } (N_2O) = 100 - 3 \times \frac{A_T}{A_S}$$

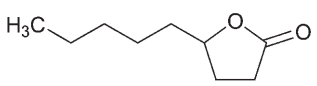
Operating Conditions

Detector: Thermal-conductivity detector.
Column: A glass tube of about 3 mm internal diameter and about 3 m length.
Column packing material: 300- to 500-μm zeolite for gas chromatography.
Column temperature: A constant temperature at about 50°C.
Carrier gas: Hydrogen or helium.
Flow rate: Adjust so that the peak of nitrogen appears about 2 minutes after injection.

γ-Nonalactone

Nonalactone
Nonano-1,4-lactone

γ-ノナラクトン



$C_9H_{16}O_2$ Mol. Wt. 156.22
5-Pentyldihydrofuran-2(3*H*)-one [104-61-0]

Content γ-Nonalactone contains not less than 98.0% of γ-nonalactone ($C_9H_{16}O_2$).

Description γ-Nonalactone is a colorless to light yellow, transparent liquid having a sweet coconut-like odor.

Identification Determine the absorption spectrum of γ-Nonalactone as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Refractive index n_D^{20}: 1.446–1.450.
(2) Specific gravity 0.965–0.970.
(3) Clarity of solution Clear (2.0 ml, 70% (vol) ethanol 4.0 ml).
(4) Acid value Not more than 2.0 (Flavoring Substances Tests).

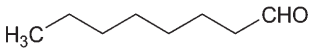
Assay Weigh accurately about 1 g of γ-Nonalactone, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 78.11 mg of $C_9H_{16}O_2$

Octanal

Caprylic Aldehyde
Octyl Aldehyde

オクタナール



$C_8H_{16}O$ Mol. Wt. 128.21
Octanal [124-13-0]

Content Octanal contains not less than 92.0% of octanal ($C_8H_{16}O$).

Description Octanal is a colorless or slightly yellowish, transparent liquid having a characteristic odor.

Identification Determine the absorption spectrum of Octanal as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Refractive index n_D^{20}: 1.417–1.425.
(2) Specific gravity 0.821–0.833.
(3) Clarity of solution Clear (1.0 ml, 70% (vol) ethanol 3.0 ml).
(4) Acid value Not more than 10.0 (Flavoring Substances Tests).

Assay Weigh accurately about 1 g of Octanal, and proceed as directed in Method 1 in the Aldehyde and Ketone Content Test in the Flavoring Substances Tests. In the test, allow the mixture to stand for 15 minutes before titrating.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 64.11 mg of $C_8H_{16}O$

Oxalic Acid

シュウ酸

$C_2H_2O_4 \cdot 2H_2O$ Mol. Wt. 126.07
Ethanedioic acid dihydrate [6153-56-6]

Content Oxalic Acid contains 99.5–101.0% of oxalic acid ($C_2H_2O_4 \cdot 2H_2O$).

Description Oxalic Acid occurs as colorless crystals. It is odorless.

Identification

(1) Oxalic Acid is sublimed by heating.

(2) To 1 ml of a solution of Oxalic Acid (1 in 10), add 2 drops of sulfuric acid, add 1 ml of potassium permanganate solution (1 in 300), and heat. The pink color of the solution disappears.

(3) Make a solution of Oxalic Acid (1 in 10) alkaline with ammonia TS, and add 1 ml of calcium chloride solution (3 in 40). A white precipitate is formed.

Purity

(1) Clarity and color of solution Colorless and almost clear.

Test Solution Weigh 1.0 g of Oxalic Acid, add 20 ml of water, and dissolve by boiling.

(2) Sulfate Not more than 0.077% as SO_4.

Sample Solution Weigh 1.0 g of Oxalic Acid, add 20 ml of water and 1 ml of anhydrous sodium carbonate solution (1 in 8), evaporate to dryness on a water bath, heat gradually, and ignite to 600–700°C. To the residue, add 10 ml of water and 0.5 ml of nitric acid, boil, add 2 ml of hydrochloric acid, and evaporate to dryness on a water bath. Add water to the residue to make 100 ml, and filter. Use 25 ml of the filtrate as the sample solution.

Control Solution To 0.40 ml of 0.005 mol/L sulfuric acid, add 1 ml of diluted hydrochloric acid (1 in 4) and water to make 50 ml.

(3) Heavy metals Not more than 20 µg/g as Pb.

Test Solution To the residue on igniting Oxalic Acid, add 1 ml of hydrochloric acid and 0.2 ml of nitric acid, and evaporate to dryness on a water bath. To the residue add 1 ml of diluted hydrochloric acid (1 in 4) and 30 ml of water, dissolve by heating, cool, add 1 drop of phenolphthalein TS, and add ammonia TS dropwise until the color of the solution changes to a slightly pink color. Add 2 ml of diluted acetic acid (1 in 20), filter if necessary, and add water to make 50 ml.

Control Solution To 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(4) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Residue on Ignition Not more than 0.30% (1 g).

Assay Weigh accurately about 1 g of Oxalic Acid, and dissolve it in water to make exactly 250 ml. Measure accurately 50 ml of this solution, add 3 ml of sulfuric acid, and warm to about 80°C. Titrate with 0.1 mol/L potassium permanganate while hot.

Each ml of 0.02 mol/L potassium permanganate = 6.303 mg of $C_2H_2O_4 \cdot 2H_2O$

Palm Oil Carotene

パーム油カロテン

Definition Palm Oil Carotene is obtained from the fruits of the oil palm *Elaeis guineensis* Jacquin and consists mainly of carotene. It may contain edible fats or oils.

Content (Color Value) Palm Oil Carotene contains the equivalent of not less than 30% of β-carotene ($C_{40}H_{56}$ = 536.87) and the equivalent of 95–115% of the labeled content; or its Color Value ($E^{10\%}_{1cm}$) is not less than 7,500 and in the range of 95–115% of the labeled value.

Description Palm Oil Carotene is red-brown to brown turbid oily solution having a slightly characteristic odor.

Identification

(1) Weigh the equivalent of 0.015 g of Palm Oil Carotene with a Color Value 7,500, and dissolve it in 5 ml of a 1:1 mixture of acetone/cyclohexane. An orange color develops.

(2) Proceed as directed in Identification (2) for Dunaliella Carotene.

(3) Proceed as directed in Identification (3) for Dunaliella Carotene.

Purity

(1) Heavy metals Not more than 20 µg /g as Pb (1.0g, Method 2, Control solution Lead standard solution 2.0 ml).

(2) Lead Not more than 10 µg /g as Pb (1.0g, Method 1).

(3) Arsenic Not more than 4.0 µg /g as As_2O_3 (0.50g, Method 3, Apparatus B).

Assay (Color Value Test) Proceed as directed in the Assay (Color Value Test) for Dunaliella Carotene.

Papain

パパイン

Definition Papain is a proteolytic enzyme derived from the fruit of the papaya *Carica papaya* Linné. It may contain lactose or dextrin.

Enzyme Activity Papain has the enzyme activity equivalent to not less than 300,000 units per gram.

Description Papain occurs as white to light yellow-brown powders. It is odorless or has slight characteristic odors.

Identification

(1) To 10 ml of a liquid including 20% of powdered skim milk, adjusted to pH 5.5 with diluted acetic acid (3 in 50), add 0.01 g of Papain, and warm to 37°C. The liquid coagulates.

(2) A solution of Papain (1 in 500) exhibits absorption maximum at a wavelength of 270–280 nm.

Purity

(1) Lead Not more than 5.0 µg/g as Pb (2.0 g, Method 1).

(2) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Microbial Limits Proceed as directed in the Microbial Limit Tests. The total bacterial count is not more than 50,000/g, and *Escherichia coli* is negative.

Enzyme Activity Determination

Sample Solution Dissolve 8.75 g of L-cysteine hydrochloride in about 800 ml of water, and then add 2.23 g of disodium ethylenediaminetetraacetate to dissolve. Adjust the pH to 4.5 with 1 mol/L sodium hydroxide TS, and add water to make 1,000 ml. Use this solution as the diluent.

Weigh accurately about 0.50 g of Papain, dissolve it in the diluent, and make exactly 100 ml. Measure exactly 1 ml of the solution, and add the diluent to make exactly 50 ml. Centrifuge this solution, if necessary. Dilute the supernatant liquid with the diluent to prepare a solution containing 20 to 100 units per ml.

Procedure Measure exactly 5 ml of casein TS (pH 8.0), transfer into a test tube, and warm for 5 minutes at 37±0.5°C. Add 1 ml of the sample solution, shake immediately, and allow to react for 10 minutes at 37±0.5°C. Add 5 ml of trichloroacetic acid TS, shake, allow to stand for 30 minutes at 37±0.5°C, and filter through a filter paper for quantitative analysis (5C). Discard the first 3 ml of the filtrate, and measure the absorbance (A_T) of the subsequent filtrate at 275 nm using water as the reference.

Separately, measure exactly 1 ml of the sample solution, add 5 ml of trichloroacetic acid TS, and shake well. Add 5 ml of casein TS (pH 8.0), shake well, allow to stand for 30 minutes at 37±0.5°C. Measure the absorbance (A_b) of this solution in the same manner. Then measure the absorbances (A_S and A_{S0}) of Tyrosine Standard Solution and 0.1 mol/L hydrochloric acid at 275 nm using water as the reference. Calculate the enzyme activity by the formula given below. One unit of the enzyme activity is the quantity of enzyme that increases the absorbance equivalent to 1 μg of tyrosine per minute when the test is performed as directed in the procedure.

$$\text{The Enzyme Activity (units/g) of the sample} = \frac{(A_T - A_b) \times 50}{A_S - A_{S0}} \times \frac{11}{10} \times \frac{1{,}000}{W}$$

W = weight (mg) of Papain in 1 ml of the sample solution.

Paprika Color

Paprika Oleoresin

トウガラシ色素

Definition Paprika Color is obtained from the fruits of the pepper *Capsicum annuum* Linné and consists mainly of capsanthins. It may contain edible fats or oils.

Color Value The Color Value ($E^{10\%}_{1cm}$) of Paprika Colour is not less than 300 and is in the range of 95–115% of the labeled value.

Description Paprika Color is a dark red, viscous liquid having a characteristic odor.

Identification

(1) Weigh the equivalent of 0.1 g of Paprika Color with a Color Value 300, and dissolve it in 100 ml of acetone. A yellow-orange color develops.

(2) Weigh 0.5 g of Paprika Color, dissolve it in 2 ml of toluene, and add 0.2 ml of sulfuric acid. A dark blue color develops.

(3) A solution of Paprika Color in acetone exhibits an absorption maximum at a wavelength of 450–460 nm or 465–475 nm or absorption maxima at both 450–460 nm and 465–475 nm.

(4) Weigh the equivalent of 0.2 g of Paprika Color with a Color Value 300, dissolve it in 20 ml of acetone, and use this solution as the test solution. Analyze a 5-μl portion of the test solution by thin-layer chromatography using a 1:1 mixture of ethanol/cyclohexane as the developing solvent. No control solution is used. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the original line, and air-dry. Two yellow-red main spots are observed: one at an R_f of approximately 0.88–0.96 and the other at an R_f of approximately 0.75–0.90. When the spots are sprayed with 5% sodium nitrite followed by 0.5 mol/L sulfuric acid, the color of the spots disappears immediately.

Purity

(1) Heavy metals Not more than 40 μg /g as Pb (0.50g, Method 2, Control solution Lead standard solution 2.0 ml).

(2) Lead Not more than 10 μg /g as Pb (1.0g, Method 1).

(3) Arsenic Not more than 4.0 μg /g as As_2O_3 (0.50g, Method 3, Apparatus B).

Color Value Test Proceed as directed in the Color Value Test, using the conditions below.

Operating Conditions

Solvent: Acetone.

Wavelength: Maximum absorption wavelength near 460nm.

Paraffin Wax

パラフィンワックス

Definition Paraffin Wax is a mixture of solid hydrocarbons obtained from petroleum atmospheric and vacuum distillates. It consists mainly of linear saturated hydrocarbons.

Description Paraffin Wax is a colorless or white, somewhat translucent solid at room temperature. It has a slight characteristic odor.

Identification Determine the absorption spectrum of Paraffin Wax as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Melting point 43–75°C (Melting Point Determination, Procedure for Class 2 Substances).

(2) Lead Not more than 3.0 μg/g as Pb (3.3 g, Method 1).

(3) Arsenic Not more than 2.0 μg/g as As_2O_3 (1.0 g, Method 3, Apparatus B).

(4) Sulfur compound To 4.0 g of Paraffin Wax, add 2 ml

of absolute ethanol and 2 drops of a transparent solution of sodium hydroxide (1 in 5) saturated with lead monoxide. Warm the mixture at 80°C for 10 minutes with occasional shaking, and allow to cool. A dark brown color does not develop.

(5) Polycyclic aromatic hydrocarbons Before use, rinse all glass instruments used in the testing with isooctane for ultraviolet absorption spectrum measurement and examine them under ultraviolet light to confirm that no fluorescent contamination is present. Because some of the polycyclic aromatic hydrocarbons sought in this test are very susceptible to photo-oxidation, the entire procedure should be performed under subdued light.

Test Solution Weigh 150 g of Paraffin Wax into a 500-ml beaker, and melt by heating to make it homogenous. Place 25 g ± 0.2 g of the melted sample into a 500-ml separating funnel, add 100 ml of dimethyl sulfoxide TS, add 50 ml of isooctane TS while warming to keep the sample from solidifying, and shake vigorously for 2 minutes. Set up three 300-ml separating funnels with each containing 30 ml of isooctane TS. After the mixture in the 500-ml separating funnel separates, allow to cool until wax is precipitated. Filter the lower layer (dimethyl sulfoxide TS phase) through glass wool fitted loosely in the separating funnel or filter paper, previously rinsed with isooctane for ultraviolet absorption spectrum measurement. Wash the filtrate in tandem with the 30 ml portions of isooctane TS contained in the 300-ml separating funnels already prepared. First, transfer the filtrate into a first separating funnel, and shake vigorously for 1 minute. Allow to stand, and use the lower layer for next washing. Repeat the same washing operation twice using the second and third separating funnels. Transfer the lower layer into a 2-L separating funnel. The individual upper layers (isooctane TS phase) should be kept in each separating funnel since they will be used later.

Conduct extraction from the isooctane TS phase in the 500-ml separating funnel with an additional 100 ml portion of dimethyl sulfoxide TS, and filter the extractive through glass wool or filter paper in the same manner as previously done. Wash the extractive in tandem with the three isooctane TS phases kept in the three 300-ml separating funnels. Collect the dimethyl sulfoxide layer washed in the 2-L separating funnel. Conduct the extraction operation again from the isooctane TS phase in the 500-ml separating funnel with an additional 100 ml portion of dimethyl sulfoxide TS, and filter. Wash the extractive in tandem with the three isooctane TS phases in the same manner. Collect the dimethyl sulfoxide layer in the 2-L separating funnel. Discard the isooctane TS layers in the 300-ml separating funnels.

Add 480 ml of water and 80 ml of isooctane for ultraviolet absorption spectrum measurement to the 2-L separating funnel containing 300 ml of the dimethyl sulfoxide TS phase, and extract by shaking vigorously for 2 minutes (first extraction). Allow to stand, transfer the lower layer into a second 2-L separating funnel, add an additional 80 ml of isooctane for ultraviolet absorption spectrum measurement, and extract by shaking vigorously for 2 minutes (second extraction). Discard the lower layer. Wash the upper layer left in the former 2-L separating funnel with 100 ml of water by shaking vigorously for 1 minute, and discard the aqueous layer. Repeat this washing operation two more times. Refer to the extractive obtained as the first isooctane extractive. Wash the upper layer obtained in the second extraction with 100 ml of water by shaking vigorously for 1 minute. Discard the aqueous layer. Repeat this washing operation two more times. Refer to the extractive obtained as the second isooctane extractive.

Place the first isooctane extractive in a 300-ml Erlenmeyer flask through a 30-ml glass filter packed with 35 g of anhydrous sodium sulfate pre-rinsed with isooctane for ultraviolet absorption spectrum measurement. Wash the first 2-L separating funnel with the second isooctane extractive, and place it in the 300-ml Erlenmeyer flask through the 30-ml glass filter packed with the anhydrous sodium sulfate. Again, wash the second and first separating funnels successively with 20 ml of isooctane for ultraviolet absorption spectrum measurement, and place into the Erlenmeyer flask through the anhydrous sodium sulfate. Transfer the isooctane extractives into a distilling flask, add 1 ml of hexadecane for ultraviolet absorption spectrum measurement, and evaporate the isooctane under nitrogen until 1 ml of residue remains. To the residue, add 10 ml of isooctane for ultraviolet absorption spectrum measurement, and evaporate again until 1 ml of residue remains. Repeat this operation.

Dissolve the residue in isooctane for ultraviolet absorption spectrum measurement, transfer into a 25-ml volumetric flask, and make exactly 25 ml with isooctane for ultraviolet absorption spectrum measurement. Use this as the test solution.

Reference Solution Prepare a reference solution in the same manner as the preparation of the test solution without using the sample.

Procedure Measure the absorbance of the test solution in a 5-cm path length cell. The corrected absorbance does not exceed the limits given below.

Wavelength (nm)	Absorbance/cm path length
280–289	0.15
290–299	0.12
300–359	0.08
360–400	0.02

(6) Readily Carbonizable Substances Test Place 5.0 g of Paraffin Wax in a Nessler tube, melt by warming in a water bath at 80ºC, and add 5 ml of 94.5–95.5% sulfuric acid. Warm again in the water bath at 80ºC for 1 minute, remove from the bath, and immediately shake vigorously for a few seconds. Repeat this operation three more times. Leave in the water bath at 80ºC for 30 seconds. The separated sulfur layer is not darker in color than a solution prepared by mixing 3.0 ml of Ferric Chloride CSSS, 1.5 ml of Cobaltous Chloride CSSS, and 0.5 ml of Cupric Sulfate CSSS in a Nessler tube.

Residue on Ignition Not more than 0.10%.

Pectin

ペクチン

Definition Pectin is obtained from citrus fruits, apples, and other plants. It consists of water-soluble polysaccharides including partially methyl-esterified polygalacturonic acid. It

may contain sucrose, glucose, lactose and dextrin.

Description Pectin occurs as a white to light brown powder or granules. It is odorless or has a slight, characteristic odor.

Identification

Test Solution Weigh 0.05 g of Pectin, and add 1 ml of 2-propanol. Next, add 50 ml of water while stirring magnetically. Adjust the pH to 12 with 0.5 mol/L sodium hydroxide solution, and allow to stand for 15 minutes. Adjust the pH to 7.0 with 0.5 mol/L hydrochloric acid, and add water to make exactly 100 ml. Use this solution as the sample solution. Place 0.5 ml of tris buffer (pH 7.0) for pectin determination in a quartz cell, add 1.0 ml of the sample solution, 0.5 ml of water, and 0.5 ml of pectate lyase solution for pectin determination (enzyme solution), and then mix.

Enzyme Blank Place 0.5 ml tris buffer (pH 7.0) for pectin determination in a quartz cell, add 1.0 ml of the sample solution and 1.0 ml of water, and mix.

Sample Blank Place 0.5 ml tris buffer (pH 7.0) for pectin determination in a quartz cell, add 1.5 ml of water and 0.5 ml of the enzyme solution, and mix.

Procedure Measure the absorbances of the test solution, enzyme blank, and sample blank at 235 nm at 0 and at 10 minutes. Calculate the absorbance (A_0) at 0 minutes and the absorbance (A_{10}) at 10 minutes by the following equation given below. The change ($A_{10} - A_0$) in absorbance is not less than 0.023.

Absorbance at 0 minutes (A_0)
= Absorbance of test solution at 0 minutes
– (Absorbance of enzyme blank at 0 minutes
+ Absorbance of sample blank at 0 minutes)

Absorbance at 10 minutes (A_{10})
= Absorbance of test solution at 10 minutes
– (Absorbance of enzyme blank at 10 minutes
+ Absorbnace of sample blank at 10 minutes)

Purity

(1) Amide group Not more than 25% of total carboxyl groups.

Weigh accurately about 5 g of Pectin, place in a beaker, and add 5 ml of hydrochloric acid and 100 ml of 60% (vol) ethanol. Stir for 10 minutes, and filter with a glass filter (1G3). Wash the residue 6 times with 15 ml of a 20:1 mixture of 60% (vol) ethanol/hydrochloric acid each time. Next, wash the residue on the glass filter with 60% (vol) ethanol until the washings are free of chlorides, wash with 20 ml of ethanol, and dry at 105°C for 2.5 hours. After cooling, weigh the residue. Weigh accurately an amount (W mg) of the residue equivalent to about one-tenth of the weight. Add 2 ml of ethanol to moisten, add 100 ml of water previously boiled and cooled, and hydrate well with occasional stirring. Add 5 drops of Phenolphthalein TS, titrate with 0.1 mol/L sodium hydroxide, and express the number of ml as V_1. Next, add exactly 20 ml of 0.5 mol/L sodium hydroxide, shake well, and allow to stand for 15 minutes. Add exactly 20 ml of 0.5 mol/L hydrochloric acid, and shake until the pink color of the solution disappears. Titrate with 0.1 mol/L sodium hydroxide, and express the number of ml as V_2. The end of titration is when a light pink color develops.

Using the apparatus for the Kjeldahl Method in Nitrogen Determination, distill the solution obtained by the titration. Transfer the solution in a 500-ml decomposition flask. Attach a spray trap and a condenser to the decomposition flask. Place 20 ml of 0.1 mol/L hydrochloric acid and 150 ml of newly boiled and cooled water in an absorption flask, and immerse the lower end of the condenser into the solution in the absorption flask. Add 20 ml of sodium hydroxide solution (1 in 10) to the decomposition flask, and heat, taking care to prevent bubble formation. Distill until 80–120 ml of distillate is obtained. Add a few drops of methyl red TS to the distillate, titrate with 0.1 mol/L sodium hydroxide, and express the number of ml as S. Perform a blank test in the same manner, and express the number of ml as B.

$$\text{Content (\%) of amido group of total carboxyl groups} = \frac{B - S}{V_1 + V_2 + (B - S)} \times 100$$

(2) Galacturonic acid Not less than 65%.

Calculate the content by the following formula from W, V_1, V_2, B, and S obtained in Purity (1).

$$\text{Content (\%) of galacturonic acid} = \frac{19.41 \times [V_1 + V_2 + (B - S)]}{W} \times 100$$

(3) Total nitrogen Not more than 2.5%.

Weigh about 2 g of Pectin, add 5 ml of hydrochloric acid and 100 ml of 60% (vol) ethanol, stir for 10 minutes, and filter through a glass filter (1G3). Wash the residue on the filter 6 times with 15 ml of a 20:1 mixture of 60% (vol) ethanol/hydrochloric acid each time. Then wash with 60% (vol) ethanol until the washings are free of chlorides and then with 20 ml of ethanol. Dry the glass filter containing the residue at 105°C for 2.5 hours. Weigh accurately about 0.2 g of the dried residue, and determine the content of nitrogen according to the semi-micro Kjeldahl method.

(4) Lead Not more than 5.0 µg/g as Pb (2.0 g, Method 1).

(5) Sulfur dioxide Not more than 50 µg/g.

Proceed as directed in Purity (4) for Quillaja Extract.

(6) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(7) Total insolubles Not more than 3.0%.

Weigh about 1 g of Pectin into a 250-ml beaker, and add 5 ml of 2-propanol to disperse the sample. While stirring magnetically, add 100 ml of 0.03 mol/L sodium hydroxide containing 0.1% disodium ethylenediaminetetraacetate, previously filtered through a glass fiber filter paper. Stir for 30 minutes, and heat until boiling. If excessive bubbling occurs, reduce heat. While the solution is hot, filter it through a 70-mm glass fiber filter under vacuum. The 70-mm glass fiber filter to be used should be previous dried in an oven at 105°C for about 1 hour, cooled in a desiccator, and weighed. Wash the beaker 5 times with five 100-ml portions of warm water, previously filtered through a glass fiber filter, and filter the washings through the glass fiber filter paper. Dry the filter paper with the residue at 105°C for about I hour, and cool in an desiccator. Weigh the filter paper.

$$\text{Total insolubles (\%)} = \frac{\left(\begin{matrix}\text{Weight (g) of}\\ \text{the residue}\end{matrix}\right) - \left(\begin{matrix}\text{Weight (g) of}\\ \text{the filter paper}\end{matrix}\right)}{\text{Weight (g) of the sample}} \times 100$$

(8) Sum of methanol and 2-propanol Not more than 1.0%.

Test Solution Weigh accurately about 0.1 g of Pectin, add exactly 10 ml of diluted internal standard solution (1 in 25), and stopper. Mix well until the sample disperses uniformly. Centrifuge it at 5,000 rpm for 30 minutes using a centrifugal ultrafiltration unit. Use the filtrate as the test solution.

Internal Standard Solution Use a solution of *tert*-butanol (1 in 1,000).

Standard Solution Weigh accurately about 0.1 g each of methanol and 2-propanol in a volumetric flask, and add water to make exactly 100 ml. Place exactly 10 ml of this solution and 4 ml of the internal standard solution into a volumetric flask, and add water to make exactly 100 ml.

Procedure Analyze 2.0 µl portions of the test solution and standard solution by gas chromatography using the operating conditions given below. Determine the peak area ratio of 2-propanol to *tert*-butanol and methanol to *tert*-butanol for the test solution and the standard solution. Express them as Q_{T1} and Q_{T2} for the test solution, and as Q_{S1} and Q_{S2} for the standard solution. Obtain the contents of methanol and 2-propanol by the following formulae:

$$\text{Content(\%) of 2 - propanol} = \frac{\text{Weight (g) of 2 - propanol}}{\text{Weight (g) of the sample}} \times \frac{Q_{T1}}{Q_{S1}}$$

$$\text{Content (\%) of methanol} = \frac{\text{Weight (g) of methanol}}{\text{Weight (g) of the sample}} \times \frac{Q_{T2}}{Q_{S2}}$$

Operating Conditions

Detector: Flame-inonization detector.

Column: A glass tube of 3 mm internal diameter and 2 m length.

Column packing material: 180- to 250-µm styrene-divinylbenzene porous polymer for gas chromatography.

Column temperature: A constant temperature at about 120°C.

Injection port temperature: A constant temperature at about 200°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust so that the retention times of methanol and 2-propanol are about 2 minutes and 10 minutes, respectively.

Loss on Drying Not more than 12.0% (105°C, 2 hours).

Acid-insoluble Ash Not more than 1.0%.

Microbial Limits Proceed as directed in the Microbial Limit Tests. The total bacterial count is not more than 5,000/g, and *Escherichia coli* is negative.

Pepsin

ペプシン

Definition Pepsin is a proteolytic enzyme obtained from animals or fish. It may contain lactose or dextrin.

Enzyme Activity Pepsin has the enzyme activity equivalent to not less than 110,000 units per gram.

Description Pepsin occurs as a hygroscopic, white to light yellowish brown powder, or as light yellowish brown to brown pastes or liquids. It is odorless or has slight characteristic odors.

Identification A solution of Pepsin in acetate buffer (pH 5.4) (1 in 500 to 1,000) exhibits absorption maximum at a wavelength of 272–278 nm.

Purity

(1) Lead Not more than 5.0 µg/g (2.0 g, Method 1).

(2) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Microbial Limits Proceed as directed in the Microbial Limit Tests. The total bacterial count is not more than 50,000/g, and *Escherichia coli* is negative.

Enzyme Activity Determination

(i) Preparation of Sample Solution and Standard Solution

Sample Solution Weigh accurately an amount of Pepsin equivalent to about 1,250 units enzyme activity, and add 0.01 mol/L hydrochloric acid, previously cooled with ice, to make exactly 50 ml.

Standard Solution Weigh accurately an amount of Saccharated Pepsin Reference Standard equivalent to about 1,250 units, and add 0.01 mol/L hydrochloric acid, previously cooled with ice, to make exactly 50 ml.

(ii) Procedure Add exactly 1 ml each of the sample solution and the standard solution, while cooling with ice, to separate 5 ml portions of casein TS (pH 2.0), exactly measured and warmed for 10 minutes at 37±0.5°C. Shake immediately, and react for exactly 10 minutes at 37±0.5°C. Add 5 ml of trichloroacetic acid solution (7.2 in 100) to each, shake, allow to stand for 30 minutes at 37±0.5°C, and separately filter through a filter paper for quantitative analysis (5C). Discard the first 3 ml of each filtrate. To the subsequent 2 ml of each filtrate, exactly measured, add 5 ml of 0.55 mol/L sodium carbonate solution and 1 ml of diluted Folin's TS (1 in 3). Allow to stand for 30 minutes at 37±0.5°C. Measure the absorbances of them at 660 nm against water as the reference. The absorbances are expressed as A_T and A_S, respectively.

Separately, measure exactly 1 ml each of the sample solution and the standard solution, add 5 ml of trichloroacetic acid solution (7.2 in 100) to each, and shake. Then add 5 ml of casein TS (pH 2.0), warm for 30 minutes at 37±0.5°C, and separately filter through a filter paper for quantitative analysis (5C). Discard the first 3 ml of each filtrate, and measure exactly 2 ml each of the subsequent filtrates. Measure absorbances (A_{TB} and A_{SB}) in the same manner. Calculate the enzyme activity by the formula:

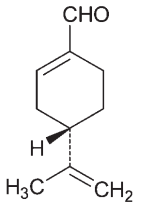
$$\text{The Enzyme Activity of Pepsin (units/g)} = \frac{U_S \times (A_T - A_{TB})}{A_S - A_{SB}} \times \frac{1}{W}$$

U_S = units in 1 ml of the standard solution,

W = weight (g) of Pepsin in 1 ml of the sample solution.

l-Perillaldehyde

l-ペリルアルデヒド



$C_{10}H_{14}O$ Mol. Wt. 150.22
(4S)-4-(1-Methylethenyl)cyclohex-1-ene-1-carbaldehyde
[18031-40-8]

Content *l*-Perillaldehyde contains not less than 90.0% of *l*-perillaldehyde ($C_{10}H_{14}O$).

Description *l*-Perillaldehyde is a colorless or slightly yellowish, transparent liquid having a strong perilla-like odor.

Identification

(1) To 0.5 ml of *l*-Perillaldehyde, add 3 ml of sodium hydrogen sulfite TS, and shake. White crystalline lumps are formed.

(2) To 0.5 ml of *l*-Perillaldehyde, add 10 ml of hydroxylamine TS, and heat under a reflux condenser in a water bath for 10 minutes. Evaporate most of the ethanol, add 50 ml of water, and allow to stand at 5°C or lower. Crystals are deposited. Collect the crystals by filtration, and recrystallize from ethanol. The melting point is 100–103°C.

Purity

(1) Refractive index n_D^{20}: 1.504–1.510.

(2) Angular rotation α_D^{20}: –110.0 to –150.0°.

(3) Specific gravity 0.965–0.975.

(4) Clarity of solution Clear (1.0 ml, 70% (vol) ethanol 3.0 ml).

(5) Acid value Not more than 3.0 (Flavoring Substances Tests).

Assay Weigh accurately about 1 g of *l*-Perillaldehyde, and proceed as directed in Method 2 in the Aldehyde and Ketone Content Test in the Flavoring Substances Tests. In the test, boil the mixture for 30 minutes before titrating.

Each ml of 0.5 mol/L hydrochloric acid = 75.11 mg of $C_{10}H_{14}O$

Perlite

パーライト

Definition Perlite is obtained by calcining mineral silicon dioxide at 800–1,200°C.

Description Perlite occurs as a white or light gray powder.

Identification Place 0.2 g of Perlite in a platinum crucible, dissolve it in 5 ml of hydrofluoric acid, and heat. It almost completely evaporates.

Purity

(1) pH 5.0–9.0.

Weigh 10.0 g of Perlite, add 100 ml of water, and heat on a water bath for 2 hours with occasional shaking while replenishing the evaporated water. Cool, and filter with suction, using a filter holder equipped with a 47-mm membrane filter (with a pore diameter of 0.45 μm). If the filtrate is turbid, repeat the filtration with suction through the same filter. Wash the container and the residue on the filter with water, combine the filtrate and the washings, and add water to make 100 ml. Refer to this solution as solution A. Measure the pH of solutrion A.

(2) Water-soluble substances Not more than 0.20%.

Measure 50 ml of solution A prepared in Purity (1), evaporate to dryness, and dry the residue at 105°C for 2 hours. Weigh the residue.

(3) Hydrochloric acid-soluble substances Not more than 2.5%.

Weigh 2.0 g of Perlite, add 50 ml of diluted hydrochloric acid (1 in 4), and heat at 50°C for 15 minutes with occasional shaking. Cool, and filter. Wash the container and the residue on the filter paper with 3 ml of diluted hydrochloric acid (1 in 4), and combine the filtrate and the washings. Add 5 ml of diluted sulfuric acid (1 in 20), evaporate to dryness, ignite at 450–550°C to constant weight, and weigh the residue.

(4) Heavy metals Not more than 50 μg/g as Pb.

Test Solution Measure 2.0 g of Perlite, add 50 ml of diluted hydrochloric acid (1 in 4), cover with a watch glass, and heat at 70°C for 15 minutes while shaking. After cooling, filter the supernatant through a filter paper for quantitative analysis (5C), wash the residue in the container three times with 10 ml of hot water each time, and filter with the previously used filter paper. Wash the filter paper and the residue on it with 15 ml of water, combine the filtrate and the washings, and add water to make 100 ml. Refer to this solution as solution A. Measure 20 ml of solution A, evaporate to dryness on a water bath, and add 2 ml of diluted acetic acid (1 in 20) and 20 ml of water to dissolve the residue. Filter if necessary, and add water to make 50 ml.

Control Solution To 2.0 ml of Lead Standard Solution, add 2.0 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(5) Lead Not more than 10 μg/g as Pb.

Test Solution Measure 25 ml of solution A prepared in Purity (4), evaporate to dryness on a water bath, and dissolve the residue in diluted hydrochloric acid (1 in 10) to make 10 ml.

Control Solution To 1.0 ml of Lead Standard Solution, add diluted hydrochloric acid (1 in 10) to make 20 ml.

Procedure Proceed as directed in Method 1 in the Lead Limit Test.

(6) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Use 25 ml of solution A prepared in Purity (4).

Apparatus Use Apparatus B.

Loss on Ignition Not more than 3.0% (105°C 2 hours, then 1,000°C 30 minutes).

Hydrofluoric Acid-insoluble Substances Not more than 37.5%.

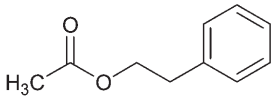
Weigh accurately about 0.2 g of Perlite into a platinum crucible, previously ignited at 1,000°C for 30 minutes, cooled in a desiccator, and weighed accurately. Weigh the crucible with the sample accurately. Add 5 ml of hydrofluoric acid and 2 drops of diluted sulfuric acid (1 in 2), evaporate to almost dryness on a water bath, and cool. Add 5 ml

of hydrofluoric acid to the residue, and evaporate gently to dryness on a sand bath. Heat at 550°C for 1 hour, raise the temperature gradually, ignite at 1,000°C for 30 minutes, allow to cool in a desiccator, and weigh accurately.

Phenethyl Acetate

Phenylethyl Acetate

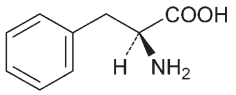
酢酸フェネチル



$C_{10}H_{12}O_2$ Mol. Wt. 164.20
2-Phenylethyl acetate [103-45-7]
Content Phenethyl Acetate contains not less than 98.0% of phenethyl acetate ($C_{10}H_{12}O_2$).
Description Phenethyl Acetate is a colorless, transparent liquid having a characteristic odor.
Identification
(1) To 1 ml of Phenethyl Acetate, add 5 ml of ethanolic 10% potassium hydroxide TS, and heat under a reflux condenser in a water bath for 20 minutes. The characteristic odor disappears. Cool, and add 8 ml of water and 1 ml of diluted hydrochloric acid (1 in 4). The solution responds to test (3) for Acetate in the Qualitative Tests
(2) To 1 ml of Phenethyl Acetate, add 0.5 g of potassium hydroxide, and boil gently. An odor of styrene is evolved.
Purity
(1) Refractive index n_D^{20}: 1.497–1.501.
(2) Specific gravity 1.033–1.037.
(3) Clarity of solution Clear (1.0 ml, 70% (vol) ethanol 2.0 ml).
(4) Acid value Not more than 1.0 (Flavoring Substances Tests).
Assay Weigh accurately about 1 g of Phenethyl Acetate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.
Each ml of 0.5 mol/L ethanolic potassium hydroxide = 82.10 mg of $C_{10}H_{12}O_2$

L-Phenylalanine

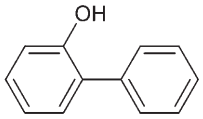
L-フェニルアラニン



$C_9H_{11}NO_2$ Mol. Wt. 165.19
(2*S*)-2-Amino-3-phenylpropanoic acid [63-91-2]
Content L-Phenylalanine, when calculated on the dried basis, contains 98.5–102.0% of L-phenylalanine ($C_9H_{11}NO_2$).
Description L-Phenylalanine occurs as white crystals or crystalline powder having a slightly bitter taste.
Identification
(1) To 5 ml of a solution of L-Phenylalanine (1 in 1,000), add 1 ml of ninhydrin solution (1 in 1,000), and heat for 3 minutes. A purple color develops.
(2) To 0.010 g of L-Phenylalanine, add 0.5 g of potassium nitrate and 2 ml of sulfuric acid, heat on a water bath for 20 minutes, and cool. Add 5 ml of hydroxylamine hydrochloride solution (1 in 10), allow to stand in ice water for 10 minutes, add 9 ml of sodium hydroxide solution (2 in 5), and allow to stand. A red-purple color develops.
(3) To 5 ml of a solution of L-Phenylalanine (1 in 100), add 1 ml of potassium dichromate solution (1 in 100), and boil. A characteristic odor is formed.
Purity
(1) Specific rotation $[\alpha]_D^{20}$: –33.0 to –35.2° (1 g, water, 50 ml, on the dried basis).
(2) Clarity and color of solution Colorless and almost clear (0.20 g, water 20 ml).
(3) pH 5.4–6.0 (1.0 g, water 100 ml).
(4) Chloride Not more than 0.021% as Cl (0.50 g, Control solution 0.01 mol/L hydrochloric acid 0.30 ml).
(5) Heavy metals Not more than 20 µg/g as Pb.
Test Solution Weigh 1.0 g of L-Phenylalanine, add 40 ml of water, dissolve by warming, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.
Control Solution To 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.
(6) Arsenic Not more than 4.0 µg/g as As_2O_3.
Test Solution Weigh 0.50 g of L-Phenylalanine, dissolve it in 5 ml of diluted hydrochloric acid (1 in 4).
Apparatus Use Apparatus B.
Loss on Drying Not more than 0.30% (105°C, 3 hours).
Residue on Ignition Not more than 0.10% .
Assay Weigh accurately about 0.3 g of DL-Phenylalanine, and proceed as directed in the Assay for DL-Alanine.
Each ml of 0.1 mol/L perchloric acid = 16.52 mg of $C_9H_{11}NO_2$

o-Phenylphenol

オルトフェニルフェノール



$C_{12}H_{10}O$ Mol. Wt. 170.21

2-Phenylphenol [90-43-7]

Content *o*-Phenylphenol contains not less than 97.0% of *o*-phenylphenol ($C_{12}H_{10}O$).

Description *o*-Phenylphenol occurs as white, light yellow or light pink powder, flakes, or lumps having a characteristic odor.

Identification

(1) To 1 ml of a solution of *o*-Phenylphenol in ethanol (1 in 100), add 4 ml of sodium borate solution (1 in 500) and small crystals of 2,6-dichloroquinonechloroimide, and shake. The solution is blue to indigo-purple.

(2) On the surface of 1 ml of a solution of *o*-Phenylphenol in ethanol (1 in 100), pour cautiously 1 ml of formalin–sulfuric acid TS to form a layer. The boundary surface of the two layers turns to pink.

Purity

(1) Melting point 57–59°C.

(2) Heavy metals Not more than 20 μg/g as Pb (powdered sample 1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(3) *p*-Phenylphenol and other organic impurities Not more than 0.1% as *p*-Phenylphenol.

Test Solution Weigh 1.0 g of *o*-Phenylphenol, and add 5 ml of ethanol and 5 ml of a solution of caffeine in ethanol (1 in 1,000) to dissolve it.

Control Solution To 5 ml of a solution of *p*-phenylphenol in ethanol (1 in 5,000), add 5 ml of a solution of caffeine in ethanol (1 in 1,000).

Analyze the test solution and the control solution by gas chromatography using the conditions given below. For the test solution, determine the ratio (A/A_s) of the sum (A) of the peak area of *p*-phenylphenol and the peak areas of all peaks appearing between the *o*-phenylphenol peak and the caffeine peak to the peak area (A_s) of caffeine, and for the control solution, determine the ratio (A'/A'_s) of the peak area (A') of *p*-phenylphenol to the peak area (A'_s) of caffeine. The ratio (A/A_s) does not exceed the ratio (A'/A'_s).

Operating Conditions

Detector: Flame ionization detector.

Column: A stainless steel tube of 3–4 mm internal diameter and 1 m length.

Column packing material

Liquid phase: 3% Diethylene glycol succinate polyester of the amount of support.

Support: 177- to 250-mm diatomaceous earth for gas chromatography.

Column temperature: A constant temperature between 195–250°C.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the peak of caffeine appears about 12 minutes after injection.

Residue on Ignition Not more than 0.05% (5 g).

Assay

Test Solution Weigh accurately about 2 g of powdered *o*-Phenylphenol, add 25 ml of sodium hydroxide solution (1 in 25), dissolve while warming if necessary, and cool. Add water to make exactly 500 ml.

Procedure Transfer exactly 25 ml of the test solution to an iodine bottle, and add exactly 30 ml of potassium bromate solution (1 in 350), 5 ml of potassium bromide solution (2 in 25), and 50 ml of methanol, and shake well. Quickly add about 10 ml of diluted hydrochloric acid (1 in 2), stopper immediately, shake gently, and allow to react for 30 seconds. Place 15 ml of potassium iodide in the upper part of the iodine bottle, allow to flow down by loosening the stopper, wash the stopper and mouth of the flask thoroughly with water, shake well, and allow to stand for 5 minutes. Titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: 4 ml of starch TS). Perform a blank test in the same manner, and calculate the content by the formula:

$$\text{Content (\%) of } o\text{-phenylphenol } (C_{12}H_{10}O) = \frac{4.255 \times (a - b)}{\text{Weight (g) of the sample} \times 50} \times 100$$

a = volume (ml) of 0.1 mol/L sodium thiosulfate consumed in the blank test,

b = volume (ml) of 0.1 mol/L sodium thiosulfate consumed in this test.

Phosphoric Acid

リン酸

H_3PO_4 Mol. Wt. 98.00

Phosphoric acid [7664-38-2]

Content Phosphoric Acid contains not less than 75.0% of phosphoric acid (H_3PO_4).

Description Phosphoric acid is a colorless, transparent syrupy liquid. It is odorless.

Identification To a solution of Phosphoric Acid (1 in 20), add 2–3 drops of phenolphthalein TS, and neutralize with sodium hydroxide (1 in 25). The solution responds to all tests for Phosphate in the Qualitative Tests.

Purity

(1) Specific gravity Not less than 1.579.

(2) Clarity and color of solution Colorless and almost clear (4.0 ml, ethanol 16 ml).

(3) Sulfate Not more than 0.14% as SO_4.

Test Solution Weigh 0.20 g of Phosphoric Acid, and add water to make 50 ml.

Control Solution To 0.60 ml of 0.005 mol/L sulfuric acid, add 1 ml of diluted hydrochloric acid (1 in 4) and water to make 50 ml.

(4) Heavy metals Not more than 10 μg/g as Pb.

Weigh 2.0 g of Phosphoric Acid, add 10 ml of water, shake, add 2 drops of phenolphthalein TS, and add ammonia TS dropwise until the solution is slightly pink. Add 8 ml of acetic acid and water to make 40 ml, add 10 ml of hydrogen

sulfide TS, and allow to stand for 5 minutes. The color of this solution is not darker than that of the solution prepared as follows: To 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 40 ml, add 10 ml of sodium sulfide TS, and allow to stand for 5 minutes.

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

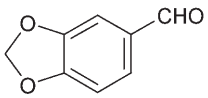
Assay Weigh accurately about 1.5 g of Phosphoric Acid, dissolve it in 25 ml of water, keep at about 15°C, and titrate with 1 mol/L sodium hydroxide (indicator: 5 drops of thymolphthalein TS) until the color of the solution changes to light blue.

Each ml of 1 mol/L sodium hydroxide = 49.00 mg of H_3PO_4

Piperonal

Heliotropin

ピペロナール



$C_8H_6O_3$ Mol. Wt. 150.13

Benzo[*d*][1,3]dioxole-5-carbaldehyde [120-57-0]

Content Piperonal, when dried, contains not less than 99.0% of piperonal ($C_8H_6O_3$).

Description Piperonal occurs as white crystals or lumps having a heliotrope-like odor.

Identification

(1) Dissolve 0.1 g of Piperonal in 2 ml of sulfuric acid, and add 2 drops of a solution of resorcinol in ethanol (1 in 20). A dark red color develops.

(2) Melt 1 g of Piperonal by warming, add 5 ml of sodium hydrogen sulfite TS, and heat in a water bath while shaking. White crystalline lumps are formed.

Purity

(1) Melting point 36–37.5°C.

(2) Clarity of solution Clear (1.0 ml, 70% (vol) ethanol 4.0 ml).

(3) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50g, Method 4, Apparatus B).

Loss on Drying Not more than 0.50% (4 hours).

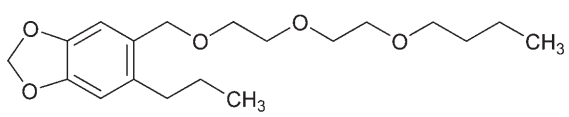
Residue on Ignition Not more than 0.05%.

Assay Weigh accurately about 1 g of Piperonal, previously dried, and proceed as directed in Method 2 in the Aldehyde and Ketone Content Test in the Flavoring Substances Tests. In the test, allow the mixture to stand for 15 minutes before titration.

Each ml of 0.5 mol/L hydrochloric acid = 75.07 mg of $C_8H_6O_3$

Piperonyl Butoxide

ピペロニルブトキシド



$C_{19}H_{30}O_5$ Mol. Wt. 338.44

5-{[2-(2-Butoxyethoxy)ethoxy]methyl}-6-propylbenzo[*d*][1,3]dioxole [51-03-6]

Description Piperonyl Butoxide is a light yellow to light brown, transparent, oily liquid. It is odorless or has a slight odor.

Identification

(1) To 0.5 ml of a solution of Piperonyl Butoxide in methanol (1 in 1,000), add 20 ml of tannic acid–acetic acid TS, and heat in a water bath with occasional shaking. A blue color develops.

(2) A solution of Piperonyl Butoxide in 90% (vol) methanol (1 in 100,000) exhibits absorption maxima at wavelengths of 236–240 nm and 288–292 nm, and the ratio of the absorbance at a wavelength 236–240 nm to that at 288–292 nm is 1.22–1.24.

Purity

(1) Refractive index n_D^{20}: 1.497–1.512.

(2) Specific gravity 1.05–1.07.

(3) Color The color of Piperonyl Butoxide is not deeper than that of the solution prepared by mixing 1.4 ml of Cobaltous Chloride Colorimetric Standard Stock Solution, 4.3 ml of Ferric Chloride Colorimetric Standard Stock Solution, and 0.3 ml of Cupric Sulfate Colorimetric Standard Stock Solution.

(4) Heavy metals Measure 15 ml of Piperonyl Butoxide, transfer into a separating funnel, add 15 ml of water and 3 drops of diluted hydrochloric acid (1 in 4), shake vigorously for 3 minutes, and allow to stand. Transfer the upper layer, add 5 ml of acetone, and add 2 drops of sodium sulfide TS. The solution is not turbid, and no dark color develops.

(5) Chlorinated compounds Not more than 0.035% as Cl.

Test Solution Weigh 0.50 g of Piperonyl Butoxide, transfer into a porcelain crucible, add 2 ml of anhydrous sodium carbonate solution (1 in 8), heat on a water bath for 1 hour with occasional shaking, and evaporate almost completely to dryness. Add 1 g of calcium carbonate, carbonize almost completely by heating weakly, and incinerate almost completely by heating to about 600°C, and cool. Dissolve the residue, by adding 35 ml of diluted nitric acid (1 in 10) gradually, and filter. Wash the insoluble residue with 10 ml of water, combine the filtrate and the washings, and add water to make 50 ml.

Control Solution Weigh 1 g of calcium carbonate, add 2 ml of anhydrous sodium carbonate solution (1 in 8), dissolve by gradually adding 35 ml of diluted nitric acid (1 in 10), and filter. Wash the insoluble residue with 10 ml of water, combine the filtrate and the washings, and add 0.5 ml of 0.01 mol/L hydrochloric acid and water to make 50 ml.

Procedure Add 0.5 ml of silver nitrate solution (1 in 50) to the test solution and the control solution, shake well, and allow to stand for 5 minutes. The test solution is not more

turbid than the control solution.

(6) Distillation test

Residue after distillation at up to 194°C Not less than 85.0%.

Residue after distillation at up to 203°C Not more than 5.0%.

Weigh 25 g of Piperonyl Butoxide into a 100-ml eggplant shaped flask, previously accurately weighed, then weigh accurately the flask containing the sample, distill to 194°C under reduced pressure of 0.53 kPa, and weigh the residue in the flask. Distill up to 203°C under reduced pressure of 0.53 kPa, and weigh the residue in the flask.

Polybutene

Polybutylene

ポリブテン

Definition Polybutene is a polymer consisting mainly of isobutylene.

Description Polybutene is a colorless to pale yellow, viscous liquid. It is odorless or has a slight, characteristic odor, and tasteless.

Identification Dissolve about 1 g of Polybutene in 5 ml of hexane, and proceed as directed in the Thin Film Method under Infrared Spectrophotometry. Polybutene exhibits absorption bands at about 1393 cm^{-1}, 1370 cm^{-1}, 1230 cm^{-1}, 950 cm^{-1}, and 920 cm^{-1}.

Purity

(1) Clarity of solution Clear (0.50 g, hexane 5.0 ml).

(2) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(4) Chlorinated compounds Not more than 0.014% as Cl.

Proceed as directed in Purity (4) for Polyisobutylene, using 0.20 ml of 0.01 mol/L hydrochloric acid.

(5) Low molecular weight polymer Not more than 0.40%.

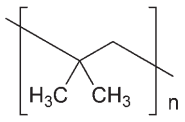
Weigh accurately about 10 g of Polybutene, and add 10 ml of methanol. Heat under a reflux condenser on a water bath for 1 hour with occasional shaking, and allow to stand in a cool place for 1 hour. Filter it into a flask, previously dried and accurately weighed, and evaporate the filtrate to dryness under reduced pressure at about 50°C. Dry in a vacuum desiccator for 20 hours, and weigh the residue accurately.

Residue on Ignition Not more than 0.05% (5 g).

Polyisobutylene

Butyl Rubber

ポリイソブチレン



$(C_4H_8)_n$

Poly(1,1-dimethylethylene) [9003-27-4]

Definition Polyisobutylene is a polymer of isobutylene. It may contain up to 2% of isoprene as a polymer component.

Description Polyisobutylene occurs as a colorless to light yellow, elastic rubbery semi-solid or viscous substance. It is odorless or has a slight, characteristic odor, and is tasteless.

Identification Dissolve about 1 g of Polyisobutylene in 5 ml of hexane, and proceed as directed in the Thin Film Method under Infrared Spectrophotometry. Polyisobutylene exhibits absorption bands at about 1393 cm^{-1}, 1370 cm^{-1}, 1230 cm^{-1}, 950 cm^{-1}, and 920 cm^{-1}.

Purity

(1) Clarity of solution Slightly turbid.

Test Solution Weigh 0.50 g of Polyisobutylene, add 50 ml of hexane, and dissolve while heating in a water bath at 80°C.

(2) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(4) Chlorinated compounds Not more than 0.028% as Cl.

Test Solution Weigh 0.50 g of Polyisobutylene and 0.7 g of calcium carbonate, transfer them into a porcelain crucible, mix with a small amount of water, dry at 100°C, and heat at about 600°C for 10 minutes. After cooling, dissolve the residue by adding 20 ml of diluted nitric acid (1 in 10), filter, and wash the insoluble residue with about 15 ml of water. Combine the filtrate and the washings, and add water to make 50 ml.

Control Solution Dissolve 0.7 g of calcium carbonate in 20 ml of diluted nitric acid (1 in 10), filter if necessary, and add 0.40 ml of 0.01 mol/L hydrochloric acid and water to make 50 ml.

Procedure Add 0.5 ml of silver nitrate solution (1 in 50) to the test solution and the control solution, shake well, and allow to stand for 5 minutes. The test solution is not more turbid than the control solution.

(5) Total unsaturated substances Not more than 2.0%.

Weigh accurately about 0.5 g of Polyisobutylene, previously prepared by cutting up in thin strips, into a flask, and add 100 ml of cyclohexane. Stopper the flask, allow to stand over night to dissolve. If insoluble substances remain, shake for about 1 hour to dissolve them completely. Transfer in a 500-ml flask, wash the flask with a small amount of cyclohexane, and add the washings to the 500-ml flask. Add exactly 15 ml of Wijs TS, and mix well. If the solution is not clear, add cyclohexane until it becomes clear. Stopper the flask, and allow to stand for 30 minutes at 20–30ºC, protected from light, with occasional shaking. Add 20 ml of

potassium iodine (1 in 10) and 100 ml of water, and shake. Titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: starch TS). Perform a blind test in the same manner, and make any necessary correction. Calculate the content of total unsaturated substances by formula:

$$\text{Total amount (\%) of unsaturated substances} = \frac{1.87 \times (a - b) \times 0.1}{\text{Weight (g) of the sample}}$$

a = volume (ml) of 0.1 mol/L sodium thiosulfate consumed in the blank test,
b = volume (ml) of 0.1 mol/L sodium thiosulfate consumed in the test.

(6) Low molecular weight polymer Not more than 1.2%.

Weigh accurately about 10 g of Polyisobutylene in a flask, and add 40 ml of cyclohexane. Dissolve by heating under a reflux condenser on a water bath for 1 hour with occasional shaking. After cooling, add 40 ml of methanol, shake well, and allow to stand in a cold place for 1 hour. Filter the liquid obtained into a flask, previously dried and accurately weighed, and evaporate the filtrate to dryness under reduced pressure at about 50°C. Dry the residue in a vacuum desiccator for 20 hours, and weigh it accurately.

Residue on Ignition Not more than 0.20%.

ε-Polylysine

ε-ポリリシン

Definition ε-Polylysine is obtained from the culture fluid of the actinomycete *Streptomyces albulus* by adsorption and isolation using ion-exchange resin. It consists mainly of ε-polylysine and may contain dextrin.

Content ε-Polylysine contains not less than 25% of ε-polylysine and 95–115% of the labeled content.

Description ε-Polylysine occurs as a light yellow liquid or highly hygroscopic powder. It has a slight bitter-taste.

Identification (1) To 1 ml of a solution of ε-Polylysine (1 in 1,000), add 1 ml of Dragendorff reagent. A red-brown precipitate is produced.

(2) To 1 ml of a solution of 0.1 g of ε-Polylysine in 100 ml of phosphate buffer (pH 6.8), add 1 ml of methyl orange TS. A red-brown precipitate is produced.

(3) To 1 ml of a solution of ε-Polylysine (1 in 100), add 1 ml of hydrochloric acid, and heat at 110°C for 24 hours. After cooling, add sodium hydroxide solution (1 in 5) to adjust its pH to 6–8. Use this solution as the test solution. Separately, prepare a control solution by dissolving 0.010 g of L-lysine monohydrochloride in 10 ml of water. Analyze 2 µl portions of the test solution and the control solution by thin-layer chromatography using a 4:2:1 mixture of 1-butanol/water/acetic acid as the developing solvent. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the original line, and air-dry. Spray evenly with ninhydrin solution (1 in 50) in acetone, and heat at 90°C for 10 minutes to allow the color to develop. Examine the plate in daylight. The spot from the test solution corresponds in color tone and R_f value to the red-violet spot from the control solution.

Purity

(1) Heavy metals Not more than 10 µg/g as Pb (sample amount: equivalent of 2.0 g of ε-polylysine, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Arsenic Not more than 4.0 µg/g as As_2O_3 (sample amount: equivalent of 0.5 g of ε-polylysine, Method 3, Apparatus B).

Residue on Ignition Not more than 1.0% (sample amount: equivalent of 0.5 g ε-polylysine).

Assay

Test Solution Weigh accurately an amount of the sample equivalent to about 0.25 g of ε-polylysine, and dissolve it in the mobile phase prepared as directed in Operating Conditions to make exactly 50 ml. To 1 ml of this solution, add 10 ml of the internal standard solution, prepared as directed below, and then add the mobile phase to make exactly 50 ml.

Internal Standard Dissolve 0.15 g of L-phenylalanine in the mobile phase to make exactly 100 ml, and dilute 5 ml of the resulting solution with the mobile phase to make exactly 100 ml.

Standard Solutions Weigh accurately about 0.3 g of ε-polylysine monohydrochloride for assay, previously dried at 105°C for 3 hours, and add the mobile phase to make exactly 100 ml. To exactly 25 ml of this solution, add the mobile phase to make exactly 100 ml (standard stock solution). Transfer 6 ml, 8 ml, and 10 ml of the standard stock solution into separate 50-ml volumetric flasks, add 10 ml of the internal standard solution to each, and dilute with the mobile phase to volume to prepare standard solutions. Calculate the concentration of ε-polylysine in each standard solution, assuming that the weight ratio of ε-polylysine to ε-polylysine monohydrochloride is 0.7785.

Procedure Analyze 100 µl portions of the test solution and the standard solutions by liquid chromatography using the operating conditions given below. Prepare a calibration curve, using the concentrations of ε-polylysine in the standard solutions and the peak area ratios of ε-polylysine to L-phenylalanine for the standard solutions. Determine the peak area ratio of ε-polylysine to L-phenylalanine for the test solution, and calculate the polylysine content using the calibration curve.

Operating Conditions

Detector: Ultraviolet absorption spectrophotometer (determination wavelength: 215 nm).

Column: A stainless steel tube of 4.6 mm internal diameter and 25 cm length.

Column packing material: 5- to 10-µm octadecylsilanized silica gel for liquid chromatography.

Column temperature: A constant temperature at about 40°C.

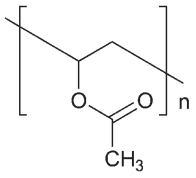
Mobile phase

Use the solution prepared as follows: Dissolve 1.74 g of dipotassium hydrogen phosphate and 1.42 g of sodium sulfate in about 800 ml of water, adjust the pH to 3.4 with phosphoric acid, and add water to make exactly 1,000 ml. To 920 ml of this solution, add 80 ml of acetonitrile.

Flow rate: Adjust so that the retention time of polylysine is about 4 minutes.

Polyvinyl Acetate

酢酸ビニル樹脂



Poly(1-acetoxyethylene)

Definition Polyvinyl Acetate is a polymer of vinyl acetate.

Description Polyvinyl Acetate occurs as colorless to light yellow granules or glassy lumps.

Identification Dissolve about 1 g of Polyvinyl Acetate in 5 ml of ethyl acetate, and proceed as directed in the Thin Film Method under Infrared Spectrophotometry. The solution exhibits absorption bands at about 1725 cm^{-1}, 1230 cm^{-1}, 1015 cm^{-1}, 937 cm^{-1}, and 785 cm^{-1}.

Purity

(1) Free acids Not more than 0.20% as CH_3COOH.

Weigh accurately about 2 g of Polyvinyl Acetate, add 50 ml of methanol, and dissolve by shaking occasionally. Add 10 ml of water, and titrate with 0.1 mol/L sodium hydroxide (indicator: 4-5 drops of phenolphthalein TS). Perform a blank test, and make any necessary correction. Calculate the amount of free acids as acetic acid (CH_3COOH) by the formula:

$$\text{Content (\%) of free acid} = \frac{\left(\begin{array}{c}\text{Volume (ml) of}\\ \text{0.1 mol/L sodium hydroxide consumed}\end{array}\right) \times 60}{\text{Weight (g) of the sample} \times 10 \times 1{,}000} \times 100$$

(2) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(4) Residual monomer Not more than 5 μg/g.

Test Solution Wrap a portion of Polyvinyl Acetate in a powder paper and then a wrapping film, and smash into fine pieces with wooden hammer. Weigh exactly 2.5 g, and dissolve it in toluene to make 25 ml.

Standard Solutions Measure exactly 0.050 g of vinyl acetate, add toluene to make exactly 50 ml, and refer to the resulting solution as solution A. Transfer 1.0 ml, 0.3 ml, 0.1 ml, 0.03 ml, and 0.01 ml of solution A into separate 100-ml volumetric flasks, and dilute each with toluene to volume.

Procedure Analyze 1 μl portions of the test solution and the standard solutions by gas chromatography using the operating conditions given below. Measure the peak height or peak area for each standard solution and prepare a calibration curve. Measure the peak height or peak area for the test solution and determine the content using the calibration curve.

Operating Conditions

Detector: Flame ionization detector.

Column: A silicate glass capillary tube (0.32 mm internal diameter and 30 cm length) coated with a 5-μm thick layer of dimethylpolysiloxane for gas chromatography.

Column temperature: Maintain the temperature at 100°C for 8 minutes, and thereafter raise at a rate of 20°C/minute. After the temperature reaches to 250°C, maintain for 5 minutes.

Temperature at the inject port: 150°C.

Injection method: Split method (8:1).

Carrier gas: Use helium.

Flow rate: Adjust so that the peak of vinyl acetate appears around 7 minutes after injection.

Loss on Drying Not more than 1.0% (not higher than 0.7 kPa, 80°C, 3 hours).

Residue on Ignition Not more than 0.05% (5 g).

Polyvinylpolypyrrolidone

ポリビニルポリピロリドン

Cross linked poly[(2-oxopyrrolidin-1-yl)ethylene] [25249-54-1]

Content Polyvinylpolypyrrolidone, when calculated on the anhydrous basis, contains 11.0–12.8% of nitrogen (N = 14.01).

Description Polyvinylpolypyrrolidone occurs a white to slightly yellowish white powder. It is odorless.

Identification Determine the absorption spectrum of Polyvinylpolypyrrolidone as directed in the Paste Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) pH 5.0–8.0 (1.0g, water 100ml).

(2) Heavy metals Not more than 10 μg/g as Pb (2.0g, Method 2, Control solution Lead Standard Solution 2.0ml).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50g, Method 2, Apparatus B).

(4) Water-soluble substances Not more than 1.5%.

Weigh accurately about 25 g of Polyvinylpolypyrrolidone, transfer to a round-bottom flask, and add 225 ml of water. Boil gently under a reflux condenser for 20 hours while stirring using a stirrer. After cooling, transfer the contents into a measuring flask, add water to make exactly 250ml, and allow to stand for 15 minutes. Take the supernatant into a centrifuge tube and centrifuge at 10,000×g for an hour. Filter the supernatant through a membrane filter with 0.45 μm in pore diameter, measure exactly 50 ml of the filtrate into a glass evaporating dish, previously weighed accurately, evaporate to dryness, and dry at 90°C for 3 hours. Allow to stand in a desiccator, and weigh the residue accurately.

(5) Vinylpyrrolidone Not more than 0.1%.

Weigh accurately about 4 g of Polyvinylpolypyrrolidone, add 30 ml of water, and shake for 15 minuets. Transfer it to a centrifuge tube, add 20 ml of water, centrifuge, and filter the supernatant a crucible-type glass filter (IG4). Wash both the residue in the centrifuge tube and residue on the filter with 50 ml each of water, combine the filtrate with the washings, and add 0.50 g of sodium acetate. Add 0.05 mol/L iodine solution until the color of iodine no longer disappear.

Add another 3.0 ml of 0.05 mol/L iodine solution, allow to stand for 10 minutes, and titrate the excess iodine with 0.1 mol/L sodium thiosulfate solution. The consumption of 0.05 mol/L iodine solution is not more than 0.72 ml (Indicator Starch TS 3 ml). Separately, perform a blank test to make any necessary correction.

Water Content Not more than 6.0% (1 g, Direct Titraion).

Residue on Ignition Not more than 0.40%.

Assay Weigh accurately about 0.2 g of Polyvinylpolypyrrolidone, determine nitrogen as directed in the Kjeldahl Method under Nitrogen Determination, and calculate on the anhydrous basis.

Each ml of 0.05 mol/L sulfuric acid = 1.401 mg of N

Potassium Alginate

アルギン酸カリウム

Potassium Alginate [9005-36-1]

Content Potassium Alginate, when dried, contains 89.2–105.5% of potassium alginate.

Description Potassium Alginate occurs in white to yellowish white filamentous, granular, or powdered form.

Identification

(1) Proceed as directed in Identification (1) for Ammonium Alginate.

(2) Ignite 1 g of Potassium Alginate at 550–600°C for 3 hours, and add 10 ml of water to the residue. The solution obtained responds to all the tests for Potassium Salt described in the Qualitative Tests.

Purity

(1) Water-insoluble substances Not more than 2.0% (on the dried basis).

Proceed as directed in Purity (1) for Ammonium Alginate in Monographs.

(2) Lead Not more than 5.0 μg/g as Pb (2.0 g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Loss on Drying Not more than 15.0% (105°C, 4 hours).

Microbial Limit Proceed as directed in the Microbial Limit Test for Ammonium Alginate.

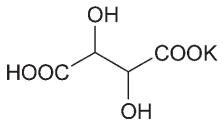
Assay Proceed as directed in the Assay for Alginic Acid.

Each ml of 0.25 mol/L sodium hydroxide = 29.75 mg of potassium alginate

Potassium DL-Bitartrate

Potassium Hydrogen *dl*-Tartrate
Potassium Hydrogen DL-Tartrate

DL-酒石酸水素カリウム



$C_4H_5KO_6$ Mol. Wt. 188.18

Monopotassium monohydrogen 2,3-dihydroxybutanedioate

Content Potassium DL-Bitartrate, when dried, contains 99.0–101.0% of potassium DL-bitartrate ($C_4H_5KO_6$).

Description Potassium DL-Bitartrate occurs as colorless crystals or as a white crystalline powder having a cool, acid taste.

Identification

(1) Dissolve 1 g of Potassium DL-Bitartrate in 10 ml of ammonia TS. The solution has no optical activity.

(2) Heat gradually 0.5 g of Potassium DL-Bitartrate. It is carbonized, emitting an odor like burning sucrose. To the residue, add 5 ml of water, and stir well. The mixture is alkaline. When neutralized with diluted hydrochloric acid (1 in 4) and filtered, the solution responds to all tests for Potassium Salt in the Qualitative Tests.

(3) Potassium DL-Bitartrate responds to all tests for Tartrate in the Qualitative Tests.

Purity

(1) Clarity of solution Colorless and almost clear (0.50 g, ammonium TS 3.0 ml).

(2) Sulfate Not more than 0.019% as SO_4.

Test Solution Weigh 0.50 g of Potassium DL-Bitartrate, add 2 ml of diluted hydrochloric acid (1 in 4) and 30 ml of water, dissolve while heating, and add water to make 50 ml.

Control Solution To 0.20 ml of 0.005 mol/L sulfuric acid, add 2 ml of diluted hydrochloric acid (1 in 4) and water to make 50 ml.

(3) Ammonium salt Weigh 0.50 g of Potassium DL-Bitartrate, add 5 ml of sodium hydroxide solution (1 in 25), and heat. No odor of ammonia is evolved.

(4) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.50 g of Potassium DL-Bitartrate, and add 10 ml of water, dissolve by heating, and cool.

Apparatus Use Apparatus B.

(6) Readily oxidizable substances Weigh 2.0 g of Potassium DL-Bitartrate, add 20 ml of water and 30 ml of diluted sulfuric acid (1 in 20) to dissolve, keep the solution at 20°C, and add 4.0 ml of 0.02 mol/L potassium permanganate. The pink color of the solution does not disappear within 3 minutes.

Loss on Drying Not more than 0.50% (105°C, 3 hours).

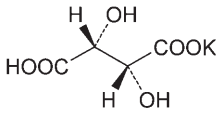
Assay Weigh accurately about 0.4 g of Potassium DL-Bitartrate, previously dried, and dissolve it in 20 ml of boiling water. Titrate the solution with 0.1 mol/L sodium hydroxide while it is hot (indicator: 2–3 drops of phenolphthalein TS).

Each ml of 0.1 mol/L sodium hydroxide = 18.82 mg of $C_4H_5KO_6$

Potassium L-Bitartrate

Potassium Acid Tartrate
Potassium Hydrogen *d*-Tartrate
Potassium Hydrogen L-Tartrate

L-酒石酸水素カリウム



$C_4H_5KO_6$ Mol. Wt. 188.18
Monopotassium monohydrogen (2*R*,3*R*)-2,3-dihydroxybutanedioate [868-14-4]
Content Potassium L-Bitartrate, when dried, contains 99.0–101.0% of potassium L-bitartrate ($C_4H_5KO_6$).
Description Potassium L-Bitartrate occurs as colorless crystals or as a white crystalline powder having a cool, acid taste.
Identification
(1) Dissolve 1 g of Potassium L-Bitartrate in 10 ml of ammonia TS. The solution is dextrorotary.
(2) Proceed as directed in Identification (2) and (3) for Potassium DL-Bitartrate.
Purity
(1) Specific rotation $[\alpha]_D^{20}$: +32.5 to +35.5°.
Weigh accurately about 5 g of the Potassium L-Bitartrate, previously dried, add 10 ml of ammonia TS and water to make exactly 50 ml, and measure the angular rotation.
(2) Clarity and color of solution Colorless and almost clear.
Proceed as directed in Purity (1) for Potassium DL-Bitartrate.
(3) Sulfate Not more than 0.019% as SO_4.
Proceed as directed in Purity (2) for Potassium DL-Bitartrate.
(4) Ammonium salt Proceed as directed in Purity (3) for Potassium DL-Bitartrate.
(5) Heavy metals Not more than 20 µg/g as Pb.
Proceed as directed in Purity (4) for Potassium DL-Bitartrate.
(6) Arsenic Not more than 4.0 µg/g as As_2O_3.
Proceed as directed in Purity (5) for Potassium DL-Bitartrate.
Loss on Drying Not more than 0.50% (105°C, 3 hours).
Assay Proceed as directed in the Assay for Potassium DL-Bitartrate.
Each ml of 0.1 mol/L sodium hydroxide = 18.82 mg of $C_4H_5KO_6$

Potassium Bromate

臭素酸カリウム

$KBrO_3$ Mol. Wt. 167.00
Potassium bromate [7758-01-2]
Content Potassium Bromate, when dried, contains 99.0–101.0% of potassium bromate ($KBrO_3$).
Description Potassium Bromate occurs as white crystals or crystalline powder.
Identification Potassium Bromate responds to all tests for Potassium Salt and for Bromate in the Qualitative Tests.
Purity
(1) Free acid and free alkali Weigh 5.0 g of Potassium Bromate, dissolve it in 60 ml of freshly boiled and cooled water while warming, cool, and add 3 drops of phenolphthalein TS. Perform the following tests on this solution:
(i) If the solution is colorless, add 1.2 ml of 0.01 mol/L sodium hydroxide. A pink color develops.
(ii) If the solution is pink, add 0.40 ml of 0.01 mol/L hydrochloric acid. The color disappears.
(2) Bromide Weigh 2.0 g of Potassium Bromate, dissolve it in 40 ml of water, add 0.25 ml of diluted sulfuric acid (3 in 100), and add 1 drop of methyl orange TS. A pink-red color develops. Shake the solution. The pink-red color does not immediately disappear.
(3) Heavy metals Not more than 10 µg/g as Pb.
Test Solution Weigh 2.0 g of Potassium Bromate, dissolve it in 10 ml of water while warming, add 10 ml of hydrochloric acid, and evaporate to dryness on a water bath. Dissolve the residue in 20 ml of water, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.
Control Solution To 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.
(4) Arsenic Not more than 4.0 µg/g as As_2O_3.
Test Solution Weigh 0.50 g of Potassium Bromate, dissolve it in 5 ml of water while warming, add 5 ml of hydrochloric acid, and evaporate to dryness on a water bath. Dissolve the residue in 5 ml of water.
Apparatus Use Apparatus B.
Loss on Drying Not more than 0.5% (105°C, 2 hours).
Assay Weigh accurately about 0.1 g of Potassium Bromate, previously dried, transfer into a 200-ml flask with a ground-glass stopper, add 50 ml of water, 1.5 g of potassium iodide, and 10 ml of diluted sulfuric acid (1 in 5), and immediately stopper tightly. Allow to stand in a dark place for 5 minutes, and titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: starch TS). Perform a blank test in the same manner, and make any necessary correction.
Each ml of 0.1 mol/L sodium thiosulfate = 2.783 mg of $KBrO_3$

Potassium Carbonate, Anhydrous

Potassium Carbonate

炭酸カリウム (無水)

K_2CO_3 Mol. Wt. 138.21

Potassium carbonate [584-08-7]

Content Potassium Carbonate, when dried, contains not less than 99.0% of potassium carbonate (K_2CO_3).

Description Potassium Carbonate occurs as a white powder or granules.

Identification A solution of Potassium Carbonate (1 in 10) responds to all tests for Potassium Salt and for Carbonate in the Qualitative Tests.

Purity

(1) Clarity and color of solution Colorless and almost clear (1.0 g, water 20 ml).

(2) Chloride Not more than 0.053% as Cl.

Sample Solution Weigh 0.20 g of Potassium Carbonate, add 3 ml of diluted nitric acid (1 in 10), boil, and cool.

Control Solution Use 0.30 ml of 0.01 mol/L hydrochloric acid.

(3) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of Potassium Carbonate, add 2 ml of water and 6 ml of diluted hydrochloric acid (1 in 4) to dissolve, and evaporate to dryness on a water bath. To the residue, add 2 ml of diluted acetic acid (1 in 20) and about 30 ml of water to dissolve, and then add water to make 50 ml.

Control Solution To 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(4) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 2.0 g of Potassium Carbonate, dissolve it in 10 ml of water, add gradually 2 ml of hydrochloric acid, and add water to make 20 ml. Use 5 ml of this solution as the test solution.

Apparatus Use Apparatus B.

Loss on Drying Not more than 5.0% (180°C 4 hours).

Assay Weigh accurately about 1 g of Potassium Carbonate, previously dried, dissolve it in 25 ml of water, and titrate with 0.25 mol/L sulfuric acid (indicator: 3 drops of bromophenol blue TS). Boil near the endpoint to let the carbon dioxide out, cool, and continue the titration.

Each ml of 0.25 mol/L sulfuric acid = 34.55 mg of K_2CO_3

Potassium Chloride

塩化カリウム

KCl Mol. Wt. 74.55

Potassium chloride [7447-40-7]

Content Potassium Chloride, when dried, contains not less than 99.0% of potassium chloride (KCl).

Description Potassium Chloride occurs as colorless crystals or as a white powder. It is odorless and has a salty taste.

Identification Potassium Chloride responds to all tests for Potassium Salt and for Chloride in the Qualitative Tests.

Purity

(1) Free acid and free alkali Weigh 5.0 g of Potassium Chloride, dissolve it in 50 ml of freshly boiled and cooled water, and add 3 drops of phenolphthalein TS. The color of the solution does not change to pink. Add 0.30 ml of 0.02 mol/L sodium hydroxide. The color of the solution changes to pink.

(2) Bromide Not more than 0.13%.

Test Solution Weigh 0.75 g of Potassium Chloride, add water to dissolve it, and make exactly 500 ml. Measure 5 ml of this solution, add 2 ml of dilute phenol red TS and 1 ml of chloramine T TS (1 in 10,000), mix immediately, and allow to stand for 2 minutes. Add 0.15 ml of 0.1 mol/L sodium thiosulfate, stir, and dilute with water to 10 ml.

Control Solution Weigh exactly 2.979 g of potassium bromide, previously dried at 110°C for 4 hours, add water to dissolve, and make exactly 1,000 ml. To exactly 1 ml of this solution, add water to make exactly 1,000 ml. Then measure exactly 5 ml of the second solution, and proceed as directed for the test solution.

Procedure Measure the absorbances of the test solution and the control solution at 590 nm. The absorbance of the test solution is not greater than that of the control solution.

(3) Iodide Moisten 5 g of Potassium Chloride by the dropwise addition of a freshly prepared mixture of 0.15 ml of sodium nitrite solution (1 in 10), 1 ml of dilute sulfuric acid, 25 ml of starch TS, and 25 ml of water. After 5 minutes, examine in daylight. No blue color is produced.

(4) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(5) Calcium or magnesium Weigh 0.20 g of Potassium Chloride, dissolve it in 20 ml of water, add 2 ml of ammonia TS, 2 ml of ammonium oxalate solution (1 in 30), and 2 ml of disodium phosphate solution (1 in 8), and allow to stand for 5 minutes. The solution does not become turbid.

(6) Sodium Weigh 0.20 g of Potassium Chloride, dissolve it in 100 ml of water, and proceed as directed in the Flame Coloration Test. No yellow color persists.

(7) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Drying Not more than 1.0% (105°C, 2 hours).

Assay Weigh accurately about 0.25 g of Potassium Chloride, previously dried, into a flask with a ground-glass stopper, dissolve it in 50 ml of water, add exactly 50 ml of 0.1 mol/L silver nitrate while shaking. Then add 3 ml of nitric acid and 5 ml of nitrobenzene while shaking, and shake vigorously. Add 2 ml of ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 mol/L ammonium thiocyanate.

Each ml of 0.1 mol/L silver nitrate = 7.455 mg of KCl

Potassium Dihydrogen Phosphate

Monopotassium Phosphate
Potassium Phosphate, Monobasic
Primary Potassium Phosphate

リン酸二水素カリウム

KH_2PO_4 Mol. Wt. 136.09
Potassium dihydrogenphosphate [7778-77-0]
Content Potassium Dihydrogen Phosphate, when dried, contains not less than 98.0% of potassium dihydrogen phosphate (KH_2PO_4).
Description Potassium Dihydrogen Phosphate occurs as colorless crystals or as a white crystalline powder.
Identification A solution of Potassium Dihydrogen Phosphate (1 in 20) responds to all tests for Potassium Salt and for Phosphate in the Qualitative Tests.
Purity
(1) Clarity and color of solution Colorless and slightly turbid (1.0 g, water 20 ml).
(2) pH 4.4–4.9 (1.0 g, water 100 ml).
(3) Chloride Not more than 0.011% as Cl (1.0 g, Control solution 0.01 mol/L hydrochloric acid 0.30 ml).
(4) Sulfate Not more than 0.019% as SO_4 (1.0 g, Control solution 0.005 mol/L sulfuric acid 0.40 ml).
(5) Heavy metals Not more than 20 μg/g as Pb.
Test Solution Weigh 1.0 g of Potassium Dihydrogen Phosphate, dissolve by adding 2 ml of diluted acetic acid (1 in 20) and about 30 ml of water, and add water to make 50 ml.
Control Solution To 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.
(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.5 g, Method 1, Apparatus B).
Loss on Drying Not more than 0.5% (105°C, 4 hours).
Assay Weigh accurately about 3 g of Potassium Dihydrogen Phosphate, previously dried, dissolve it in 30 ml of water, add 5 g of sodium chloride, and dissolve by shaking well. While keeping at about 15°C, titrate with 1 mol/L sodium hydroxide (indicator: 3–4 drops of thymol blue TS).
Each ml of 1 mol/L sodium hydroxide = 136.1 mg of KH_2PO_4

Potassium Ferrocyanide

Potassium Hexacyanoferrate(II)

フェロシアン化カリウム

$K_4[Fe(CN)_6]\cdot 3H_2O$ Mol. Wt.422.39
Potassium hexacyanoferrate(II) trihydrate [13943-58-3]
Content Potassium Ferrocyanide contains not less than 99.0% of potassium ferrocyanide ($K_4[Fe(CN)_6]\cdot 3H_2O$).
Description Potassium Ferrocyanide occurs as yellow crystals or crystalline powder.
Identification
(1) To 10 ml of a solution of Potassium Ferrocyanide (1 in 100), add 1 ml of iron(III) chloride TS. A dark blue precipitate is formed.
(2) Potassium Ferrocyanide responds to all tests for Potassium Salt in the Qualitative Tests.
Purity
(1) Cyanide To 0.010 g of copper sulfate, add 8 ml of water and 2 ml of ammonia TS to dissolve the sample. Immerge a strip of filter paper in this solution, and expose to hydrogen sulfite. A brown color develops. When 1 drop of a solution of Potassium Ferrocyanide (1 in 100) is dropped on the browned strip, no white circle is produced.
(2) Ferricyanide Dissolve 0.010 g of Potassium Ferrocyanide in 10 ml of water. To 1 drop of this solution, add 1 drop of lead nitrate solution (1 in 100) and then a few drops of 2 mol/L acetic acid saturated with benzidine. A blue color does not develop.
Assay Weigh accurately about 1 g of Potassium Ferrocyanide, and dissolve it in 200 ml of water. To this solution, add 10 ml of sulfuric acid, and titrate with 0.02 mol/L potassium permanganate. The endpoint is when the red color of the solution persists for 30 seconds.
Each ml of 0.02 mol/L potassium permanganate = 42.24 mg of $K_4[Fe(CN)_6]\cdot 3H_2O$

Potassium Gluconate

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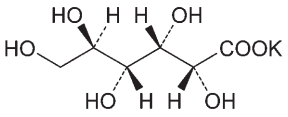


$C_6H_{11}KO_7$ Mol. Wt. 234.23
Monopotassium D-gluconate [299-27-4]
Content Potassium Gluconate, when dried, contains 97.0–103.0% of potassium gluconate ($C_6H_{11}KO_7$).
Description Potassium Gluconate occurs as a white to yellowish white crystalline powder or granules. It is odorless.
Identification
(1) Potassium Gluconate responds to all tests for Potassium Salt in the Qualitative Tests.
(2) Measure 5 ml of a solution of Potassium Gluconate (1 in 10), and proceed as directed in Identification (2) for Glucono-δ-Lactone.
Purity
(1) Clarity and color of solution Colorless and almost clear (1.0 g, water 10 ml).
(2) pH 7.3–8.5 (1.0 g, water 10 ml).
(3) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).
(4) Lead Not more than 10 μg/g (1.0 g, Method 1).
(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).
(6) Reducing sugars Not more than 0.50% as D-glucose.
Weigh 1.0 g of Potassium Gluconate and proceed as di-

rected in Purity (3) for Zinc Gluconate. Titrate excess iodine with 0.1 mol/L sodium thiosulfate. The volume of the sodium thiosulfate solution consumed is not less than 8.15 ml.
Loss on Drying Not more than 3.0% (105°C, 4 hours).
Assay Weigh accurately about 0.15 g of Potassium Gluconate, previously dried, and dissolve it in 75 ml of acetic acid, and titrate with 0.1 mol/L perchloric acid until the red color of the solution disappears (indicator: 10 drops of quinaldine red TS). Perform a blank test in the same manner.

Each ml of 0.1 mol/L perchloric acid = 23.43 mg of $C_6H_{11}KO_7$

Potassium Hydrogen Sulfite Solution

亜硫酸水素カリウム液

Content Potassium Hydrogen Sulfite Solution contains not less than 25.0% of potassium hydrogen sulfite ($KHSO_3$ = 120.17).
Description Potassium Hydrogen Sulfite Solution is a light yellow liquid having an odor of sulfur dioxide.
Identification Diluted Potassium Hydrogen Sulfite Solution (1 in 5) responds to all tests for Potassium Salt and for Sulfite in the Qualitative Tests.
Purity

(1) Clarity of solution Slightly turbid (3.0 g, water 20 ml).

(2) Heavy metals Not more than 4.0 μg/g as Pb.

Test Solution Weigh 5.0 g of Potassium Hydrogen Sulfite Solution, add 15 ml of boiling water and 5 ml of hydrochloric acid, and evaporate to dryness on a water bath. To the residue, add 10 ml of boiling water and 2 ml of hydrochloric acid, and evaporate to dryness on a water bath again. To this residue, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml to dissolve, and filter if necessary.

Control Solution To 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(3) Arsenic Not more than 2.0 μg/g as As_2O_3.

Test Solution Weigh 10 g of Potassium Hydrogen Sulfite Solution, and add water to make 25 ml. Measure 5 ml of this solution, add 1 ml of sulfuric acid, and heat on a water bath until sulfur dioxide is no longer evolved. Evaporate to about 2 ml, and add water to make 10 ml. Use 5 ml of this solution.

Apparatus Use Apparatus B.
Assay Weigh accurately about 0.5 g of Potassium Hydrogen Sulfite Solution, and proceed as directed under Sulfite Determination.

Each ml of 0.05 mol/L iodine = 6.009 mg of $KHSO_3$

Potassium Hydroxide

Caustic Potash

水酸化カリウム

KOH Mol. Wt. 56.11
Potassium hydroxide [1310-58-3]
Content Potassium Hydroxide contains not less than 85.0% of potassium hydroxide (KOH).
Description Potassium Hydroxide occurs as white lumps having various shapes including pellets, flakes, and rods, or as a white powder.
Identification

(1) A solution of Potassium Hydroxide (1 in 50) is strongly alkaline.

(2) Potassium Hydroxide responds to all tests for Potassium Salt in the Qualitative Test.

Purity

(1) Clarity and Color of Solution Colorless and almost clear.

Test Solution Weigh 50 g of Potassium Hydroxide, dissolve it in newly boiled and cooled water to make 250 ml, and use this solution as the sample solution. To 5 ml of the sample solution, add 20 ml of water, and mix.

(2) Potassium Carbonate The content of potassium Carbonate (K_2CO_3) determined in the Assay is not more than 2.0%.

(3) Heavy metals Not more than 30 μg/g as Pb.

Test Solution Measure exactly 5 ml of the sample solution prepared in Purity (1) above, neutralize by gradually adding diluted hydrochloric acid (1 in 4), and add 2 ml of diluted acetic acid solution (1 in 20) and water to make 50 ml.

Control Solution To 3.0 ml of Lead Standard Solution, add 2 ml of acetic acid solution (1 in 20) and water to make 50 ml.

Procedure Proceed as directed in Method 1 in the Lead Limit Test.

(4) Lead Not more than 10 μg/g as Pb.

Test Solution Weigh 5.0 g of Potassium Hydroxide, neutralize by gradually adding diluted hydrochloric acid (2 in 3), add again 1 ml of diluted hydrochloric acid (2 in 3) and water to make 50 ml.

Control Solution To 5.0 ml of the Lead Standard Solution, add 1 ml of diluted hydrochloric acid (2 in 3) and water to make 50 ml.

Procedure Proceed as directed in Method 1 in the Lead Limit Test.

(5) Mercury Not more than 0.10 μg/g as Hg.

Test Solution Measure exactly 10 ml of the sample solution prepared in Identification (1) above, add 1 ml of potassium permanganate solution (3 in 50) and about 30 ml of water, and shake well. Neutralize by gradually adding purified hydrochloric acid, add 5 ml of diluted sulfuric acid (1 in 2), and cool.

Procedure To the test solution, add hydroxylamine hydrochloride solution (1 in 5) until the purple color of the potassium permanganate disappears and the precipitate of manganese dioxide dissolves, and then add water to make 100 ml. Transfer the mixture into the gas washing bottle of the atomic absorption spectrophotometer. Add 10 ml of stannous

chloride TS, immediately connect with the atomic absorption spectrophotometer, and set off the diaphragm pump to circulate the air. When the recorder reading increases rapidly and then indicates a constant value, measure the absorbance. The absorbance is not more than that of the following solution: Measure 2.0 ml of Mercury Standard Solution, add 1 ml of potassium permanganate solution (3 in 50), 30 ml of water, and the same amount of purified hydrochloric acid as used for preparing the test solution, and proceed in the same manner as the preparation of the test solution.

(6) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Measure 2.5 ml of the sample solution prepared in Purity (1) above, add 5 ml of water, and neutralize by gradually adding hydrochloric acid.

Apparatus Use Apparatus B.

Assay Weigh accurately about 50 g of Potassium Hydroxide, and dissolve it in freshly boiled and cooled water to make exactly 1,000 ml. Use this solution as the sample solution.

Measure exactly 25 ml of the sample solution, add 10 ml of freshly boiled and cooled water, and titrate with 1 mol/L hydrochloric acid (indicator: 1 ml of bromophenol blue TS). When the solution reaches neutral, add exactly 1 ml of 1 mol/L hydrochloric acid, and boil for about 5 minutes. After cooling, titrate the excess acid with 0.1 mol/L sodium hydroxide, and determine the volume (a ml) of 1 mol/L hydrochloric acid consumed.

Separately, measure exactly 25 ml of the sample solution, transfer into a flask with a ground-glass stopper, and add 25 ml of freshly boiled and cooled water. To the solution, add 10 ml of the barium chloride solution (3 in 25), stopper, shake gently, and titrate with 1 mol/L hydrochloric acid (indicator: 1 ml of phenolphthalein TS). Record the volume consumed as b (ml).

$$\text{Content (\%) of potassium hydroxide (KOH)} = \frac{0.05611 \times b \times 40}{\text{Weight (g) of the sample}} \times 100$$

$$\text{Content (\%) of potassium carbonate } (K_2CO_3) = \frac{0.06910 \times (a - b) \times 40}{\text{Weight (g) of the sample}} \times 100$$

Potassium Hydroxide Solution

水酸化カリウム液

Content Potassium Hydroxide Solution contains 95–120% of the labeled content of potassium hydroxide (KOH = 56.11).

Description Potassium Hydroxide Solution is a colorless or slightly colored liquid.

Identification

(1) Diluted Potassium Hydroxide Solution (1 in 50) is strongly alkaline.

(2) Potassium Hydroxide Solution responds to all tests for Potassium Salt in the Qualitative Tests.

Purity

(1) Clarity and color of solution Colorless and almost clear.

Test Solution To Potassium Hydroxide Solution, add freshly boiled and cooled water to prepare a 20% (w/v) solution of KOH, calculated from the labeled content, and use this solution as the sample solution. Mix 5 ml of the sample solution with 20 ml of water.

(2) Potassium Carbonate Not more than 2.0% as K_2CO_3 per KOH.

Proceed as directed in Purity (2) for Potassium Hydroxide.

(3) Heavy metals Not more than 30 μg/g of KOH as Pb.

Proceed as directed in Purity (3) for Potassium Hydroxide.

(4) Lead Not more than 10 μg/g of KOH as Pb.

Proceed as directed in Purity (4) for Potassium Hydroxide.

(5) Mercury Not more than 0.10 μg/g of KOH as Hg.

Proceed as directed in Purity (5) for Potassium Hydroxide.

(6) Arsenic Not more than 4.0 μg/g of KOH as As_2O_3.

Proceed as directed in Purity (6) for Potassium Hydroxide.

Assay Weigh accurately an amount of Potassium Hydroxide Solution equivalent to about 5 g of potassium hydroxide (KOH). Add freshly boiled and cooled water to make 100 ml, and use this solution as the sample solution. Measure exactly 25 ml of the sample solution, and proceed as directed in the Assay for Potassium Hydroxide.

$$\text{Content (\%) of potassium hydroxide (KOH)} = \frac{0.05611 \times b \times 4}{\text{Weight (g) of the sample}} \times 100$$

$$\text{Content (\%) of potassium carbonate } (K_2CO_3) \text{ per potassium hydroxide (KOH)} = \frac{0.06910 \times (a - b) \times 4}{\text{Weight (g) of the sample}} \times \frac{100}{\text{Content (\%) of the potassium hydroxide}}$$

Potassium Metaphosphate

メタリン酸カリウム

Content Potassium Metaphosphate, when dried, contains the equivalent of 53.0–80.0% of phosphorus(V) oxide (P_2O_5 = 141.94).

Description Potassium Metaphosphate occurs as white fibrous crystals or powder or as colorless to white glassy flakes or lumps.

Identification

(1) Dissolve 0.1 g of Potassium Metaphosphate by adding 0.4 g of sodium acetate and 10 ml of water, make the solution slightly acidic with diluted acetic acid (1 in 20) or sodium hydroxide solution (1 in 20), and add 5 ml of egg white TS. A white precipitate is formed.

(2) Potassium Metaphosphate responds to all tests for Potassium Salt in the Qualitative Tests.

Purity

(1) Clarity and color of solution Colorless and slight turbid.

Test Solution Weigh 1.0 g of powdered Potassium Metaphosphate, add 50 ml of water, heat in a water bath, and dissolve by vigorously shaking. Add gradually 50 ml of sodium

hydroxide solution (1 in 25), heat in a water bath for 10 minutes with occasional stirring, and cool to 35–45°C.

(2) Chloride Not more than 0.11% as Cl (0.10 g in powder form, Control solution 0.01 mol/L hydrochloric acid 0.30 ml).

(3) Orthophosphate Weigh 1.0 g of powdered Potassium Metaphosphate, and add 2–3 drops of silver nitrate solution (1 in 50). No brilliant yellow color develops.

(4) Sulfate Not more than 0.096% as SO_4.

Test Solution Weigh 0.20 g of powdered Potassium Metaphosphate, add 30 ml of water and 2 ml of diluted hydrochloric acid (1 in 4), and dissolve by boiling for 1 minute. Cool, and add water to make 50 ml.

Control Solution To 0.40 ml of 0.005 mol/L sulfuric acid, add 1 ml of diluted hydrochloric acid (1 in 4) and water to make 50 ml.

(5) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of Potassium Metaphosphate, and dissolve it in 30 ml of water. If it does not dissolve easily, add 2–3 drops of nitric acid. Neutralize the solution with diluted acetic acid (1 in 20) or ammonia TS, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Measure exactly 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Drying Not more than 5.0% (110°C, 4 hours).

Assay Proceed as directed in the Assay for Potassium Polyphosphate.

Potassium Nitrate

硝酸カリウム

KNO_3 Mol. Wt. 101.10

Potassium nitrate [7757-79-1]

Content Potassium Nitrate, when dried, contains not less than 99.0% of potassium nitrate (KNO_3).

Description Potassium Nitrate occurs as colorless prismatic crystals or as a white crystalline powder. It is odorless and has a salty and cool taste.

Identification Potassium Nitrate responds to all tests for Potassium Salt and for Nitrate in the Qualitative Tests.

Purity

(1) Clarity and color of solution Colorless and clear (1.0 g, water 10 ml).

(2) Chloride Not more than 0.021% as Cl (0.50 g, Control solution 0.01 mol/L hydrochloric acid 0.30 ml).

(3) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.50 g of Potassium Nitrate, dissolve it in 3 ml of water, add 2 ml of sulfuric acid, and heat until white fumes are evolved. Then add a small amount of water to dissolve, heat until white fumes are evolved, cool, and add 5 ml of water to dissolve.

Apparatus Use Apparatus B.

Loss on Drying Not more than 1.0% (105°C, 4 hours).

Assay Weigh accurately about 0.4 g of Potassium Nitrate, previously dried, into a 500-ml round-bottom flask, and dissolve it in about 300 ml of water. Add 3 g of powdered Devarda's alloy and 15 ml of sodium hydroxide solution (2 in 5). Connect the flask immediately to the distillation apparatus that is fit to a spray trap and a receiver with a condenser, containing exactly 50 ml of 0.05 mol/L sulfuric acid. Allow to stand for 2 hours, and distill until about 250 ml of the distillate is obtained. Titrate the excess acid with 0.1 mol/L sodium hydroxide (indicator: 3 drops of methyl red–methylene blue mixture TS). Perform a blank test in the same manner.

Each ml of 0.05 mol/L sulfuric acid = 10.11 mg of KNO_3

Potassium Polyphosphate

Potassium Tripolyphosphate
Pentapotassium Triphosphate

ポリリン酸カリウム

Content Potassium Polyphosphate, when dried, contains the equivalent of 43.0–76.0% of phosphorus(V) oxide (P_2O_5 = 141.94).

Description Potassium Polyphosphate occurs as white fibrous crystals or powder or as colorless to white glassy flakes or lumps.

Identification

(1) To 0.1 g of Potassium Polyphosphate, add 0.4 g of sodium acetate and 10 ml of water to dissolve the sample, add diluted acetic acid (1 in 20) to make the solution slightly acidic, and add 3 ml of silver nitrate solution (1 in 50). A white precipitate is formed.

(2) Potassium Polyphosphate responds to all tests for Potassium Salt in the Qualitative Tests.

Purity

(1) Clarity and color of solution Colorless and slightly turbid (1.0 g, sodium acetate 4.0 g and water 100 ml).

(2) Chloride Not more than 0.11% as Cl (0.10 g, Control solution 0.01 mol/L hydrochloric acid 0.30 ml).

(3) Orthophosphate Weigh 1.0 g of Potassium Polyphosphate, and add 2–3 drops of silver nitrate solution (1 in 50). No brilliant yellow color develops.

(4) Sulfate Not more than 0.096% as SO_4.

Test Solution Weigh 0.20 g of Potassium Polyphosphate, and add 30 ml of water and 2 ml of diluted hydrochloric acid (1 in 4), and dissolve by boiling for 1 minute. Cool, and add water to make 50 ml.

Control Solution To 0.40 ml of 0.005 mol/L sulfuric acid, add 1 ml of diluted hydrochloric acid (1 in 4) and water to make 50 ml.

(5) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of Potassium Polyphosphate, dissolve by adding 30 ml of water and 3–4 drops of nitric acid, neutralize with diluted acetic acid (1 in 20) or ammonia TS, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution To 2.0 ml of Lead Standard Solution, add 2 ml of diluted hydrochloric acid (1 in 20) and water to

make 50 ml.

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Drying Not more than 5.0% (110°C, 4 hours).

Assay

Test Solution Weigh accurately about 0.2 g of Potassium Polyphosphate, previously dried, dissolve by adding 5 ml of nitric acid and 25 ml of water, boil for 30 minutes while replenishing the lost water, and cool. Add water to make exactly 500 ml, and filter through a dry filter paper if necessary.

Procedure Measure exactly 5 ml of the test solution, add 20 ml of vanadic acid–molybdic acid TS and water to make exactly 100 ml, shake well, and allow to stand for 30 minutes. Measure the absorbance of this solution at a wavelength of 400 nm against a reference solution prepared in the same manner as for the test solution, using 5 ml of water.

Separately, measure exactly 10 ml of Monopotassium Phosphate Standard Solution, add 20 ml of diluted nitric acid (1 in 25), and add water to make exactly 250 ml. Measure exactly 10 ml, 15 ml, and 20 ml, respectively, of this solution, and proceed in the same manner as for the test solution. Measure the absorbance of each solution, and prepare a calibration curve.

Determine the weight (g) of phosphorus (P) in 5 ml of the test solution from the calibration curve and the absorbance of the test solution, and calculate the content of phosphorus(V) oxide (P_2O_5) by the formula:

$$\text{Content (\%) of phosphorus (V) oxide } (P_2O_5) = \frac{\left(\begin{array}{c}\text{Weight (g) of phosphorus (P)}\\ \text{in 5 ml of the test solution}\end{array}\right) \times 2.291 \times 100}{\text{Weight (g) of the sample}} \times 100$$

Potassium Pyrophosphate

Tetrapotassium Pyrophosphate
Tetrapotassium Diphosphate

ピロリン酸四カリウム

K_4P_2O7 Mol. Wt. 330.34

Potassium diphosphate [7320-34-5]

Content Potassium Pyrophosphate, when dried, contains 98.0–101.0% of potassium pyrophosphate ($K_4P_2O_7$).

Description Potassium Pyrophosphate occurs as a colorless to white crystalline powder or lumps or as a white powder.

Identification

(1) Dissolve 0.1 g of Potassium Pyrophosphate by adding 10 ml of water and 2–3 drops of nitric acid, and add 1 ml of silver nitrate solution (1 in 50). A white precipitate is formed.

(2) Potassium Pyrophosphate responds to all tests for Potassium Salt in the Qualitative Tests.

Purity

(1) Clarity and color of solution Colorless and slightly turbid (0.50 g, water 20 ml).

(2) pH 10.0–10.7 (1.0 g, water 100 ml).

(3) Chloride Not more than 0.011% as Cl (1.0 g, Control solution 0.01 mol/L hydrochloric acid 0.30 ml).

(4) Orthophosphate Weigh 1.0 g of Potassium Pyrophosphate, and add 2–3 drops of silver nitrate solution (1 in 50). No brilliant yellow color develops.

(5) Sulfate Not more than 0.019% as SO_4 (1.0 g, Control solution 0.005 mol/L sulfuric acid 0.40 ml).

(6) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of Potassium Pyrophosphate, dissolve by adding 30 ml of water and 3–4 drops of nitric acid, neutralize with diluted acetic acid (1 in 20) or ammonia TS, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution To 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(7) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Drying Not more than 7.0% (110°C, 4 hours).

Assay Weigh accurately about 3 g of Potassium Pyrophosphate, previously dried, dissolve it in 75 ml of water, keep at about 15°C, and titrate with 1 mol/L hydrochloric acid (indicator: 3–4 drops of methyl orange–xylene cyanol FF TS).

Each ml of 0.05 mol/L hydrochloric acid = 165.2 mg of $K_4P_2O_7$

Potassium Pyrosulfite

Potassium Metabisulfite

ピロ亜硫酸カリウム

$K_2S_2O_5$ Mol. Wt. 222.33

Potassium disulfite [16731-55-8]

Content Potassium Pyrosulfite contains not less than 93.0% of potassium pyrosulfite ($K_2S_2O_5$).

Description Potassium Pyrosulfite occurs as white crystals or crystalline powder having an odor of sulfur dioxide.

Identification Potassium Pyrosulfite responds to all tests for Potassium Salt and for Sulfite in the Qualitative Tests.

Purity

(1) Clarity of solution Almost clear (1.0 g, water 10 ml).

(2) Heavy metals Not more than 10 μg/g as Pb.

Test Solution Weigh 2.0 g of Potassium Pyrosulfite, dissolve it in 15 ml of hot water, add 5 ml of hydrochloric acid, and evaporate to dryness on a water bath. To the residue, add 10 ml of hot water and 2 ml of hydrochloric acid, and evaporate to dryness on a water bath again. Dissolve this residue by adding 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml, and filter if necessary.

Control Solution To 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(3) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 5.0 g of Potassium Pyrosulfite, and dissolve it in water to make 25 ml. Measure 5 ml of this solution, add 1 ml of sulfuric acid, evaporate to about 2 ml, and add water to make 10 ml. Use 5 ml of this solution as the test solution.

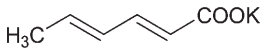
Apparatus Use Apparatus B.

Assay Weigh accurately about 0.2 g of Potassium Pyrosulfite, and proceed as directed under Sulfite Determination.

Each ml of 0.05 mol/L iodine = 5.558 mg of $K_2S_2O_5$

Potassium Sorbate

ソルビン酸カリウム



$C_6H_7KO_2$ Mol. Wt. 150.22

Monopotassium (2*E*,4*E*)-hexa-2,4-dienoate [24634-61-5]

Content Potassium Sorbate, when dried, contains 98.0–102.0% of potassium sorbate ($C_6H_7KO_2$).

Description Potassium Sorbate occurs as white to light yellow-brown, flaky crystals, crystalline powder, or granules. It is odorless or has a slight odor.

Identification

(1) To a solution of Potassium Sorbate (1 in 100), add 1 ml of acetone. Add diluted hydrochloric acid (1 in 4) dropwise to make the solution slightly acidic, add 2 drops of bromine TS, and shake. The color of the solution disappears immediately.

(2) Potassium Sorbate responds to all tests for Potassium Salt in the Qualitative Tests.

Purity

(1) Clarity and color of solution Weigh 0.20 g of Potassium Sorbate, and dissolve it in 5.0 ml of water. The solution is not darker in color than Matching Fluid F.

(2) Free alkali Weigh 1.0 g of Potassium Sorbate, and dissolve it in 20 ml of freshly boiled and cooled water. When 2 drops of phenolphthalein TS are added, a pink color develops. The color disappears on the addition of 0.40 ml of 0.05 mol/L sulfuric acid.

(3) Chloride Not more than 0.018% as Cl.

Test Solution Weigh 1.0 g of Potassium Sorbate, dissolve it in about 30 ml of water, and add 11 ml of diluted nitric acid (1 in 10) while shaking well. Filter, wash with water, combine the filtrate and the washings, and add water to make 50 ml.

Control Solution To 0.50 ml of 0.01 mol/L hydrochloric acid, add 6 ml of diluted nitric acid (1 in 10) and water to make 50 ml.

(4) Sulfate Not more than 0.038% as SO_4.

Test Solution Weigh 0.50 g of Potassium Sorbate, dissolve it in about 30 ml of water, and add 3 ml of diluted hydrochloric acid (1 in 4) while shaking well. Filter, wash with water, combine the filtrate and the washings, and add water to make 50 ml.

Control Solution To 0.40 ml of 0.005 mol/L sulfuric acid, add 1 ml of diluted hydrochloric acid (1 in 4) and water to make 50 ml.

(5) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Drying Not more than 1.0% (105°C, 3 hours).

Assay Weigh accurately about 0.3 g of Potassium Sorbate, previously dried, add 50 ml of acetic acid for nonaqueous titration, and titrate with 0.1 mol/L perchloric acid (indicator: 10 drops of α-naphtholbenzein TS) until the brown color of the solution changes to green.

Each ml of 0.1 mol/L perchloric acid = 15.02 mg of $C_6H_7KO_2$

Powdered Cellulose

粉末セルロース

Definition Powdered Cellulose is obtained by decomposing pulp and consists mainly of cellulose.

Description Powdered Cellulose occurs as a white powder. It is odorless.

Identification

(1) To 10 g of Powdered Cellulose, add 290 ml of water, and mix in a high-speed (12,000 rpm or more) power blender for 5 minutes. Transfer 100 ml of the mixture to a 100-ml measuring cylinder, and allow to stand for 1 hour. The suspension separates into a clear or white supernatant liquid and a precipitate.

(2) Determine the absorption spectrum of Powdered Cellulose, previously dried, as directed in the Potassium Bromide Disk Method under the Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) pH 5.0–7.5.

Weigh accurately 10.0 g of Powdered Cellulose, add 90 ml of water, allow to stand with occasional stirring for 1 hour, and centrifuge. Use the supernatant liquid for measurement.

(2) Water-soluble substances Not more than 1.5%.

Weigh accurately about 6 g of Powdered Cellulose, previously dried, add 90 ml of water freshly boiled and cooled. Allow to stand for 10 minutes with occasional stirring, filter though a glass filter (1G4), and discard the initial 10 ml of filtrate. If necessary, filter again, using the same filter to obtain a clear filtrate. Place 15 ml of the filtrate in an evaporation dish, previously dried and weighed, heat on a water bath, taking care not to scorch it, and evaporate to dryness. Dry the residue at 105°C for 1 hour, and accurately weigh the evaporation dish containing the residue. Separately, perform blank test for correction.

(3) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 2, Apparatus B).

(5) Starch To 20 ml of the liquid obtained in Identification (1), add a few drops of iodine TS, and mix. No bluish purple or blue color develops.

Loss on drying Not more than 10.0% (105°C, 3 hours).

Ash Not more than 0.30% (about 800°C, 2 hours).

Preparations of Tar Colors

タール色素の製剤

Identification To preparations of the tar colors given in Column 1 in the following table, apply the corresponding procedures specified in Column 2 in the table. Perform the test as directed under Other Coloring Matters in the Coloring Matter Tests, and compare the spots obtained between each sample and the corresponding reference standard.

Purity

(1) Chromium

When the content of the coloring matter(s) is more than 50%: Not more than 50 μg/g as Cr.

When the content of the coloring matter(s) is not more than 50%: Not more than 25 μg/g as Cr.

Apply this test to the preparation containing Food Red No. 106, Food Green No. 3, or Food Blue No. 1.

Sample Solution and Blank Test Solution Use 5.0 ml each of the sample solution and blank test solution prepared in Purity (2) below.

Procedure Proceed as directed in Heavy Metals (2) in the

Column 1	Column 2
Food Red No. 2 Food Red No. 3 Food Red No. 40 Food Red No. 102 Food Red No. 104 Food Red No. 105 Food Yellow No. 4 Food Yellow No. 5 Food Blue No. 2	*Test Solution* A solution of the sample equivalent to 0.1% of the tar color under test (when an insoluble matter remains, centrifuge at 3,000–3,500 rpm to remove it). *Procedure* Develop as directed in Other Coloring Matters (1) in the Coloring Matter Tests. If the tar color cannot be separated well, proceed as directed in Other Coloring Matters (2).
Food Red No. 106	*Test Solution* A solution of the sample equivalent to 0.03% of the tar color under test (when an insoluble matter remains, centrifuge by 3,000–3,500 rpm to remove it). *Procedure* Develop as directed in Other Coloring Matters (1) in the Coloring Matter Tests. If the tar color cannot be separated well, proceed as directed in Other Coloring Matters (2).
Food Green No. 3 Food Blue No. 1	*Test Solution* A solution of the sample equivalent to 0.05% of the tar color under test (when an insoluble matter remains, centrifuge by 3,000–3,500 rpm to remove it). *Procedure* Develop as directed in Other Coloring Matters (1) in the Coloring Matter Tests. If the tar color cannot be separated well, proceed as directed in Other Coloring Matters (2).
Food Red No. 2 Aluminum Lake Food Red No. 40 Aluminum Lake Food Yellow No. 4 Aluminum Lake Food Yellow No. 5 Aluminum Lake Food Green No. 3 Aluminum Lake Food Blue No. 1 Aluminum Lake	*Test Sample* Weigh a quantity of the sample equivalent to 0.5 g of the tar color aluminum lake under test into a centrifuge tube, add 50 ml of water, shake well, and centrifuge for about 10 minutes at 3,000–3,500 rpm. Remove the supernatant, add 50 ml of water to the residue, shake well, and centrifuge again. Repeat this procedure three times. Use the residue as the test sample. *Procedure* Develop as directed in Other Coloring Matter Lakes (1) in the Coloring Matter Aluminum Lake Tests. If the tar color cannot be separated well, proceed as directed in Other Coloring Matter Lakes (2).
Food Red No. 3 Aluminum Lake	*Test Sample* Weigh a quantity of the sample equivalent to 0.5 g of the tar color aluminum lake under test into a centrifuge tube, add 50 ml of water, shake well, and centrifuge for about 10 minutes at 3,000–3,500 rpm. Remove the supernatant, add 50 ml of water to the residue, shake well, and centrifuge again. Repeat this procedure three times. Use the residue as the test sample. *Procedure* Develop as directed in Other Coloring Matter Lakes (2) in the Coloring Matter Aluminum Lake Tests.
Food Blue No. 2 Aluminum Lake	*Test Sample* Weigh a quantity of the sample equivalent to 0.5 g of the tar color aluminum lake under test into a centrifuge tube, add 50 ml of water, shake well, and centrifuge for about 10 minutes at 3,000–3,500 rpm. Remove the supernatant, add 50 ml of water to the residue, shake well, and centrifuge again. Repeat this procedure three times. Use the residue as the test sample. *Procedure* Develop as directed in Other Coloring Matter Lakes (4) in the Coloring Matter Aluminum Lake Tests. If the tar color cannot be separated well, proceed as directed in Other Coloring Matter Lakes (2).

Coloring Matter Tests (if the preparation under test contains not more than 50% of the tar color(s), use 10.0 ml each of the solutions).

(2) Heavy metals Not more than 20 μg/g as Pb.

In the case of the Preparation of Tar Color(s) not containing tar color aluminum lakes, proceed as directed in test (5) for Heavy Metals in the Coloring Matter Tests. In the case of the Preparation of Tar Color(s) containing tar color aluminum lakes, proceed as directed in test (3) for Heavy Metals in the Coloring Matter Aluminum Lake Tests.

(3) Arsenic Not more than 4.0 μg/g as As_2O_3.

In the case of the Preparation of Tar Color(s) not containing tar color aluminum lakes, proceed as directed in the test for Arsenic in the Coloring Matter Tests. In the case of the Preparation of Tar Color(s) containing tar color aluminum lakes, proceed as directed in the test for Arsenic in the Coloring Matter Aluminum Lake Tests.

(4) Manganese

When the content of the coloring matter is more than 50%: Not more than 50 μg/g as Mn.

When the content of the coloring matter is not more than 50%: Not more than 25 μg/g as Mn.

Apply this test to the preparation containing Food Red No. 106, Food Green No. 3, or Food Blue No. 1.

Sample Solution and Blank Test Solution Use 4.0 ml each of the sample solution and the blank test solution prepared in Purity (2) above.

Procedure Proceed as directed in test (4) for Heavy Metals in the Coloring Matter Tests (if the content of the coloring matter is not more than 50%, use 8.0 ml each of the solutions).

Processed Eucheuma Algae

Processed Red Algae
Semirefined Carrageenan

加工ユーケマ藻類

Definition Processed Eucheuma Algae is one of carrageenans. "Carrageenan"* is defined as a substance that is obtained from the whole algae of the genus *Hypnea*, *Eucheuma*, *Iridaea*, *Gigartina,* or *Chondrus* and that consists mainly of ι-carrageenan, κ-carrageenan, and λ-carrageenan.

Description Processed Eucheuma Algae occurs as a white to light-brown powder or granules. It has no or slight odor.

Identification

(1) To 4 g of Processed Eucheuma Algae, add 200 ml of water. Keep at 80°C in a water bath while stirring to make a homogeneous viscous liquid. Replenish the lost water, and cool to room temperature. A viscous solution or gel is formed.

(2) To 20 ml of water, add 0.1 g of Processed Eucheuma Algae and 5 ml of diluted hydrochloric acid (1 in 5), boil for 5 minutes, and remove the precipitate if necessary. When 3 ml of barium chloride solution (3 in 25) is added to the resulting liquid, a white turbidity or white crystalline precipitate is formed.

Purity

(1) Viscosity Not less than 5.0 mPa·s.

Weigh 7.5 g of Processed Eucheuma Algae on the dried basis, add 450 ml of water, agitate for 10–20 minutes to make a suspension, and add water to make the content 500 g. Heat to 80°C in a water bath while agitating continuously. Replenish the water lost by evaporation, measure the viscosity at 75°C as directed in Method 2 for Viscosity in the General Tests. To a viscometer, fit rotor No.1 and an adapter, heated to about 75°C, and sink the rotor into the specified position. Start the measurement at 30 rpm, and take the readings after 6 rounds (12 seconds). If the viscosity is too low, use an adapter for low viscosity, and if too high, use rotor No. 2.

(2) Calcium Not more than 1.5 %.

Test Solution Weigh accurately about 10 g of Processed Eucheuma Algae, previously dried, and transfer into a crucible. Heat gently to carbonize, and incinerate at 400–500°C for about 5 hours. Add 10 ml of water and 5 ml of 1 mol/L nitric acid to the incinerated ash, and boil for 3 minutes. Filter it, and add water to make exactly 50 ml. Measure exactly 1 ml of the solution, add 1 ml of 1 mol/L nitric acid, and add water to make exactly 100 ml.

Standard Solution Weigh exactly 2.497 g of calcium carbonate, previously dried at 180°C for 1 hour, add 20 ml of diluted hydrochloric acid (1 in 4) to dissolve, and add water to make 1,000 ml exactly. Measure exactly a suitable amount of this solution, and dilute by 1 mol/L nitric acid to make a solution containing exactly 1–3 μg of calcium (Ca = 40.08) per ml.

Procedure Perform the tests on the test solution and standard solution as directed under Flame Atomic Absorption Spectrophotometry using the operating conditions below. Determine the calcium amount in test solution from the calibration curve prepared from the standard solution.

Operating Conditions

Light Source: Calcium hollow cathode lamp.
Wavelength: 422.7 nm.
Supporting gas: Air.
Combustible gas: Acetylene.

(3) Sodium Not more than 1.0 %.

Test Solution Weigh accurately about 1 g of Processed Eucheuma Algae, previously dried, and transfer into a crucible. Heat gently to carbonize, and incinerate at 400–500°C for about 5 hours. To the ash obtained, add 5 ml of 3 mol/L hydrochloric acid to disperse, and boil for 3 minutes. Using a little amount of 3 mol/L hydrochloric acid, wash out the contents in the crucible completely into a chromatography column (70 mm in height and 12 mm in inner diameter), stuffed with glass fiber and attached to a 50-ml volumetric flask as a receiver. Elute with 3 mol/L hydrochloric acid to gain about 45 ml of eluate, and add water to make exactly 50 ml. Measure exactly 2 ml of this solution, and add 0.02 mol/L hydrochloric acid to make exactly 500 ml.

Standard Solution Weigh exactly 0.2542 g of sodium chloride, previously dried at 130°C for 2 hour, and dissolve it in 0.02 mol/L hydrochloric acid to make exactly 1,000 ml. Measure exactly a suitable amount of this solution, and dilute with 0.02 mol/L hydrochloric acid to make a solution containing exactly 1–3 μg of sodium (Na = 22.99) per ml.

Procedure Perform the tests on the test solution and the standard solution as directed under Flame Atomic Absorp-

* "Carrageenan" is defined in the List of Existing Food Additives.

tion Spectrophotometry using the operating conditions below. Determine the sodium amount in the test solution from the calibration curve prepared from the standard solution.

Operating Conditions

Light Source: Sodium hollow cathode lamp.

Wavelength: 589.0 nm.

Supporting gas: Air.

Combustible gas: Acetylene.

(4) Sulfate 15–40 % (on the dried basis).

Weigh accurately about 1 g of Processed Eucheuma Algae, and transfer into a 100-ml Kjeldahl flask. Add 50 ml of diluted hydrochloric acid (1 in 10), attach a reflux condenser, and boil for 1 hour. Add 25 ml of 10% (vol) hydrogen peroxide solution, and boil for 5 hours. Filter the separate solution if necessary, and transfer the filtrate into a 500-ml beaker. Add gradually 10 ml of barium chloride solution (3 in 25) while boiling. Heat for 2 hours in a water bath, and cool. Filter using filter paper for the quantitative analysis (5C), and wash the residue on the filter paper with warm water until the washings are free of chlorides. Dry the residue with the filter paper, and place into a porcelain crucible. Incinerate the content to white ash, weigh as barium sulfate. Calculate the content of Sulfate (SO_4) by the formula, and determine on the dried basis.

$$\text{Content (\%) of sulfate } (SO_4) = \frac{\left(\text{Weight (g) of barium sulfate}\right) \times 0.4116}{\text{Weight (g) of the sample}} \times 100$$

(5) Acid-insoluble substances 8–18%.

Weigh accurately about 2 g of Processed Eucheuma Algae, and transfer into a 300-ml beaker containing 150 ml of water and 1.5 ml of sulfuric acid. Cover the beaker with a watch glass, and heat for 6 hours in a water bath. Occasionally, rub down the adhered matter on the wall of the beaker with a glass rod, and wash down with water to replenish the water lost by evaporation. Weigh accurately about 0.5 g of diatomaceous earth for chromatography, dried for 3 hours at 105°C, add to the sample solution, and mix well. Weigh a glass filter (1G3), dried for 3 hours at 105°C. Filter, with suction, the mixture of diatomaceous earth and the sample solution, using the glass filter, and wash down the residue into the glass filter with warm water. Dry the glass filter with the residue for 3 hours at 105°C. Allow to cool in the desiccator, and measure the total weight. Calculate the amount of the acid-insoluble substances by the formula:

$$\text{Acid-insoluble substances (\%)} = \frac{\text{Total weight (g)} - \left[\left(\text{Weight (g) of diatomaceous earth}\right) + \left(\text{Weight (g) of the glass filter}\right)\right]}{\text{Weight (g) of the sample}} \times 100$$

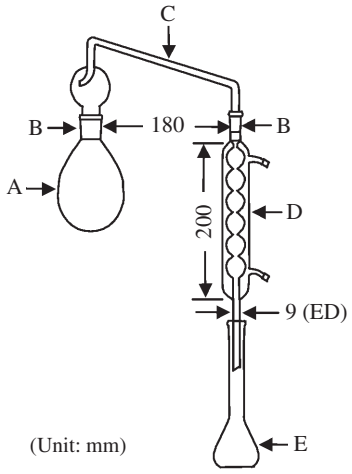
(6) Heavy metals Not more than 40 µg/g as Pb (0.50 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(7) Lead Not more than 5.0 µg/g as Pb (2.0 g, Method 1).

(8) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(9) Total amount of 2-propanol and methanol Not more than 0.10%.

(i) Apparatus Use the apparatus illustrated in the right column.



A: Eggplant-shaped flask (300 ml)
B: Ground glass joint
C: Delivery tube with a spray trap
D: Condenser
E: Volumetric flask (100 ml)

(ii) Method

Test Solution Weigh accurately about 2 g of Processed Eucheuma Algae in eggplant-shaped flask A, add 200 ml of water, a few boiling chips, and 1 ml of silicon resin, and stir well. Place exactly 4 ml of internal standard solution in volumetric flask E, and set up the apparatus. Moisten the joint parts with water. Distill it at a rate of 2 to 3 ml/minute, being careful not to allow bubbles to come in delivery tube C, and collect about 90 ml of distillate. To the distillate, add water to make exactly 100 ml. Use *tert*-butanol solution (1 in 1,000) as the internal standard solution.

Standard Solution Weigh accurately about 0.5 g each of 2-propanol and methanol, and add water to make exactly 50 ml. Measure exactly 5 ml of this solution, and add water to make exactly 50 ml. Then measure exactly 2 ml of the second solution and 4 ml of the internal standard solution in a 100-ml volumetric flask, and add water to volume.

Procedure Analyze 2.0 µl portions of the test solution and the standard solution by gas chromatography using the operating conditions below. Determine the peak area ratios of each of 2-propanol and methanol to *tert*-butanol for each solution, and express as Q_{T1} and Q_{T2} for the test solution and as Q_{S1} and Q_{S2} for the standards solution. Calculate each content by the following formulae, and obtain the sum of both substances.

$$\text{Content (\%) of 2-propanol} = \frac{\text{Weight (g) of 2-propanol}}{\text{Weight (g) of the sample}} \times \frac{Q_{T1}}{Q_{S1}} \times 0.4$$

$$\text{Content (\%) of methanol} = \frac{\text{Weight (g) of methanol}}{\text{Weight (g) of the sample}} \times \frac{Q_{T2}}{Q_{S2}} \times 0.4$$

Operating Conditions

Detector: Flame-ionization detector.

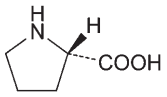
Column: A glass tube of 3 mm internal diameter and 2 m length.

Column packing material: 180- to 250-μm styrene-divinylbenzene porous polymer for gas chromatography.
Column temperature: A constant temperature at about 120°C.
Injection port: A constant temperature at about 200°C.
Carrier gas: Nitrogen or helium.
Flow rate: Adjust so that the retention times of methanol and for 2-propanol are about 2 minutes and about 10 minutes, respectively.

Loss on Drying Not more than 12.0% (105°C, 4 hours).
Ash 15.0–35.0% (on the dried basis).
Acid-insoluble Ash Not more than 2.0% (on the dried basis).
Microbial Limits Proceed as directed in the Microbial Limit Tests. The total bacterial count is not more than 10,000/g, and *Escherichia coli* is negative.

L-Proline

L-プロリン



$C_5H_9NO_2$ Mol. Wt. 115.13
(2*S*)-Pyrrolidine-2-carboxylic acid [147-85-3]
Content L-Proline, when calculated on the dried basis, contains 98.0–102.0% of L-proline ($C_5H_9NO_2$).
Description L-Proline occurs as white crystals or crystalline powder. It is odorless, or has a very slight characteristic odor, and has a very slight sweet taste.
Identification
(1) To 5 ml of a solution of L-Proline (1 in 1,000), add 1 ml of ninhydrin solution (1 in 50), and heat for 1 minute in a water bath. A yellow color develops.
(2) To 1 ml of a solution of L-Proline (1 in 500), add 1 ml of sodium carbonate solution (1 in 50), 1 ml of sodium nitroprusside solution (1 in 100), and 1 ml of acetaldehyde solution (1 in 10). A blue color develops.
Purity
(1) Specific rotation $[\alpha]_D^{20}$: – 84.0 to – 86.0°.
Weigh accurately about 4 g of L-Proline, and dissolve it in water to make exactly 100 ml. Measure the angular rotation of this solution, and calculate on the dried basis.
(2) Clarity and color of solution Colorless and clear (1.0 g, water 10 ml).
(3) pH 5.9–6.9 (1.0 g, water 10 ml).
(4) Chloride Not more than 0.1% as Cl (0.07 g, Control solution 0.01 mol/L hydrochloric acid 0.20 ml).
(5) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).
(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).
Loss on Drying Not more than 0.30% (105°C, 3 hours).
Residue on Ignition Not more than 0.10%.
Assay Weigh accurately about 0.25 g of L-Proline and proceed as directed in the Assay for L-Asparagine.

Each ml of 0.1 mol/L perchloric acid = 11.51 mg of $C_5H_9NO_2$

L-Proline Solution

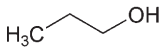
L-プロリン液

Content L-Proline Solution contains not more than 50% of L-proline ($C_5H_9NO_2$ = 115.13) and 95.0–110.0% of the labeled content.
Description L-Proline Solution is a colorless liquid. It is odorless or has a very slight characteristic odor, and has a very slight sweet taste.
Identification
(1) To 5 ml of diluted L-Proline Solution (1 in 200), add 1 ml of ninhydrin solution (1 in 50), and heat for 1 minute in a water bath. A yellow color develops.
(2) To 4 g of L-Proline Solution, add 100 ml of water, and mix. It shows levorotatory.
Purity
(1) Heavy metals Not more than 20 μg/g of L-proline ($C_5H_9NO_2$) as Pb.
Test Solution Weigh an amount of L-Proline Solution equivalent to 1.0 g of L-proline ($C_5H_9NO_2$), add 40 ml of water, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.
Control Solution To 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.
(2) Arsenic Not more than 4.0 μg/g of L-proline ($C_5H_9NO_2$) as As_2O_3.
Test Solution Weigh an amount of L-Proline Solution equivalent to 0.50 g of L-proline ($C_5H_9NO_2$), add 5 ml of water, and dissolve by heating if necessary.
Apparatus Use Apparatus B.
Residue on Ignition Not more than 0.10% on the basis of L-proline ($C_5H_9NO_2$).
Assay Weigh accurately an amount of L-Proline Solution equivalent to about 0.25 g of L-proline ($C_5H_9NO_2$), and proceed as directed in the Assay for L-Asparagine.
Each ml of 0.1 mol/L perchloric acid = 11.51 mg of $C_5H_9NO_2$

Propanol

Propyl Alcohol

プロパノール



C_3H_8O Mol. Wt. 60.09

Propan-1-ol [71-23-8]

Content Propanol contains not lass than 99.0% of propanol (C_3H_8O).

Description Propanol is a colorless, transparent liquid having a characteristic odor.

Identification Determine the absorption spectrum of Propanol as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

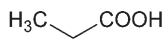
(1) Refractive index n_D^{20}: 1.383–1.388.

(2) Specific gravity d_{25}^{25}: 0.800–0.805.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay in Flavoring Agents in the Flavor Substances Tests, using operating conditions (2).

Propionic Acid

プロピオン酸



$C_3H_6O_2$ Mol. Wt. 74.08

Propanoic acid [79-09-4]

Content Propionic Acid contains not less than 99.5% of propionic acid ($C_3H_6O_2$).

Description Propionic Acid is an oily, clear liquid having a characteristic odor.

Identification To 1 ml of Propionic Acid, add 3 drops of sulfuric acid and 1 ml of ethanol, and heat. An aroma is evolved.

Purity

(1) Specific gravity 0.993–0.997.

(2) Distillation test Not less than 95% (vol) is distilled at 138.5–142.5°C (Method 2).

(3) Heavy metals Not more than 10 μg/ml as Pb.

Test Solution Measure 2.0 ml of Propionic Acid, neutralize with 10 ml of water and ammonia TS, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution To 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.5 ml, Method 1, Apparatus B).

(5) Aldehyde Not more than 0.2% as propionic aldehyde.

Measure 10 ml of Propionic Acid into a 250-ml Erlenmeyer flask with a ground-glass stopper containing 50 ml of water and 10 ml of sodium hydrogen sulfite solution (1 in 80), stopper, shake vigorously, allow to stand for 30 minutes, and titrate with 0.05 mol/L iodine until the color of the solution changes to yellow-brown. The volume of the iodine consumed is not more than 7 ml. Perform a blank test in the same manner, and make any necessary correction.

(6) Residue on evaporation Not more than 0.01%.

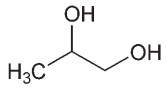
Weigh 20 g of Propionic Acid, evaporate at 140°C to constant weight, and weigh the residue.

Assay Weigh accurately about 3 g of Propionic Acid, dissolve it in 40 ml of freshly boiled and cooled water, and titrate with 1 mol/L sodium hydroxide (indicator: 2 drops of phenolphthalein TS).

Each ml of 1 mol/L sodium hydroxide = 74.08 mg of $C_3H_6O_2$

Propylene Glycol

プロピレングリコール



$C_3H_8O_2$ Mol. Wt. 76.09

Propane-1,2-diol [57-55-6]

Content Propylene Glycol contains not less than 98.0% of propylene glycol ($C_3H_8O_2$).

Description Propylene Glycol is a colorless, clear, viscous liquid. It is odorless and has a slightly bitter-sweet taste.

Identification

(1) To 1 ml of Propylene Glycol, add 0.5 g of potassium hydrogen sulfate, and heat. A fruity odor is evolved.

(2) With 2–3 drops of Propylene Glycol, mix 0.7 g triphenylchloromethane, add 1 ml of pyridine, heat under a reflux condenser on a water bath for an hour, and cool. Add 20 ml of acetone, and dissolve by heating. Add 0.02 g of active carbon, stir, and filter. Concentrate the filtrate to about 10 ml, and cool. Collect the deposited crystals by filtration, and dry for 4 hours in a desiccator. The melting point of the crystals obtained is 174–178°C.

Purity

(1) Specific gravity 1.036–1.040.

(2) Distillation test Not less than 95% (vol) is distilled at 185–189°C (Method 2).

(3) Free acid To 50 ml of water, add 1 ml of phenolphthalein TS, then add sodium hydroxide solution (1 in 2,500) until the pink color of the solution persists for 30 seconds. To the prepared water, add 10 ml of Propylene Glycol, mix, and add 0.20 ml of 0.1 mol/L sodium hydroxide. A pink color persists for not less than 30 seconds.

(4) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g,

Method 1, Apparatus B).
Water Content Not more than 0.20% (10 g, Direct Titration).
Residue on Ignition Not more than 0.05% (10 g).
Assay Weigh accurately about 1 g of Propylene Glycol, and add water to make exactly 250 ml. Measure exactly 10 ml of this solution, transfer into a flask with a ground-glass stopper, add exactly 10 ml of sodium metaperiodate solution TS and 4 ml of diluted sulfuric acid (1 in 2), shake well, and allow to stand for 40 minutes. To this solution, add 5 g of potassium iodide, immediately stopper tightly, shake well, and allow to stand in a dark place for 5 minutes. Titrate with 0.1 mol/L sodium thiosulfate (indicator: 1 ml of starch TS). Perform a blank test in the same manner, and calculate the content by the formula:

$$\text{Content (\%) of propylene glycol } (C_3H_8O_2) = \frac{(a-b)\times 3.805\times 25}{\text{Weight (g) of the sample}\times 1{,}000}\times 100$$

a = volume (ml) of 0.1 mol/L sodium thiosulfate consumed in the blank test,
b = volume (ml) of 0.1 mol/L sodium thiosulfate consumed in the test.

Propylene Glycol Alginate

Propan-1,2-diol Alginate

アルギン酸プロピレングリコールエステル

Description Propylene Glycol Alginate occurs as a white to yellowish white coarse or fine powder. It is almost odorless.
Identification Prepare a test solution as follows: To 1 g of Propylene Glycol Alginate, add 100 ml of water to produce a pasty solution.
(1) To 5 ml of the test solution, add 5 ml of lead acetate TS. It immediately becomes gelled.
(2) To 10 ml of the test solution, add 1 ml of sodium hydroxide solution (1 in 25), heat the mixture in a water bath for 5–6 minutes, cool it, and add 1 ml of diluted sulfuric acid (1 in 20). It immediately becomes gelled.
(3) To 1 ml of the test solution, add 4 ml of water, and shake vigorously. Effervescence persists.
Purity
(1) Esterification value Not less than 40.0%.
Calculate the esterification value of Propylene Glycol Alginate by the formula:

$$\text{Esterification value (\%)} = 100-(a+b+c)$$

a = content (%) of free alginic acid,
b = content (%) of sodium alginate,
c = content (%) of insoluble ash.

Determine a, b, and c as directed in (1)(i), (1)(ii), and (2), respectively.
(i) *Free alginic acid* Weigh accurately about 0.5 g of Propylene Glycol Alginate, previously dried, dissolve it in 200 ml of freshly boiled and cooled water, add 2 drops of phenolphthalein TS, and titrate with 0.02 mol/L sodium hydroxide to the first pink color that persists for about 20 seconds. Calculate the content by the formula below. Perform a blank test in the same manner, and make any necessary correction.

$$\text{Content (\%) of free alginic acid} = \frac{\left(\begin{array}{c}\text{Volume (ml) of 0.02 mol/L}\\ \text{sodium hydroxide consumed}\end{array}\right)\times 0.00352}{\text{Weight (g) of the sample}}\times 100$$

(ii) *Sodium alginate* Weigh accurately about 1 g of Propylene Glycol Alginate, previously dried, transfer into a porcelain or platinum crucible (diameter: 20–30 mm), heat very gently at first, then gradually raise the temperature, and heat at 300–400°C for about 2 hours until completely carbonized. After cooling, crush the carbonized substance with a glass rod, transfer together with the crucible into a beaker, add about 50 ml of water, and then add 20 ml of 0.05 mol/L sulfuric acid. Cover the beaker with a watch glass, heat on a water bath for 1 hour, and filter. If the filtrate is colored, take a new sample, carbonize thoroughly, and repeat the procedure in the same manner. Wash thoroughly with hot water the beaker, the crucible, and the residue on the filter paper until the washings do not turn the litmus paper red, then combine the filtrate and the washings. Titrate the excess sulfuric acid with 0.1 mol/L sodium hydroxide (indicator: 3 drops of methyl red TS) and calculate the content by the formula:

$$\text{Content (\%) of sodium alginate} = \frac{\left(\begin{array}{c}\text{Volume (ml) of 0.05 mol/L}\\ \text{sulfuric acid consumed}\end{array}\right)\times 0.0198}{\text{Weight (g) of the sample}}\times 100$$

(2) Insoluble ash Not more than 1.5%.
Dry the residue on the filter paper obtained under (1)(ii), ignite to constant weight, cool, and weigh accurately.
(3) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).
(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).
Loss on Drying Not more than 20.0% (105°C, 4 hours).

Propylene Glycol Esters of Fatty Acids

Propylene Glycol Mono- and Diesters
Propane-1,2-diol Esters of Fatty Acids

プロピレングリコール脂肪酸エステル

Definition Propylene Glycol Esters of Fatty Acids are esters of fatty acids and propylene glycol, or transesterified substances of fats and oils and propylene glycol.
Description Propylene Glycol Esters of Fatty Acids occur as a white to light yellow-brown powder, flakes, granules, or waxy lumps or as a white to light yellow-brown, viscous liq-

uid. They are odorless or have a slight, characteristic odor.

Identification

(1) To 0.1 g of the sample, add 2 ml of ethanol, dissolve by warming, add 5 ml of diluted sulfuric acid (1 in 20), heat in a water bath for 30 minutes, and cool. Oil drops or white to yellow-white solids are formed. To the separated oil drops or solids, add 3 ml of diethyl ether, and shake. They dissolve.

(2) *Test Solution* To about 5 g of the sample, add 50 ml of ethanolic potassium hydroxide TS, and heat under a reflux condenser in a water bath for 1 hour. Dilute the mixture with methanol (1 in 5), and use the resulting solution as the test solution.

Control Solutions Use both a 9:1 mixture of methanol/propylene glycol and a 9:1 mixture of methanol/glycerol.

Procedure Analyze 5 µl portions of the test solution and the control solutions by thin-layer chromatography using a 9:1 mixture of acetone/water as the developing solvent. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point 15 cm above the original line. Air-dry the plate, and heat at 110°C for 10 minutes to remove the solvent. After cooling, spray with thymol–sulfuric acid TS, and heat at 110°C for 20 minutes to develop a color. A yellow spot is observed at the position corresponding to propylene glycol in the control solution. A yellow-brown spot may be observed at the position corresponding to glycerol in the control solution.

Purity

(1) Acid value Not more than 8.0 (Fats and Related Substances Tests).

(2) Heavy metals Not more than 20 µg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

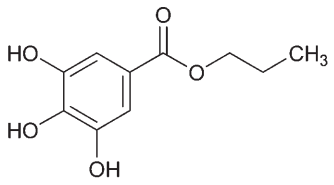
(3) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(4) Polyoxyethylene Proceed as directed in Purity (4) for Sorbitan Esters of Fatty Acids.

Residue on Ignition Not more than 1.5%.

Propyl Gallate

没食子酸プロピル



$C_{10}H_{12}O_5$ Mol. Wt. 212.20

Propyl 3,4,5-trihydroxybenzoate [121-79-9]

Content Propyl Gallate, when dried, contains 98.0–102.0% of propyl gallate ($C_{10}H_{12}O_5$).

Description Propyl Gallate occurs as a white to light brown-yellow crystalline powder. It is odorless and has a slightly bitter taste.

Identification

(1) Dissolve 0.5 g of Propyl Gallate in 10 ml of sodium hydroxide solution (1 in 25), distill, and collect the initial about 4 ml of distillate. The solution is clear, and it emits an odor of propanol when heated.

(2) To 5 ml of a solution of Propyl Gallate in ethanol (1 in 50), add 1 drop of iron(III) chloride solution (1 in 500). A purple color develops.

Purity

(1) Melting point 146–150°C (dried sample).

(2) Clarity and color of solution Weigh 0.50 g of Propyl Gallate, and dissolve it in 10 ml of ethanol. The color of the solution is not darker than that of Matching Fluid C.

(3) Chloride Not more than 0.028% as Cl.

Sample Solution Weigh 1.50 g of Propyl Gallate, add 75 ml of water, warm for 5 minutes at about 70°C, cool to about 20°C, and filter. Use 25 ml of the filtrate as the sample solution.

Control Solution Use 0.40 ml of 0.01 mol/L hydrochloric acid for the preparation.

(4) Sulfate Not more than 0.048% as SO_4.

Sample Solution Use 25 ml of the filtrate obtained in Purity (3) as the sample solution.

Control Solution Use 0.50 ml of 0.005 mol/L sulfuric acid for the preparation.

(5) Heavy metals Not more than 20 µg/g as Pb.

Test Solution To the residue obtained when the residue on ignition test has been performed on Propyl Gallate, add 1 ml of hydrochloric acid and 0.2 ml of nitric acid, and evaporate to dryness on a water bath. To the residue, add 1 ml of diluted hydrochloric acid (1 in 4) and 15 ml of water, dissolve by heating, and cool. Add 1 drop of phenolphthalein TS, and then add ammonia TS dropwise until the color of the solution changes to a slightly pink color. Add water to make 50 ml. Measure 25 ml of the resulting solution, add 2 ml of diluted acetic acid (1 in 20), filter if necessary, and add water to make 50 ml.

Control Solution To 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(6) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Loss on Drying Not more than 1.5% (105°C, 2 hours).

Residue on Ignition Not more than 0.10% .

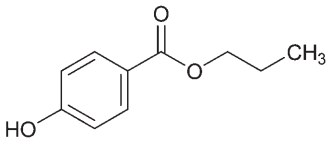
Assay Dry a glass filter (1G4) at 110°C for 30 minutes, allow to cool in a desiccator, and weigh accurately. Weigh accurately about 0.2 g of Propyl Gallate, previously dried, add 150 ml of water, and boil. To the mixture, add 50 ml of bismuth nitrate TS while stirring vigorously, stir for an additional several minutes, and filter the precipitate through the glass filter, previously prepared. Wash twice with two 5-ml portions of diluted nitric acid (1 in 300) cooled in ice, and wash with icy water until the blue litmus paper does not change to red. Dry at 110° for 3 hours, allow to cool in a desiccator, weigh accurately, and calculate the content by the formula:

$$\text{Content (\%) of propyl gallate } (C_{10}H_{12}O_5) = \frac{\text{Weight (g) of the precipitate} \times 0.4865}{\text{Weight (g) of the sample}} \times 100$$

Propyl *p*-Hydroxybenzoate

Propylparaben

パラオキシ安息香酸プロピル



$C_{10}H_{12}O_3$ Mol. Wt. 180.20
Propyl 4-hydroxybenzoate [94-13-3]

Content Propyl *p*-Hydroxybenzoate, when dried, contains not less than 99.0% of propyl *p*-hydroxybenzoate ($C_{10}H_{12}O_3$).

Description Propyl *p*-Hydroxybenzoate occurs as colorless crystals or as a white crystalline powder. It is odorless.

Identification

(1) Proceed as directed in Identification (1) for Butyl *p*-Hydroxybenzoate.

(2) To 0.05 g of Propyl *p*-Hydroxybenzoate, add 2 drops of acetic acid and 5 drops of sulfuric acid, and warm for 5 minutes. An odor of propyl acetate is evolved.

Purity

(1) Melting point 95–98°C.

(2) Free acid Not more than 0.55% as *p*-hydroxybenzoic acid.

Proceed as directed in Purity (2) for Butyl *p*-Hydroxybenzoate.

(3) Sulfate Not more than 0.024% as SO_4.

Proceed as directed in Purity (3) for Butyl *p*-Hydroxybenzoate.

(4) Heavy metals Not more than 10 μg/g as Pb.

Proceed as directed in Purity (4) for Butyl *p*-Hydroxybenzoate.

(5) Arsenic Not more than 4.0 μg/g as As_2O_3.

Proceed as directed in Purity (5) for Butyl *p*-Hydroxybenzoate.

Loss on Drying Not more than 0.50% (5 hours).

Residue on Ignition Not more than 0.05% (5 g).

Assay Proceed as directed in the Assay for Butyl *p*-Hydroxybenzoate.

Each ml of 1 mol/L sodium hydroxide = 180.2 mg of $C_{10}H_{12}O_3$

Psyllium Seed Gum

サイリウムシードガム

Definition Psyllium Seed Gum is obtained from the seed coats of the blonde psyllium *Plantago ovata* Forsskål and consists mainly of polysaccharides. It may contain sucrose, glucose, lactose, dextrin, or maltose.

Description Psyllium Seed Gum occurs as an off-white or light yellow-brown powder or granules. It is odorless or has a slight characteristic odor.

Identification Place 2 g of Psyllium Seed Gum in a 400-ml beaker, add 200 ml of water, and dissolve while stirring at 80°C for 10 minutes. When cooled to room temperature, the solution becomes a characteristic, flowable substance in a partial gel or partial sol state.

Purity

(1) Heavy metals Not more than 40 μg/g as Pb (0.5 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 10 μg/g as Pb (1.0 g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(4) Protein Not more than 2.0%.

Weigh accurately about 1 g of Psyllium Seed Gum, and proceed as directed in the Semi-micro Kjeldahl Method under Nitrogen Determination.

Each ml of 0.005 mol/L sulfuric acid = 0.8754 mg of protein

Loss on Drying Not more than 12.0% (105°C, 5 hours).

Ash Not more than 5.0% (on the dried basis).

Microbial Limits Proceed as directed in the Microbial Limit Tests. The total bacterial count is not more than 10,000/g, and *Escherichia coli* is negative.

Pullulan

プルラン

Definition Pullulan is obtained by isolation from the culture fluid of the filamentous fungus *Aureobasidium pullulans*. It consists of the polysaccharide pullulan.

Description Pullulan occurs as a white to light yellowish white powder. It is odorless or has a slight characteristic odor.

Identification

(1) Dissolve 10 g of Pullulan in 100 ml of water by adding in small amounts while stirring. A viscous solution is produced.

(2) To 10 ml of the solution prepared in Identification (1), add 0.1 ml of pullulanase TS, mix, and allow to stand. It is not viscous.

(3) To 10 ml of a solution of Pullulan (1 in 50), add 2 ml of polyethylene glycol 600. A white precipitate forms immediately.

Purity

(1) Kinematic viscosity 15–180 mm^2/s.

Weigh exactly 10.0 g of Pullulan, previously dried, dissolve it in water, and make exactly 100 g of solution. Measure the viscosity at 30 ± 0.1°C.

(2) Heavy metals Not more than 5.0 μg/g as Pb (4.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(3) Lead Not more than 2.0 μg/g as Pb (1.0 g, Method 1).

(4) Arsenic Not more than 2.0 μg/g as As_2O_3 (1.0 g, Method 3, Apparatus B).

(5) Total nitrogen Not more than 0.05%.

Weigh accurately about 3 g of Pullulan, and determine the total nitrogen by the Semi-micro Kjeldahl Method. Use 12 ml of sulfuric acid to decompose the sample. The amount of sodium hydroxide solution added in the distillation procedure is 40 ml.

(6) Mono- and oligo-saccharides Not more than 12.0%.

Test Solution Dissolve accurately 0.800 g of Pullulan, dried previously, in 100 ml of water, and use this solution as the sample stock solution. Place 1 ml of the stock solution in a centrifuge tube, add 0.1 ml of saturated potassium chloride solution and 3 ml of methanol, mix vigorously, and centrifuge. Use the supernatant as the sample solution. Gently add exactly 0.2 ml of the sample solution to 5 ml of a solution (1 in 500) of anthrone in diluted sulfuric acid (3 in 4), previously cooled in icy water, and mix immediately. Warm the mixture at 90ºC for 10 minutes, and then cool immediately.

Standard Solution and Blank Test Solution To exactly 1 ml of the sample stock solution, add water to make exactly 50 ml. With exactly 0.2 ml each of the resulting solution and water in place of 0.2 ml of the sample solution, proceed in the same manner as the preparation of the test solution, and use the solutions obtained as a standard solution and a blank test solution, respectively.

Procedure Measure the absorbances (A_T, A_S, and A_0) of the test solution, standard solution, and blank test solution at a wavelength of 620 nm. Use water as the reference.
Calculate the content by the formula:

$$\text{Content (\%) of mono - and oligo - saccharides} = \frac{A_T - A_0}{A_S - A_0} \times 8.2$$

Loss on Drying Not more than 8.0% (90°C, reduced pressure, 6 hours).
Residue on Ignition Not more than 5.0%.
Microbial Limits Proceed as directed in the Microbial Limit Tests. The total bacterial count is not more than 10,000/g, and *Escherichia coli* is negative.

Purified Carrageenan

Refined Carrageenan

精製カラギナン

Definition Purified Carrageenan is one of carrageenans. "Carrageenan"* is defined as a substance that is obtained from the whole algae of the genus *Hypnea*, *Eucheuma*, *Iridaea*, *Gigartina,* or *Chondrus* and that consists mainly of ι-carrageenan, κ-carrageenan, and λ-carrageenan. It may contain sucrose, glucose, maltose, lactose, or dextrin.
Description Purified Carrageenan occurs as a white to light-brown powder or granules. It has no or slight odor.
Identification

(1) Proceed as directed in Identification (1) for Processed Eucheuma Algae.

(2) To 20 ml of water, add 0.1 g of Purified Carrageenan, then add 3 ml of barium chloride solution (3 in 25) and 5 ml of diluted hydrochloric acid (1 in 5), stir well, and remove the precipitate if necessary. Boil the solution for 5 minutes, and a white crystalline precipitate is formed.

* "Carrageenan" is defined in the List of Existing Food Additives.

Purity

(1) Viscosity Not less than 5.0 mPa·s.

Proceed as directed in Purity (1) for Processed Eucheuma Algae.

(2) Sulfate 15–40 % (on the dried basis).

Weigh accurately about 8 g, and disperse in 400 ml of 60% 2-propanol. Stir gently for 4 hours, and filter through a filter paper (5C) for quantitative analysis. Wash the residue on the filter paper four times with two 10-ml portions of 60% 2-propanol and then with two 10-ml portions of 2-propanol. Dry the residue at 105ºC to constant weight, and use as the test sample. Weigh accurately about 1 g of the test sample into a 100-ml Kjeldahl flask, and add 50 ml of diluted hydrochloric acid (1 in 10). Boil under a reflux condenser for 1 hour. Add 25 ml of 10% (vol) hydrogen peroxide solution, and boil for an additional 5 hours. Filter to remove the precipitate if necessary, transfer the filtrate to a 500-ml beaker, and gently add 10 ml of barium chloride solution (3 in 25) while boiling. Heat for 2 hours in a water bath, cool, and filter through a filter paper (5C) for quantitative analysis. Wash the residue on the filter paper with warm water until the filtrate is free from chloride. Dry the residue with the filter paper, place in a ceramic crucible, and incinerate. Weigh the crucible containing ash, determine the amount of ash (as barium sulfate), and calculate the percentage of sulfate group (SO_4) by formula:

$$\text{Amount (\%) of sulfate group} = \frac{\text{Weight (g) barium sulfate} \times 0.4116}{\text{Weight (g) of the sample}} \times 100$$

(3) Acid-insoluble substances Not more than 2.0%.

Weigh accurately about 2 g of the test sample prepared in Purity (2), and proceed as directed in Purity (5) for Processed Eucheuma Algae.

(4) Heavy metals Not more than 40 μg/g as Pb (0.50 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(5) Lead Not more than 5.0 μg/g as Pb (2.0 g, Method 1).

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(7) 2-Propanol and methanol Not more than 0.10% as the sum of 2-propanol and methanol.

Proceed as directed in Purity (9) for Processed Eucheuma Algae.

Loss on Drying Not more than 12.0% (105ºC, 4 hours).
Ash 15.0–40.0 % (2.0 g of the sample prepared in Purity (2)).
Acid-insoluble Ash Not more than 1.0%.
Microbial Limits Proceed as directed in the Microbial Limit Tests. The total bacterial count is not more than 10,000/g, and *Escherichia coli* is negative.

Purple Corn Color

ムラサキトウモロコシ色素

Definition Purple Corn Color is obtained from the seeds of the corn plant *Zea mays* Linné and consists mainly of cyanidin 3-glucoside. It may contain dextrin or lactose.

Color Value The Color Value ($E^{10\%}_{1cm}$) of Purple Corn Color is not less than 30 and is in the range of 90–120% of the labeled value.

Description Purple Corn Color occurs as a dark red powder, paste, or liquid. It has a slight characteristic odor.

Identification

(1) Weigh the equivalent of 1 g of Purple Corn Color with a Color Value 30, and dissolve it in 100 ml of citrate buffer (pH 3.0). A red to dark red-orange color develops.

(2) To the solution prepared in Identification (1), add sodium hydroxide solution (1 in 25) to make it alkaline. The solution turns dark green.

(3) A solution of Purple Corn Color in citrate buffer (pH 3.0) exhibits an absorption maximum at a wavelength of 505–525 nm.

(4) Prepare a test solution by diluting 10 ml of the solution prepared in Identification (1) to 100 ml with citrate buffer (pH 3.0). Separately, prepare a standard solution by dissolving 1 mg of cyanidin 3-glucoside chloride in citrate buffer (pH 3.0) to make 5 ml. Analyze 10 µl portions of the test solution and the standard solution by high-pressure liquid chromatography using the operating conditions given below. The main peak of the test solution corresponds to the retention time of cyanidin 3-glucoside chloride.

Operating Conditions

Detector: Visible absorption spectrophotometer (determination wavelength: 515 nm).

Column: A stainless steel tube of 4–5 mm internal diameter and 15–30 cm length.

Column packing material: 5-µm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 40°C.

Mobile phase: A 73:27 mixture of 4 % phosphoric acid solution/methanol.

Flow rate: Adjust the flow rate so that the retention time of cyanidin 3-glucoside chloride is about 10 minutes.

Purity

(1) Heavy metals Not more than 40 µg/g as Pb (0.50 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 8.0 µg/g (1.25 g, Method 1).

(3) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(4) Fumonisin B_1 Not more than 0.3 µg/g (on the basis of a Color Value 30).

Test Solution Set up a glass or polypropylene column (15 mm internal diameter). Pack it with about 2 g of trimethylaminopropyl-bonded silica gel, and rinse with methanol and then with a 3:1 mixture of methanol/water. Weigh accurately an equivalent of about 5 g of Purple Corn Color with a Color Value 30, add 80 ml of a 3:1 mixture of methanol/water, and shake. Adjust the pH to 8–9 with sodium hydroxide solution (1 in 10), and add a 3:1 mixture of methanol/water to make exactly 100 ml (sample solution). Pour 10 ml of the sample solution into the column, and discard the effluent. Rinse the column with 20 ml of a 3:1 mixture of methanol/water and then with 10 ml of methanol. Pour 20 ml of a 99:1 mixture of methanol/acetic acid onto the column, and collect the effluent. Evaporate it to dryness under reduced pressure at less than 40°C, and dissolve the residue by adding 0.2 ml of a 1:1 mixture of water/acetonitrile.

Standard Solution Weigh accurately about 0.01 g of fumonisin B_1, and add a 1:1 mixture of water/acetonitrile to make exactly 100 ml. Measure exactly 10 ml, 5 ml, and 1 ml of this solution into separate 200-ml volumetric flasks, and add a 1:1 mixture of water/acetonitrile to volume.

Procedure To 0.1 ml of each of the test solution and the standard solutions, add 0.1 ml of phthalaldehyde TS, and shake. Measure exactly 20 µl of each solution, inject each into the liquid chromatograph within one minute after the addition of phthalaldehyde TS, and analyze by liquid chromatography according to the operating conditions given below. Measure the fumonisin B_1 peak areas for the test solution and the standard solutions, and calculate the content using the calibration curve prepared.

Operating Conditions

Detector: Fluorescence spectrophotometer (excitation wavelength: 335 nm, fluorescent wavelength: 440 nm).

Column: A stainless steel tube of 4.6 mm internal diameter and 15 cm length.

Column packing material: 5-µm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 25°C.

Mobile phase: A 7:3 mixture of methanol/phosphate buffer (pH 3.3).

Flow rate: Adjust the flow rate so that the retention time of fumonisin B_1 is about 17 minutes.

Color Value Test Proceed as directed in the Color Value Test, using the following operating conditions.

Operating Conditions

Solvent: Citrate buffer (pH 3.0).

Wavelength: Maximum absorption wavelength of 505–525 nm.

Purple Sweet Potato Color

ムラサキイモ色素

Definition Purple Sweet Potato Color is obtained from the tuberous roots of the sweet potato plant *Ipomoea batatas* Poiret and consists mainly of cyanidin acylglucosides and peonidin acylglucosides. It may contain dextrin or lactose.

Color Value The Color Value ($E^{10\%}_{1cm}$) of Purple Sweet Potato Color is not less than 50 and is in the range of 90–110% of the labeled value.

Description Purple Sweet Potato Color occurs as a dark red powder, paste, or liquid. It has a slight characteristic odor.

Identification

(1) Weigh the equivalent of 1.0 g of Purple Sweet Potato Color with a Color Value 50, and dissolve it in 100 ml of citrate buffer (pH 3.0). A red to dark purple-red color develops.

(2) To the solution prepared in Identification (1), add sodium hydroxide solution (1 in 25) to make it alkaline. The solution turns dark green.

(3) A solution of Purple Sweet Potato Color in citrate buffer (pH 3.0) exhibits an absorption maximum at a wavelength of 515–535 nm.

Purity

(1) Heavy metals Not more than 40 µg/g as Pb (0.50 g , Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 8.0 µg/g (1.25 g , Method 1).

(3) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g,

Method 3, Apparatus B).

Color Value Test Proceed as directed in the Color Value Test, using the following operating conditions.

Operating Conditions

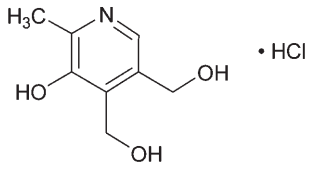
Solvent: Citrate buffer (pH 3.0).

Wavelength: Maximum absorption wavelength of 515–535 nm.

Pyridoxine Hydrochloride

Vitamin B_6

ピリドキシン塩酸塩



$C_8H_{11}NO_3$·HCl Mol. Wt. 205.64

(5-Hydroxy-6-methylpyridine-3,4-diyl)dimethanol monohydrochloride [58-56-0]

Content Pyridoxine Hydrochloride, when calculated on the dried basis, contains not less than 98.0% of pyridoxine hydrochloride ($C_8H_{11}NO_3$·HCl).

Description Pyridoxine Hydrochloride occurs as white to light yellow crystals or crystalline powder. It is odorless.

Identification

(1) To 1 ml of a solution of Pyridoxine Hydrochloride (1 in 10,000), add 2 ml of a solution of 2,6-dibromoquinonechloroimide in ethanol (1 in 4,000) and 1 drop of ammonia TS. A blue color develops. When the same test is performed using Pyridoxine Hydrochloride to which 1 ml of boric acid saturated solution is previously added, no blue color develops.

(2) Pyridoxine Hydrochloride responds to all tests for Chloride in the Qualitative Tests.

Purity

(1) Melting point 203–209°C (decomposition).

(2) pH 2.5–3.5 (0.50 g, water 25 ml).

(3) Heavy metals Not more than 30 μg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 3.0 ml).

Loss on Drying Not more than 0.50% (4 hours).

Residue on Ignition Not more than 0.10%.

Assay Weigh accurately about 0.4 g of Pyridoxine Hydrochloride, add 5 ml of acetic acid and 5 ml of acetic anhydride, and dissolve by boiling gently. After cooling, add 30 ml of acetic anhydride, and titrate with 0.1 mol/L perchloric acid (indicator: 1 ml of crystal violet–acetic acid TS) until the color of the solution changes from purple through blue to green. Perform a blank test in the same manner, and make any necessary correction. Calculate on the dried basis.

Each ml of 0.1 mol/L perchloric acid = 20.56 mg of $C_8H_{11}NO_3$·HCl

Quillaia Extract

Quillaja Extract

キラヤ抽出物

Definition Quillaia Extract is obtained from the bark of the soapbark tree *Quillaja saponaria* Molina and consists mainly of saponins.

Content Quillaia Extract, when dried, contains not less than 30.0% of partially hydrolyzed saponins.

Description Quillaia Extract occurs as a reddish light brown powder or brown liquid having a characteristic pungent taste.

Identification

(1) To 1.0 g of the powder-form sample, add the same quantity of water, and mix at room temperature. It dissolves, making a slightly suspended solution.

(2) Weigh 0.50 g of the powder-form sample or previously dried liquid-form sample, dissolve it in 20 ml of water, and use the resulting solution as the test solution. Analyze a 2 μl portion of the test solution by thin-layer chromatography using a 30:16:8:1 mixture of ethyl acetate/ethanol/water/acetic acid as the developing solvent. No control solution is used. Use a thin-layer plate, coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 15 cm above the initial spot. Air-dry the plate, spray with *p*-anisaldehyde–sulfuric acid TS uniformly, heat at 110°C for 10 minutes, and examine. Four successive purple-brown spots are observed at R_f values of about 0.1–0.5.

Purity

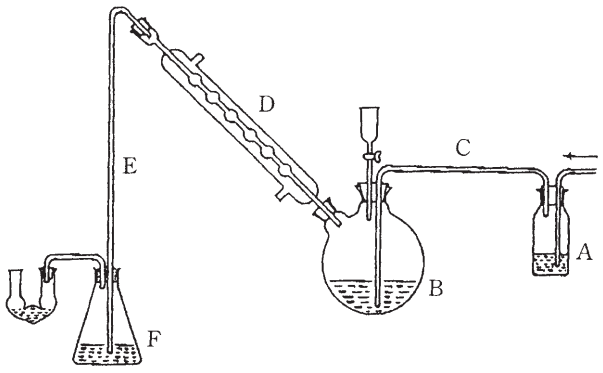
(1) pH 4.5–5.5 (4.0 g of the powder-form sample or previously dried liquid-form sample, water 100 ml).

(2) Lead Not more than 5.0 μg/g (2.0 g of the powder-form sample or previously dried liquid-form sample, Method 1).

(3) Arsenic Not more than 2.6 μg/g As_2O_3 (0.75 g of the powder-form sample or previously dried liquid-form sample, Method 3, Apparatus B).

(4) Sulfur dioxide Not more than 30 μg/g.

(i) Apparatus Use the apparatus as illustrated below.



A: Gas washing bottle
B: Round-bottom flask
C: Gas introducing tube
D: Reflux condenser
E: Glass joint tube
F: Absorption flask

(ii) Procedure Weigh accurately about 100 g of Quillaia Extract, place in 1,000-ml round-bottom flask B, and add 500 ml of methanol to produce a suspension. Equip gas-introducing tube C, so that the tube can reach the flask bottom, and join reflux condenser D to flask B. Place in absorption flask F 10 ml of hydrogen peroxide TS, previously confirmed to be neutral with methyl red TS. Equip glass joint tube E between D and F. Allow carbon dioxide or nitrogen gas to flow through introducing tube C, and remove air in the apparatus. After air is removed, immediately add 30 ml of diluted hydrochloric acid (1 in 3) to round-bottom flask B, connect reflux condenser D to joint glass tube E. Heat round-bottom flask B slowly until methanol begins to be refluxed, and keep heating mildly for 2 hours. Detach absorption flask F from the apparatus and cool. Titrate the solution in flask F with 0.01 mol/L sodium hydroxide solution (indicator, 3 drops of methyl red TS).

Each ml of 0.01 mol/L sodium hydroxide solution = 0.3203 mg of SO_2

Water Content Powder-form sample Not more than 6.0% (1.0 g, Direct Titration).

Loss on Drying Liquid-form sample 50.1–70.0% (1.0 g, 105°C, 5 hours).

Residue on Ignition Not more than 10.0% (1.0 g of the powder-form sample or previously dried liquid-form sample).

Assay

Test Solution Weigh accurately about 2 g of the powder-form sample or previously dried liquid-form sample, dissolve it in water to make exactly 100 ml. Measure exactly 10 ml of this solution, add 10 ml of 2% sodium hydroxide solution, attach a reflux condenser, and heat in a water bath for 2 hours. After cooling, dissolve it in 25 ml of ethanol, add 0.5 ml of phosphoric acid, and add water to make exactly 50 ml.

Standard Solution Weigh accurately about 0.02 g of partially hydrolyzed saponin for quantitative analysis, previously dried at 105°C for 3 hours, dissolve it in 50% (vol) ethanol to make exactly 50 ml.

Procedure Analyze 20 µl portions of the test solution and the standard solution by high performance liquid chromatography using the operating conditions given below. Measure the peak area (A_{T1}) of partially hydrolyzed saponin and the peak area (A_{T2}) of analogous saponin (the relative retention time of the analogous saponin to partially hydrolyzed saponin is about 0.95) for the test solution, and the peak area (A_S) of partially hydrolyzed saponin for the standard solution.

$$\text{Content (\%) of partially hydrolyzed saponin} = \frac{\text{Weight (g) of partially hydrolyzed saponin}}{\text{Weight (g) of the sample}} \times \frac{(A_{T1} + A_{T2}) \times 10}{A_S} \times 100$$

Operating Conditions

Detector: Ultraviolet spectrophotometer (determination wavelength: 210 nm).

Column: Stainless steel tube of 4–6 mm internal diameter and 15–30 cm length.

Column packing material: 5- to 10-µm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 40°C.

Mobile phase: A 13:7 mixture of 0.1% phosphoric acid/acetonitrile.

Flow rate: Adjust so that the retention time of partially hydrolyzed saponin is about 10 minutes.

Red Cabbage Color

アカキャベツ色素

Definition Red Cabbage Color is obtained by extraction from the leaves of the cabbage plant *Brassica oleracea* Linné with a weakly acidic aqueous solution. It consists mainly of cyanidin acylglucosides. It may contain dextrin or lactose.

Color Value The Color Value ($E^{10\%}_{1cm}$) of Red Cabbage Color is not less than 50 and is in the range of 90–110% of the labeled value.

Description Red Cabbage Color occurs as a dark red powder, paste, or liquid. It has a slight characteristic odor.

Identification

(1) Weigh the equivalent of 0.1 g of Red Cabbage Color with a Color Value 50, and dissolve it in 100 ml of citrate buffer (pH 3.0). A red to dark purple-red color develops.

(2) To the solution prepared in Identification (1), add sodium hydroxide solution (1 in 25) to make it alkaline. The solution turns dark green to light yellow-green.

(3) A solution of Red Cabbage Color in citrate buffer (pH 3.0) exhibits an absorption maximum at a wavelength of 520–540 nm.

Purity

(1) Heavy metals Not more than 40 µg/g as Pb (0.50 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 8.0 µg/g (1.25 g, Method 1).

(3) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Color Value Test Proceed as directed in the Color Value Test, using the following operating conditions.

Operating Conditions

Solvent: Citrate buffer (pH 3.0).

Wavelength: Maximum absorption wavelength of 520–540 nm.

Rhamsan Gum

ラムザンガム

Definition Rhamsan Gum is obtained from the culture fluid of *Sphingomonas* sp. and consists mainly of polysaccharides. It may include sucrose, glucose, lactose, dextrin, or maltose.

Description Rhamsan Gum occurs as a whitish or brownish powder having a slight odor.

Identification

(1) Add 0.3 g of Rhamsan Gum gradually to 100 ml of water with vigorous stirring. A viscous solution is produced. When the solution is heated to 80°C, its viscosity remains almost unchanged.

(2) Heat the solution obtained in Identification (1) to 80°C, add 0.3 g of carob bean gum gradually with vigorous stirring, and continue to stir for an additional 10 minutes. When the solution is cooled to about 10°C, it is not gelatinized.

Purity

(1) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 5.0 μg/g as Pb (2.0 g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(4) Total nitrogen Not more than 5.0% (on the dried basis).

Proceed as directed in the Kjeldahl method in Nitrogen Determination, using about 1 g of Rhamsan Gum, weighed accurately.

(5) 2-Propanol Not more than 0.10%.

Proceed as directed in Purity (9) for Processed Eucheuma Algae in the Monographs. Methanol determination is not conducted.

Loss on Drying Not more than 15.0% (105°C, 2.5 hours).

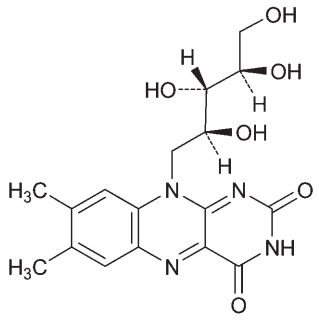
Ash Not more than 16.0% (on the dried basis).

Microbial Limits Proceed as directed in the Microbial Limit Tests. The total bacterial count is not more than 10,000/g, and *Escherichia coli* is negative. Prepare a test sample for *Escherichia coli* using 1 g of Rhamsan Gum.

Riboflavin

Vitamin B_2

リボフラビン



$C_{17}H_{20}N_4O_6$ Mol. Wt. 376.36

7,8-Dimethyl-10-[(2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl]benzo[*g*]pteridine-2,4(*3H*,*10H*)-dione [83-88-5]

Content Riboflavin, when dried, contains 98.0–102.0% of riboflavin ($C_{17}H_{20}N_4O_6$).

Description Riboflavin occurs as yellow to orange-yellow crystals or crystalline powder having a slight odor and a bitter taste.

Identification A solution of Riboflavin (1 in 100,000) is light yellow-green and emits a strong yellowish green fluorescence, which disappears on the addition of diluted hydrochloric acid (1 in 4) or sodium hydroxide solution (1 in 25).

Purity

(1) Specific rotation $[\alpha]_D^{20}$: –128.0 to –142.0°.

Weigh accurately about 0.1 g of Riboflavin, previously dried, dissolve it in 4 ml of potassium hydroxide solution (1 in 150), add 10 ml of freshly boiled and cooled water, and add 4 ml of ethanol with sufficient shaking. Add freshly boiled and cooled water to make exactly 20 ml, and measure the angular rotation of the resulting solution within 30 minutes.

(2) Lumiflavin Weigh 0.025 g of Riboflavin, add 10 ml of ethanol-free chloroform, shake for 5 minutes, and filter. The color of the filtrate is not darker than that of the solution prepared by adding water to 3.0 ml of 1/60 mol/L potassium dichromate to make 1,000 ml.

Loss on Drying Not more than 1.5% (105°C, 2 hours).

Residue on Ignition Not more than 0.30%.

Assay Throughout this assay, all procedures should be protected from direct light and the apparatus used should be light-resistant.

Test Solution Weigh accurately about 0.015 g of Riboflavin, previously dried, add 800 ml of diluted acetic acid (1 in 400), dissolve by warming, cool, and add water to make exactly 1,000 ml.

Standard Solution Proceed as directed for the test solution, using Riboflavin Reference Standard.

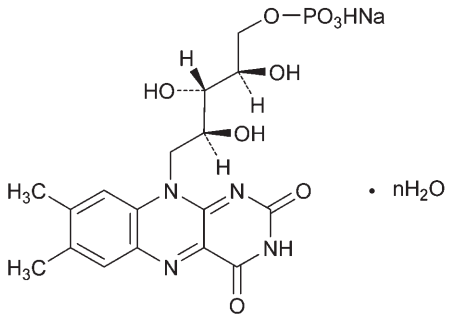
Procedure Using water as the reference solution, measure the absorbances (A_T and A_S) of the test solution and standard solution, respectively, at a wavelength of 445 nm. Add 0.02 g of sodium hydrosulfite to 5 ml of each solution, and shake well to discolor, and immediately measure the absorbances (A_T' and A_S'). Calculate the content by the formula:

$$\text{Content(\%) of riboflavin } (C_{17}H_{20}N_4O_6) = \frac{\text{Weight (g) of Riboflavin Standard Refernce}}{\text{Weight (g) of the sample}} \times \frac{A_T - A_T'}{A_S - A_S'} \times 100$$

Riboflavin 5′-Phosphate Sodium

Sodium Riboflavin Phosphate
Sodium Vitamin B_2 Phosphate

リボフラビン 5′-リン酸エステルナトリウム



n = 2 or 0

$C_{17}H_{20}N_4NaO_9P{\cdot}nH_2O$ (n = 2 or 0)

Mol. Wt. dihydrate 514.36
anhydrous 478.33

Monosodium (2*R*,3*S*,4*S*)-5-(7,8-dimethyl-2,4-dioxo-3,4-dihydrobenzo[*g*]pteridin-10 (2*H*)-yl)-2,3,4-trihydroxypenthyl monohydrogenphosphate dihydrate

Monosodium (2*R*,3*S*,4*S*)-5-(7,8-dimethyl-2,4-dioxo-3,4-dihydrobenzo[*g*]pteridin-10(2*H*)-yl)-2,3,4-trihydroxypenthyl monohydrogenphosphate [130-40-5]

Content Riboflavin 5′-Phosphate Sodium, when calculated on the anhydrous basis, contains not less than 95.0% of riboflavin 5′-phosphate sodium ($C_{17}H_{20}N_4NaO_9P$).

Description Riboflavin 5′-Phosphate Sodium occurs as yellow to orange crystals or crystalline powder. It is almost odorless and has a bitter taste.

Identification

(1) Proceed as directed under Identification for Riboflavin.

(2) To 0.050 g of Riboflavin 5′-Phosphate Sodium, add 10 ml of nitric acid, evaporate to dryness on a water bath, and ignite. To the residue, add 10 ml of diluted nitric acid (1 in 50), and boil for 5 minutes. Cool, neutralize with ammonia TS, and filter if necessary. The solution responds to all tests for Sodium Salt and for Phosphate in the Qualitative Tests.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +38.0 to +43.0° (0.30 g, diluted hydrochloric acid (9 in 20), 20 ml, on the anhydrous basis).

(2) Clarity of solution Clear (0.20 g, water 10 ml).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(4) Lumiflavin Proceed as directed in Purity (2) for Riboflavin, using 0.035 g of Riboflavin 5′-Phosphate Sodium.

Water Content Not more than 10.0% (0.100 g, Back Titration).

Instead of 20 ml of methanol for water determination, use 25 ml of a 1:1 mixture of methanol for water determination/ethylene glycol for water determination.

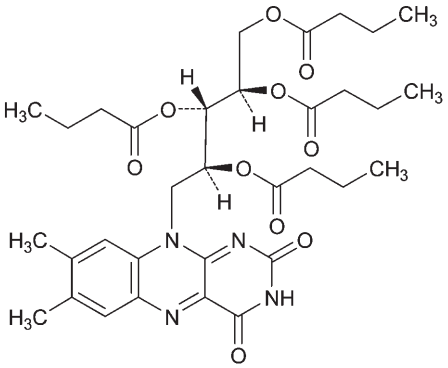
Assay Weigh accurately about 0.02 g of Riboflavin 5′-Phosphate Sodium, and proceed as directed in the Assay for Riboflavin. Calculate the content by the formula:

$$\text{Content (\%) of riboflavin 5′-phosphate sodium } (C_{17}H_{20}N_4NaO_9P) = \frac{\text{Weight (g) of Riboflavin Reference Standard}}{\text{Anhydrous basis weight (g) of the sample}} \times \frac{A_T - A_T'}{A_S - A_S'} \times 1.271 \times 100$$

Riboflavin Tetrabutyrate

Vitamin B_2 Tetrabutyrate

リボフラビン酪酸エステル



$C_{33}H_{44}N_4O_{10}$ Mol. Wt. 656.72

(2*R*,3*S*,4*S*)-5-(7,8-Dimethyl-2,4-dioxo-3,4-dihydrobenzo[*g*]pteridin-10(2*H*)-yl)pentane-1,2,3,4-tetrayl tetrabutanoate [752-56-7]

Content Riboflavin Tetrabutyrate, when dried, contains 97.0–102.0% of riboflavin tetrabutyrate ($C_{33}H_{44}N_4O_{10}$).

Description Riboflavin Tetrabutyrate occurs as yellow-orange crystals or crystalline powder. It is almost tasteless and has a slight, characteristic odor.

Identification

(1) To 5 ml of a solution of Riboflavin Tetrabutyrate in ethanol (1 in 500), add 2 ml of a 1:1 mixture of hydroxylamine hydrochloride solution (3 in 20)/sodium hydroxide solution (3 in 20), shake well, and add 0.8 ml of hydrochloric acid, 0.5 ml of iron(III) chloride solution (1 in 10), and 8 ml of ethanol. A deep red-brown color develops.

(2) A solution of Riboflavin Tetrabutyrate in ethanol (1 in 100,000) is light yellow-green and emits a strong yellowish green fluorescence, which disappears on the addition of diluted hydrochloric acid (1 in 4) or sodium hydroxide solution (1 in 25).

Purity

(1) Clarity of solution Clear (0.10 g, chloroform 10 ml).

(2) Absorbance ratio Weigh 0.10 g of Riboflavin Tetrabutyrate, and dissolve it in ethanol to make 200 ml. Measure 10 ml of this solution, and add ethanol to make 200 ml. The solution obtained exhibits absorption maxima at wavelengths

of 270 nm, 350 nm, and 445 nm. When the absorbances at the respective maximum wavelengths are expressed as A_1, A_2, and A_3, A_1/A_3 is 2.47–2.77, A_1/A_2 is 3.50–3.90, and A_2/A_3 is 0.65–0.75.

Loss on Drying Not more than 1.0% (reduced pressure, 4 hours).

Residue on Ignition Not more than 0.50%.

Assay Throughout this assay, all procedures should be protected from direct light and the apparatus used should be light-resistant.

Test Solution Weigh accurately about 0.04 g of Riboflavin Tetrabutyrate, previously dried, and dissolve it in ethanol to make exactly 500 ml. Measure exactly 10 ml of this solution, and add ethanol to make exactly 50 ml.

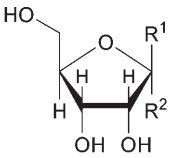
Standard Solution Weigh accurately about 0.05 g of Riboflavin Reference Standard, previously dried at 105°C for 2 hours, add 160 ml of diluted acetic acid (1 in 40), dissolve by heating, cool, and add water to make exactly 500 ml. Measure exactly 5 ml of this solution, and add ethanol to make exactly 50 ml.

Procedure Measure the absorbances (A_T and A_S) of the test solution and the standard solution at a wavelength of 445 nm against ethanol as the reference solution. Calculate the content by the formula:

$$\text{Content(\%) of riboflavin tetrabutyrate } (C_{33}H_{44}N_4O_{10}) = \frac{\text{Weight (g) of Riboflavin Standard Reference}}{\text{Weight (g) of the sample} \times 2} \times \frac{A_T \times 1.745}{A_S} \times 100$$

D-Ribose

D-リボース



α-D-Ribose: R^1=H, R^2=OH
β-D-Ribose: R^1=OH, R^2=H

$C_5H_{10}O_5$ Mol. Wt. 150.13
D-Ribofuranose [50-69-1]

Definition D-Ribose is obtained by isolation from the fermentation culture fluid of D-glucose by the Gram-positive bacterium *Bacillus pumilus* or *Bacillus subtilis*. It consists mainly of D-ribose.

Content D-Ribose, when calculated on the anhydrous basis, contains 90.0–102.0% of D-ribose ($C_5H_{10}O_5$).

Description D-Ribose occurs as white or light brown crystals or powder. It is odorless or has a slight characteristic odor.

Identification

(1) Add 2–3 drops of a solution of D-Ribose (1 in 20) to 5 ml of boiling Feling's TS. A red precipitation is produced.

(2) A solution of D-Ribose (1 in 50) is levorotatory.

Purity

(1) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 10 μg/g (1.0 g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

(4) Other Sugars Analyze the test solution and the standard solution by liquid chromatography as directed in the Assay. The sum of the peak areas of all solutes, other than D-ribose, in the test solution that appear within two times the retention time of D-ribose is not greater than 10.0% of the total area of all the peaks.

Water Content Not more than 5.0% (1 g, Direct Titration).

Residue on Ignition Not more than 1.0%.

Assay

Test Solution and Standard Solution Weigh accurately about 1 g each of D-Ribose and D-ribose for assay, separately dissolve them in water, and make 2 solutions of exactly 50 ml each. Use them as the test solution and the standard solution, respectively.

Procedure Analyze 10 μl portions of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Measure the peak areas (A_T and A_S) of D-ribose for the test solution and the standard solution. Calculate the content by the following formula:

$$\text{Content (\%) of D-ribose } (C_5H_{10}O_5) = \frac{\text{Anhydrous basis weight (g) of D-ribose for assay}}{\text{Anhydrous basis weight (g) of the sample}} \times \frac{A_T}{A_S} \times 100$$

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube of 8 mm internal diameter and 25–35 cm length.

Column packing material: About 6-μm gel strongly acidic anion exchange resin for liquid chromatography.

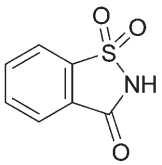
Column temperature: 80°C.

Mobile phase: Water.

Flow rate: Adjust so that the retention time of D-ribose is about 14 minutes.

Saccharin

サッカリン



$C_7H_5NO_3S$ Mol. Wt. 183.19
1,2-Benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide [81-07-2]

Content Saccharin, when dried, contains not less than

99.0% of saccharin ($C_7H_5NO_3S$).

Description Saccharin occurs as colorless to white crystals or as a white crystalline powder. It is odorless or has a slight aroma, and has a strongly sweet taste.

Identification

(1) Mix 0.02 g of Saccharin with 0.040 g of resorcinol, add 10 drops of sulfuric acid, and heat gently until the color of the mixture changes to dark green. Cool, and dissolve it adding 10 ml of water and 10 ml of sodium hydroxide solution (1 in 25). The solution emits a green fluorescence.

(2) Dissolve 0.1 g of Saccharin in 5 ml of sodium hydroxide solution (1 in 25), evaporate to dryness while gently heating, and fuse the residue, being careful not to carbonize it. Continue heating until the odor of ammonia is no longer evolved, and cool. Dissolve the residue by adding about 20 ml of water, neutralize with diluted hydrochloric acid (1 in 10), filter, and then add 1 drop of iron(III) chloride solution (1 in 10) to the filtrate. A purple to red-purple color develops.

Purity

(1) Melting point 226–230°C.

(2) Clarity and color of solution

Colorless and clear (1.0 g, hot water 30 ml).

Colorless and clear (1.0 g, ethanol 35 ml).

(3) Heavy metals Not more than 10 μg/g as Pb.

Weigh 2.0 g of Saccharin, and dissolve it in 40 ml of ethanol. Proceed as directed in Method 1 in the Heavy Metals Limit Tests in the General Tests, using this solution. Prepare a control solution with 2.0 ml of Lead Standard Solution.

(4) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 5.0 g of Saccharin, transfer into a Kjeldahl flask, add 10 ml of nitric acid and 5 ml of sulfuric acid, and heat. If the solution is still brown, cool the solution, add 1 ml of nitric acid, and heat. Repeat this procedure until the solution becomes colorless or light yellow, and heat until white fumes are evolved. After cooling, add 10 ml of water and 15 ml of ammonium oxalate saturated solution, and heat until white fumes are evolved again. After cooling, add water to make 50 ml. Use 5 ml of this solution as the test solution.

Apparatus Use Apparatus B.

Standard Color Measure 10 ml of Arsenic Standard Solution, transfer into a Kjeldahl flask, add 10 ml of nitric acid and 5 ml of sulfuric acid, and proceed in the same manner as the preparation of the test solution. Use 10 ml of the resulting solution as the standard color solution.

(5) Benzoic acid and Salicylic acid Weigh 0.5 g of Saccharin, dissolve it in 15 ml of hot water, and add 3 drops of iron(III) chloride solution (1 in 10). No precipitate is formed, and no purple to red-purple color develops.

(6) *o*-Toluenesulfonamide Not more than 25 μg/g as *o*-toluenesulfonamide.

Test Solution Weigh 10 g of Saccharin, dissolve it in 70 ml of sodium hydroxide solution (1 in 25), and extract three times with 30 ml of ethyl acetate each time. Combine all the ethyl acetate layers, and wash with 30 ml of sodium chloride solution (1 in 4), and add about 10 g of anhydrous sodium sulfate, and shake. Transfer the ethyl acetate layer quantitatively to an eggplant-shaped flask, evaporate the ethyl acetate, and dissolve the residue in 1.0 ml of a solution of caffeine in ethyl acetate (1 in 4,000).

Control Solution Measure 1.0 ml of a solution of *o*-toluenesulfonamide in ethyl acetate (1 in 4,000), remove the ethyl acetate while heating on a water bath, and dissolve the residue in 1.0 ml of a solution of caffeine in ethyl acetate (1 in 4,000).

Procedure Analyze the test solution and the control solution by gas chromatography using the conditions given below. The peak height ratio H/Hs of *o*-toluenesulfonamide (H) to caffeine (Hs) for the test solution does not exceed the peak height ratio H′/H′s of *o*-toluenesulfonamide (H′) to caffeine (H′s) for the control solution.

Operating Conditions

Detector: Flame ionization detector.

Column: A glass or stainless steel tube of 3–4 mm internal diameter and 1 m length.

Column packing material

Liquid phase: 3% Diethylene glycol succinate polyester of the amount of support.

Support: 177- to 250-μm diatomaceous earth for gas chromatography.

Column temperature: A constant temperature of 195–205°C.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the caffeine peak appears after about 6 minutes.

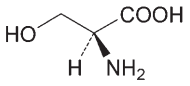
Loss on Drying Not more than 1.0% (105°C, 2 hours).

Assay Weigh accurately about 0.3 g of Saccharin, previously dried, dissolve it in 75 ml of boiling water, cool, and titrate with 0.1 mol/L sodium hydroxide (indicator: 3 drops of phenolphthalein TS).

Each ml of 0.1 mol/L sodium hydroxide = 18.32 mg of $C_7H_5NO_3S$

L-Serine

L-セリン



$C_3H_7NO_3$ Mol. Wt. 105.09

(2*S*)-2-Amino-3-hydroxypropanoic acid [56-45-1]

Content L-Serine, when calculated on the dried basis, contains 98.0–102.0% of L-serine ($C_3H_7NO_3$).

Description L-Serine occurs as white crystals or crystalline powder. It is odorless, and has a very slight sweet taste.

Identification

(1) To 5 ml of a solution of L-Serine (1 in 1,000), add 1 ml of ninhydrin solution (1 in 50), and heat for 3 minutes in a water bath. A bluish purple color develops.

(2) To 10 ml of a solution of L-Serine (1 in 20), add 0.2 g of periodic acid, and heat. The odor of formalin is evolved.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +13.5 to +16.0°.

Weigh accurately about 10 g of L-Serine, and dissolve it in 2 mol/L hydrochloric acid to make exactly 100 ml. Measure the angular rotation of this solution, and calculate on the dried basis.

(2) Clarity and color of solution Colorless and clear (1.0 g, water 20 ml).

(3) pH 5.2–6.2 (1.0 g, water 10 ml).

(4) Chloride Not more than 0.1% as Cl (0.07 g, Control solution 0.01 mol/L hydrochloric acid 0.20 ml).

(5) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Drying Not more than 0.30% (105°C, 3 hours).

Residue on Ignition Not more than 0.10%.

Assay Weigh accurately about 0.2 g of L-Serine, and proceed as directed in the Assay for L-Asparagine.

Each ml of 0.1 mol/L perchloric acid = 10.51 mg of $C_3H_7NO_3$

Shellac

シェラック

Definition Shellac is obtained from the secretion of lac scale insects, *Laccifer* spp., and consists mainly of esters of aleuritic acid and shellolic acid or esters of aleuritic acid and jaralic acid. There are two types of products: White Shellac and Purified Shellac. These products are also divided into two types: wax-containing shellac, from which wax is not removed, and wax-free shellac, from which wax is removed.

White Shellac

白シェラック

Description White Shellac occurs as white to light yellow granules or small granular flakes. It is odorless or has a slight, characteristic odor .

Identification

(1) To 12 g of White Shellac, add 60 ml of ethanol, and shake. It dissolves within 3 hours at ordinary temperature. To 12 g of White Shellac, add 60 ml of toluene, and proceed in the same manner. It does not dissolve. A wax-containing product makes a solution containing dispersed fine particles of wax.

(2) Heat and melt 0.05 g of White Shellac on the hot plate at 170°C, and continue heating. Gummy materials are formed by thermal polymerization. After cooling, add 1 ml of ethanol and shake. It does not dissolve.

Purity

(1) Acid value 73–89.

Test Solution Weigh accurately about 1 g of White Shellac, dissolve it in 50 ml of neutralized ethanol.

Procedure Proceed as directed in the Acid Value Test in the Fats and Related Substances Tests. In titration, confirm the endpoint, using a potentiometer, or, visually by checking that a pink color persists for 30 seconds.

(2) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(3) Arsenic Not more than 2.0 μg/g as As_2O_3 (1.0 g, Method 3, Apparatus B).

(4) Wax

Wax-containing shellac : Not more than 5.5%.

Wax-free shellac : Not more than 0.2%.

To 10.0 g of White Shellac, add 150 ml of sodium carbonate solution (1 in 60). Shake in a water bath to dissolve, and cover with a watch glass. Allow to stand and heat in a water bath for 3 hours. Cool with water more than 1 hour. Filter the floating wax, wash the wax and the filter paper with water. Transfer the wax and the filter into a beaker, and dry until almost all the water evaporate under 65°C. Transfer the wax and the filter paper into an extraction thimble in a Soxhlet extractor. Pour a suitable amount of hexane into the beaker, warm and dissolve the wax, and transfer into the extraction thimble. Extract with hexane for 2 hours. Evaporate hexane to dryness, and dry the residue for 3 hours at 105°C and weigh.

(5) Rosin Dissolve 2.0 g of White Shellac in 10 ml of absolute ethanol and dissolve. Add gradually 50 ml of hexane while shaking. Transfer the solution into a 200 ml separating funnel, and wash twice with 50 ml of water each time. Transfer the upper layer solution, and filter. Evaporate the filtrate to dryness on a water bath. To the residue, add 5 ml of acetic anhydride, and dissolve the residue while warming on a water bath if necessary. Transfer 20 ml of the resulting solution to a test tube, and add 1 drop of sulfuric acid. The solution does not show a color change from purple-red through purple to khaki.

Loss on Drying Not more than 6.0% (dry for 4 hours at 40°C, then desiccate 15 hours in the desiccator).

Ash Not more than 1.0%.

Purified Shellac

精製シェラック

Description Purified Shellac occurs as a yellow to dark brown small flakes. It is odorless or has slight characteristic odor.

Identification Proceed as directed in Identification (1) and (2) for White Shellac.

Purity

(1) Acid value 60–80.

Proceed as directed in Purity (1) for White Shellac. Use a potentiometer to confirm the endpoint.

(2) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(3) Arsenic Not more than 2.0 μg/g as As_2O_3 (1.0 g, Method 3, Apparatus B).

(4) Wax

Wax-containing shellac: Not more than 5.5%.

Wax-free shellac: Not more than 0.2%.

Proceed as directed in Purity (4) for White Shellac.

(5) Rosin Proceed as directed in Purity (5) for White Shellac.

Loss on Drying Not more than 2.0% (dry for 4 hours at 40°C, then desiccate 15 hours in the desiccator).

Ash Not more than 1.0%.

Silicon Dioxide

Silica Gel

二酸化ケイ素

SiO_2 Mol. Wt. 60.08
Silicon dioxide

Content Silicon Dioxide, when ignited, contains not less than 94.0% of silicon dioxide (SiO_2).

Description Silicon Dioxide occurs as a white powder or granules or as a white, colloidal liquid. It is odorless.

Identification Place 0.2 g of Silicon Dioxide in a platinum crucible, add 5 ml of hydrofluoric acid to dissolve, and heat. It almost completely evaporates.

Purity

(1) Water-soluble substances Not more than 5.0% of the dried substance.

Weigh 5.0 g of Silicon Dioxide, previously dried at 105°C for 2 hours, in a flask, add 150 ml of water, and stir thoroughly for 15 minutes with a magnetic stirrer. Filter with suction, using a filter holder equipped with a 47-mm diameter membrane filter (0.45 μm in pore size). If the filtrate is turbid, repeat the filtration with suction through the same filter. Wash the flask and the residue on the filter with water, combine the filtrate and the washings, and add water to make 250 ml. Measure 50 ml of this solution, evaporate to dryness, dry the residue at 105°C for 2 hours, and weigh the residue.

(2) Heavy metals Not more than 30 μg/g of the dried substance as Pb.

Test Solution Weigh 5.0 g of Silicon Dioxide, previously dried at 105°C for 2 hours, add 50 ml of diluted hydrochloric acid (1 in 4), heat on a water bath for 1 hour with occasional shaking while replenishing the lost water, cool, and filter. Wash the container and the residue on the filter paper with water, combine the filtrate and the washings, and add water to make 100 ml. Refer to this solution as solution A. Measure 20 ml of solution A, evaporate to dryness on a water bath, add 2 ml of diluted acetic acid (1 in 20) and 20 ml of water to dissolve the residue, and filter if necessary. Then add water to make 50 ml.

Control Solution To 3.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(3) Arsenic Not more than 4.0 μg/g of the dried substance as As_2O_3.

Test Solution Use exactly 10 ml of solution A.

Apparatus Use Apparatus B.

Loss on Ignition Not more than 70.0% (83.0% in the case of colloidal liquid) (105°C, 2 hours, then 1,000°C, 30 minutes).

Assay Weigh accurately about 1 g of Silicon Dioxide, previously ignited, and transfer into a platinum crucible, previously ignited at 1,000°C for 30 minutes, and cooled in a desiccator. Accurately weigh the crucible containing the sample, W (g). Add 4 drops of ethanol and 2 drops of sulfuric acid, then add a sufficient amount of hydrofluoric acid, and evaporate to dryness on a water bath. After cooling, add 5 ml of hydrofluoric acid to the residue, and evaporate to dryness. Heat at 550°C for 1 hour, gradually raise the temperature, ignite at 1,000°C for 30 minutes, and allow to cool in a desiccator. Weigh accurately the crucible with the residue, w (g), and calculate the content by the formula:

$$\text{Content (\%) of silicon dioxide } (SiO_2) = \frac{W\text{ (g)} - w\text{ (g)}}{\text{Weight (g) of the sample}} \times 100$$

Silicon Dioxide (fine)

微粒二酸化ケイ素

SiO_2 Mol. Wt. 60.08
Silicon dioxide

Content Silicon Dioxide (fine), when ignited, contains not less than 99.0% of silicon dioxide (SiO_2).

Description Silicon Dioxide (fine) occurs as a white fine powder of less than 15 μm in average particle diameter having a smooth touch, and is odorless and tasteless.

Identification Place 0.2 g of Silicon Dioxide (fine) into a platinum crucible, dissolve it in 5 ml of hydrofluoric acid, and heat. It almost evaporates.

Purity

(1) Water-soluble substances Not more than 5.0% of the dried substance.

Weigh 2.0 g of Silicon Dioxide (fine), dried at 105°C for 2 hours, add 60 ml of water, mix thoroughly for 15 minutes with a magnetic stirrer, and filter with suction, using a filter holder equipped with a membrane filter (0.45 μm in pore diameter). If the filtrate is turbid, repeat the filtration with suction through the same filter. Wash the container and the residue on the filter with water, combine the filtrate and the washings, and add water to make 100 ml. Measure 50 ml of this solution, evaporate to dryness, dry the residue at 105°C for 2 hours, and accurately weigh.

(2) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 5.0 g of Silicon Dioxide (fine), dried at 105°C for 2 hours, in a flask, add 50 ml of diluted hydrochloric acid (1 in 4), and warm on a water bath for 1 hour with occasional shaking while replenish the lost water. Cool, and filter through a filter paper. Wash the flask and the residue on the filter paper with water, combine the filtrate and the washings, and add water to make 100 ml. Refer to this solution as solution A. Measure 20 ml of solution A, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution To 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(3) Arsenic Not more than 2.0 μg/g as As_2O_3.

Test Solution 20 ml of solution A prepared in Purity (2).

Apparatus Use Apparatus B.

(4) Sodium Not more than 0.20% as Na_2O.

Test Solution To 5 ml of solution A prepared in Purity (2), add water to make 100 ml.

Control Solution Weigh exactly 1.886 g of sodium chloride, dried at 130°C for 2 hours, dissolve it in water to make exactly 1,000 ml. Measure exactly 5.0 ml of this solution, and add water to make exactly 1,000 ml.

Procedure Determine the atomic absorbance under the

following operating conditions. The atomic absorbance of the test solution is not more than that of the control solution.

Operating Conditions

Light source: Sodium hollow cathode lamp.
Wavelength of analysis line: 589.0 nm.
Supporting gas: Air.
Combustible gas: Acetylene.

(5) Aluminum Not more than 0.20% as Al_2O_3.

Test Solution To 20 ml of solution A prepared in Purity (2), add water to make 100 ml.

Control Solution Weigh exactly 2.33 g of aluminum potassium sulfate dodecahydrate, and dissolve by adding 5 ml of hydrochloric acid and water to make exactly 100 ml. Measure 2.0 ml of this solution, and add water to make exactly 250 ml.

Procedure Determine the atomic absorbance using the operating conditions below. The atomic absorbance of the test solution is not more than that of the control solution.

Operating Conditions

Light source: Aluminum hollow cathode lamp.
Wavelength of analysis line: 309.3 nm.
Supporting gas: Dinitrogen monoxide.
Combustible gas: Acetylene.

(6) Iron Not more than 0.50 mg/g as Fe_2O_3.

Test Solution To 20 ml of solution A prepared in Purity (2), add water to make 100 ml.

Control Solution Weigh exactly 6.04 g of ferric ammonium sulfate dodecahydrate, and dissolve by adding 20 ml of hydrochloric acid and water to make exactly 1,000 ml. Measure 5.0 ml of this solution, and add 10 ml of hydrochloric acid and water to make exactly 1,000 ml.

Procedure Determine the atomic absorbance using the operating conditions below. The atomic absorbance of the test solution is not more than that of the control solution.

Operating Conditions

Light source: Iron hollow cathode lamp.
Wavelength of analysis line: 248.3 nm.
Supporting gas: Air.
Combustible gas: Acetylene.

Loss on Drying Not more than 7.0% (105°C, 2 hours).

Loss on Ignition Not more than 8.5% (105°C, 2 hours, then 1,000°C, 30 minutes).

Assay Weigh accurately about 1 g of Silicon Dioxide (fine), previously ignited, place into a platinum crucible, ignited at 1,000°C for 30 minutes and allowed to cool in a desiccator previously. Weigh accurately the crucible, W (g), containing the sample. Add 4 drops of ethanol and 2 drops of sulfuric acid, then add a sufficient amount of hydrofluoric acid, and evaporate to dryness on a water bath. After cooling, add 5 ml of hydrofluoric acid to the residue, and evaporate to dryness. Heat at 550°C for 1 hour, gradually raise the temperature, ignite at 1,000°C for 30 minutes, and allow to cool in a desiccator. Weigh accurately the crucible, w (g), containing the residue, and calculate the content by the formula:

$$\text{Content (\%) of silicon dioxide } (SiO_2) = \frac{W(g) - w(g)}{\text{Weight (g) of the sample}} \times 100$$

Silicone Resin

Dimethylpolysiloxane
Polydimethylsiloxane

シリコーン樹脂

Description Silicone Resin occurs as a colorless to light gray, transparent or translucent, viscous liquid or pasty substance. It is almost odorless.

Identification Determine the absorption spectrum of Silicone Resin as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Refractive index of extracted silicone oil n_D^{25}: 1.400–1.410.

Test Solution Weigh 15 g of Silicone Resin, transfer to a Soxhlet extractor, and extract with 150 ml of carbon tetrachloride for 3 hours. Evaporate the extract on a water bath.

Procedure Determine the refractive index of the test solution.

(2) Viscosity of extracted silicone oil 100–1,100 mm^2/s.

Measure the viscosity at 25°C of the test solution prepared in Purity (1).

(3) Specific gravity 0.96–1.02.

(4) Silicon dioxide Not more than 15.0%.

Dry the residue obtained after the extraction in Purity (1) at about 100°C for 1 hour, and weigh.

Sodium Acetate

酢酸ナトリウム

$H_3C-COONa \cdot nH_2O$
n = 3 or 0

$C_2H_3NaO_2 \cdot nH_2O$ (n = 3 or 0) Mol. Wt. trihydrate 136.08
anhydrous 82.03

Monosodium acetate trihydrate [6131-90-4]
Monosodium acetate [127-09-3]

Definition Sodium Acetate occurs in two forms: the crystalline form (trihydrate) called Sodium Acetate (crystal) and the anhydrous form called Sodium Acetate (anhydrous).

Content Sodium Acetate, when dried, contains not less than 98.5% of sodium acetate ($C_2H_3NaO_2$).

Description Sodium Acetate (crystal) occurs as colorless, transparent crystals or as a white crystalline powder. Sodium Acetate (anhydrous) occurs as white crystalline powder or lumps. They are odorless.

Identification

(1) Heat the Sodium Acetate gradually. It fuses, and then decomposes, emitting an odor of acetone. The aqueous solution of the residue is alkaline.

(2) Sodium Acetate responds to all tests for Sodium Salt

and for Acetate in the Qualitative Tests.

Purity

(1) Clarity and color of solution Colorless and clear (1.0 g, water 20 ml).

(2) Free acid and free alkali Weigh 2.0 g of Sodium Acetate (crystal) or 1.2 g of Sodium Acetate (anhydrous), and dissolve it in 20 ml of freshly boiled and cooled water. Add 2 drops of phenolphthalein TS, and while keeping the solution at 10°C, perform the following test:

(i) If the solution is colorless, add 0.10 ml of 0.1 mol/L sodium hydroxide. A pink color develops.

(ii) If the solution is pink, add 0.10 ml of 0.1 mol/L hydrochloric acid. The color disappears.

(3) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Drying

Crystal: 36.0–42.0% (120°C, 4 hours).

Anhydrous: Not more than 2.0% (120°C, 4 hours).

Assay Weigh accurately about 0.2 g of Sodium Acetate, previously dried, dissolve it in 40 ml of acetic acid, and titrate with 0.1 mol/L perchloric acid. The endpoint is usually confirmed using a potentiometer. When crystal violet–acetic acid TS (1 ml) is used as the indicator, the endpoint is when the color of the solution changes from purple through blue to green. Perform a blank test in the same manner, and make any necessary correction.

Each ml of 0.1 mol/L perchloric acid = 8.203 mg of $C_2H_3NaO_2$

Sodium Alginate

アルギン酸ナトリウム

Sodium Alginate [9005-38-3]

Content Sodium Alginate, when dried, contains 90.8–106.0% of sodium alginate.

Description Sodium Alginate occurs as a white to yellowish-white powder. It is almost odorless.

Identification

(1) Prepare a test solution as follows: To 0.5 g of Sodium Alginate, add 50 ml of water in small portions while stirring, warm the mixture at 60–70°C for 20 minutes with occasional shaking to make it homogenous, and cool.

(i) To 5 ml of the test solution, add 1 ml of calcium chloride solution (3 in 40). A gelatinous precipitate is formed immediately.

(ii) To 10 ml of the test solution, add 1 ml of diluted sulfuric acid (1 in 20). A gelatinous precipitate is formed immediately.

(iii) To 1 ml of the test solution, add 1 ml of ammonium sulfate saturated solution. No precipitate is formed.

(2) The residue on ignition of Sodium Alginate responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) pH 6.0–8.0.

Add 0.50 g of Sodium Alginate to 50 ml of water gradually while stirring, and warm the mixture at 60–70°C for 20 minutes with occasional stirring to make it homogenous. Cool, and measure the pH.

(2) Sulfate Not more than 0.96% as SO_4.

To 0.10 g of Sodium Alginate, add 20 ml of water to make it pasty, then add 1 ml of hydrochloric acid, shake vigorously, heat in a water bath for several minutes, and proceed as directed in Purity (3) for Alginic acid.

(3) Phosphate Add 0.10 g of Sodium Alginate to 20 ml of water gradually while stirring, and warm the mixture at 60–70°C for 20 minutes with occasional stirring to make it homogenous. Then proceed as directed in Purity (4) for Alginic acid.

(4) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Loss on Drying Not more than 15.0% (105°C, 4 hours).

Residue on Ignition 33.0–37.0% (calculated on the dried basis).

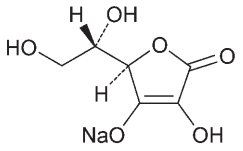
Assay Proceed as directed in the Assay for Alginic acid.

Each ml of 0.25 mol/L sodium hydroxide solution = 27.75 mg of sodium alginate

Sodium L-Ascorbate

Sodium Ascorbate
Vitamin C Sodium

L-アスコルビン酸ナトリウム



$C_6H_7NaO_6$ Mol. Wt. 198.11

Monosodium (2*R*)-2[(1*S*)-1,2-dihydroxyethyl]-4-hydroxy-5-oxo-2,5-dihydrofuran-3-olate [134-03-2]

Content Sodium L-Ascorbate, when dried, contains not less than 99.0% of sodium L-ascorbate ($C_6H_7NaO_6$).

Description Sodium L-Ascorbate occurs as white to yellowish white crystalline powder, granules, or fine granules. It is odorless and has a slightly salty taste.

Identification

(1) Proceed as directed in Identification (1) and (2) for L-Ascorbic Acid.

(2) Sodium L-Ascorbate responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +103.0 to +108.0° (1 g, freshly boiled and cooled water, 10 ml, on the dried basis).

(2) pH 6.5–8.0 (2.0 g, water 20 ml).

(3) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

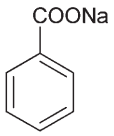
Loss on Drying Not more than 0.50% (reduced pressure, 24 hours).

Assay Weigh accurately about 0.2 g of Sodium L-Ascorbate, previously dried, dissolve it in 50 ml of metaphosphoric acid solution (1 in 50), and titrate with 0.05 mol/L iodine (indicator: starch TS).

Each ml of 0.05 mol/L iodine = 9.905 mg of $C_6H_7NaO_6$

Sodium Benzoate

安息香酸ナトリウム



$C_7H_5NaO_2$ Mol. Wt. 144.10

Monosodium benzenecarboxylate [532-32-1]

Content Sodium Benzoate, when dried, contains not less than 99.0% of sodium benzoate ($C_7H_5NaO_2$).

Description Sodium Benzoate occurs as white crystalline powder or granules. It is odorless.

Identification Sodium Benzoate responds to all tests for Sodium Salt and for Benzoate in the Qualitative Tests.

Purity

(1) Clarity and color of solution Colorless and clear (1.0 g, water 5.0 ml).

(2) Free acid and free alkali Weigh 2.0 g of Sodium Benzoate, dissolve it in 20 ml of boiling water, and add 2 drops of phenolphthalein TS and 0.20 ml of 0.05 mol/L sulfuric acid. The solution is colorless. To this solution, add 0.40 ml of 0.1 mol/L sodium hydroxide. The color of the solution changes to red.

(3) Sulfate Not more than 0.30% as SO_4.

Test Solution Weigh 0.20 g of Sodium Benzoate, and dissolve it in water to make 100 ml. To 40 ml of this solution, add 2.5 ml of diluted hydrochloric acid (1 in 4) dropwise while shaking well. Filter, wash with water, combine the filtrate and the washings, and add water to make 50 ml.

Control Solution To 0.50 ml of 0.005 mol/L sulfuric acid, add 1 ml of diluted hydrochloric acid (1 in 4) and water to make 50 ml.

(4) Heavy metals Not more than 10 μg/g as Pb.

Test Solution Weigh 2.0 g of Sodium Benzoate, dissolve it in about 30 ml of water, and add 3 ml of diluted hydrochloric acid (1 in 4) dropwise while shaking well. Filter, wash with water, and combine the filtrate and the washings. To this solution, add 1 drop of phenolphthalein TS, and add ammonia TS dropwise until the color of the solution changes to a slightly pink color. Add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(5) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.50 g of Sodium Benzoate, mix with 0.20 g of calcium hydroxide thoroughly, and ignite. Dissolve the residue in 10 ml of diluted hydrochloric acid (1 in 4).

Apparatus Use Apparatus B.

(6) Readily oxidizable substances Proceed as directed in Purity (4) for Benzoic Acid.

(7) Chlorinated compounds Not more than 0.014% as Cl.

Test Solution Weigh 0.50 g of Sodium Benzoate, transfer into a porcelain crucible, add 2.5 ml of diluted nitric acid (1 in 10), and mix thoroughly. Dry at 100°C, add 0.8 g of calcium carbonate and a small amount of water, mix, and dry at 100°C. Heat at about 600°C for 10 minutes and cool. Dissolve the residue in 20 ml of diluted nitric acid (1 in 10), filter, wash the insoluble substances with about 15 ml of water, combine the filtrate and the washings, and add water to make 50 ml.

Control Solution Weigh 0.8 g of calcium carbonate, dissolve it in 22.5 ml of diluted nitric acid (1 in 10), filter if necessary, and add 0.20 ml of 0.01 mol/L hydrochloric acid and water to make 50 ml.

Procedure Add 0.5 ml each of silver nitrate solution (1 in 50) to each solution, shake well, and allow to stand for 5 minutes. The test solution is not more turbid than the control solution.

(8) Phthalate Not more than 50 μg/g as phthalate.

Test Solution Weigh 1.0 g of Sodium Benzoate, and dissolve it in a 7:3 mixture of diluted acetic acid (1 in 100)/methanol to make exactly 50 ml.

Control Solution Prepared the control solution as directed in Purity (6) for Benzoic Acid, using a 7:3 mixture of diluted acetic acid (1 in 100)/methanol.

Procedure Proceed as directed in Purity (6) for Benzoic Acid.

Loss on Drying Not more than 1.5% (105°C, 4 hours).

Assay Weigh accurately about 1.5 g of Sodium Benzoate, previously dried and transfer into a 300-ml flask with a ground-glass stopper. Dissolve it in 25 ml of water, add 75 ml of diethyl ether, and titrate with 0.5 mol/L hydrochloric acid (indicator: 10 drops of bromophenol blue TS). Perform the titration while mixing the water and diethyl ether layers well by shaking. Titrate until the aqueous layer produces a persistent light green color.

Each ml of 0.5 mol/L hydrochloric acid = 72.05 mg of $C_7H_5NaO_2$

Sodium Bicarbonate

Sodium Hydrogen Carbonate
Bicarbonate of Soda

炭酸水素ナトリウム

$NaHCO_3$ Mol. Wt. 84.01

Sodium hydrogencarbonate [144-55-8]

Content Sodium Bicarbonate, when dried, contains not less than 99.0% of sodium bicarbonate ($NaHCO_3$).

Description Sodium Bicarbonate occurs as a white crystalline powder or crystalline lumps.

Identification Sodium Bicarbonate responds to all tests for Sodium Salt and for Bicarbonate in the Qualitative Tests.

Purity

(1) Clarity of solution Clear (1.0 g, water 20 ml).

(2) Chloride Not more than 0.021% as Cl.

Sample Solution Weigh 0.50 g of Sodium Bicarbonate, add 5 ml of diluted nitric acid (1 in 10), boil, and cool.

Control Solution Use 0.30 ml of 0.01 mol/L hydrochloric acid.

(3) Carbonate Weigh 1.0 g of Sodium Bicarbonate, add carefully 20 ml of freshly boiled and cooled water, and dissolve at 15°C or lower while shaking horizontally. Add 2.0 ml of 0.1 mol/L hydrochloric acid, and add 2 drops of phenolphthalein TS. No pink color develops immediately.

(4) Ammonium salt Weigh 1.0 g of Sodium Bicarbonate, and heat. No odor of ammonia is evolved.

(5) Heavy metals Not more than 10 μg/g as Pb.

Test Solution Weigh 2.0 g of Sodium Bicarbonate, dissolve by adding 5 ml of water and 20 ml of diluted hydrochloric acid (1 in 4), and evaporate to dryness on a water bath. Dissolve the residue by adding 2.0 ml of diluted acetic acid (1 in 20) and about 30 ml of water, and add water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(6) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.50 g of Sodium Bicarbonate, and dissolve by adding 3 ml of water and 2 ml of hydrochloric acid.

Apparatus Use Apparatus B.

Loss on Drying Not more than 0.25% (4 hours).

Assay Weigh accurately about 2 g of Sodium Bicarbonate, previously dried, dissolve it in 25 ml of water, and titrate with 0.5 mol/L sulfuric acid (indicator: 3 drops of bromophenol blue TS). Just before the endpoint, boil to let the carbon dioxide out, cool, and continue the titration.

Each ml of 0.5 mol/L sulfuric acid = 84.01 mg of $NaHCO_3$

Sodium Carbonate

Crystal: Soda Carbonate
Anhydrous: Soda Ash

炭酸ナトリウム

$Na_2CO_3 \cdot nH_2O$ (n = 1 or 0) Mol. Wt. monohydrate 124.00
anhydrous 105.99

Sodium carbonate monohydrate [5968-11-6]
Sodium carbonate [497-19-8]

Definition Sodium Carbonate occurs as two forms: the crystalline from (monohydrate) called Sodium Carbonate (crystal) and the anhydrous form called Sodium Carbonate (anhydrous).

Content Sodium Carbonate, when dried, contains not less than 99.0% of sodium carbonate (Na_2CO_3).

Description Sodium Carbonate (crystal) occurs as a white crystalline powder or as colorless to white crystalline lumps. Sodium Carbonate (anhydrous) occurs as a white powder or granules.

Identification Sodium Carbonate responds to all tests for Sodium Salt and to tests (1) and (3) for Carbonate in the Qualitative Tests.

Purity

(1) Clarity and color of solution Colorless and very slightly turbid (1.0 g, water 20 ml).

(2) Chloride Not more than 0.35% as Cl.

Sample Solution Weigh 0.50 g of Sodium Carbonate, add 6 ml of diluted nitric acid (1 in 10), boil, and cool. Add water to make 100 ml and perform the test, using 10 ml of the solution as the sample solution.

Control Solution 0.50 ml of 0.01 mol/L hydrochloric acid.

(3) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of Sodium Carbonate, dissolve it in 10 ml of water, add 7.5 ml of diluted hydrochloric acid (1 in 4), and evaporate to dryness on a water bath. Dissolve the residue with 2 ml of diluted acetic acid (1 in 20) and 30 ml of water. Add water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Drying Not more than 17.0% (105°C, 4 hours).

Assay Weigh accurately about 0.6 g of Sodium Carbonate, previously dried, dissolve it in 50 ml of water, and titrate with 0.5 mol/L hydrochloric acid (indicator: 3 drops of bromophenol blue TS). Soon before the titration reaches the endpoint, boil to expel carbon dioxide, cool, and continue the titration.

Each ml of 0.5 mol/L hydrochloric acid = 26.50 mg of Na_2CO_3

Sodium Carboxymethylcellulose

Sodium Cellulose Glycolate
Cellulose Gum

カルボキシメチルセルロースナトリウム

[9004-32-4]

Description Sodium Carboxymethylcellulose occurs as a white to light yellow powder, or granular or fibrous substance. It is odorless.

Identification

(1) Determine the absorption spectrum of Sodium Carboxymethylcellulose, previously dried, as directed in the Potassium Bromide Disk Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

(2) Ignite 1 g of Sodium Carboxymethylcellulose at 550–600°C for 3 hours. The resulting residue responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) pH 6.0–8.5.

Weigh 0.50 g of Sodium Carboxymethylcellulose, and add in small portions to 50 ml of water while stirring. Warm at 60–70°C for 20 minutes while stirring occasionally to make the solution homogeneous, and allow to cool.

(2) Chloride Not more than 0.64% as Cl.

Sample Solution Weigh 0.10 g of Sodium Carboxymeth-

ylcellulose, add 20 ml of water and 0.5 ml of hydrogen peroxide, and heat in a water bath for 30 minutes. After cooling, add water to make 100 ml, and filter through a dry filter paper. Use 25 ml of the filtrate as the sample solution.

Control Solution Use 0.45 ml of 0.01 mol/L hydrochloric acid.

(3) Sulfate Not more than 0.96% as SO_4.

Sample Solution 20 ml of the filtrate obtained in Purity (2).

Control Solution 0.40 ml of 0.005 mol/L sulfuric acid.

(4) Lead Not more than 2.0 μg/g as Pb (5.0 g, Method 1).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Loss on Drying Not more the 12.0% (105°C, 4 hours).

Sodium Carboxymethylstarch

デンプングリコール酸ナトリウム

Description Sodium Carboxymethylstarch occurs as a white powder. It is odorless.

Identification

(1) To 5 ml of a solution of Sodium Carboxymethylstarch (1 in 1,000), add 5 drops of diluted hydrochloric acid (1 in 4) and 1 drop of iodine TS, and shake. A blue to red-purple color develops.

(2) To 1 ml of a solution of Sodium Carboxymethylstarch (1 in 500), add 5 ml of chromotropic acid TS, and heat in a water bath for 10 minutes. A purple to purple-pink color develops.

(3) To 5 ml of a solution Sodium Carboxymethylstarch (1 in 500), add 5 ml of cupric sulfate solution (1 in 20), and shake. A light blue precipitate is formed.

(4) Ignite 1 g of Sodium Carboxymethylstarch at 450–550°C for 3 hours. The resulting residue responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) pH 6.0–8.5 (1.0 g, water 50 ml).

(2) Chloride Not more than 0.43% as Cl.

Sample Solution Weigh 0.10 g of Sodium Carboxymethylstarch, add 10 ml of water and 1 ml of nitric acid, heat in a water bath for 10 minutes, cool, and filter if necessary. Wash the residue with a small amount of water, combine the filtrate and the washings, and add water to make 100 ml. Use 25 ml of this solution as the sample solution.

Control Solution 0.30 ml of 0.01 mol/L hydrochloric acid.

(3) Sulfate Not more than 0.96% as SO_4.

Sample Solution Weigh 0.10 g of Sodium Carboxymethylstarch, add 10 ml of water and 1 ml of hydrochloric acid, heat in a water bath for 10 minutes, cool, and filter if necessary. Wash the residue with a small amount of water, combine the filtrate and the washings, and add water to make 50 ml. Use 10 ml of this solution as the sample solution.

Control Solution 0.40 ml of 0.005 mol/L sulfuric acid.

(4) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Loss on Drying Not more than 10.0% (105°C, 4 hours).

Sodium Caseinate

カゼインナトリウム

[9005-46-3]

Content Sodium Caseinate, when dried, contains 14.5–15.8% of nitrogen (N = 14.01).

Description Sodium Caseinate occurs as a white to light yellow powder, granules, or flakes. It is odorless and tasteless or has a slight, characteristic odor and taste.

Identification

(1) Proceed as directed in Identification (1), (2), and (3) for Casein.

(2) The residue on ignition of Sodium Caseinate responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Clarity and color of solution Colorless and slightly turbid.

Proceed as directed in Purity (1) for Casein.

(2) pH 6.0–7.5 (1.0 g, water 50 ml).

(3) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 2.0 μg/g as As_2O_3 (1.0 g, Method 3, Apparatus B).

(5) Fat Not more than 1.5%.

Proceed as directed in Purity (5) for Casein.

Loss on Drying Not more than 15.0% (100°C, 3 hours).

Residue on Ignition Not more than 6.0% (dried sample).

Assay Weigh accurately about 0.15g of Sodium Caseinate, previously dried, and proceed as directed in the Kjeldahl Method in Nitrogen Determination.

Each ml of 0.05 mol/L sulfuric acid = 1.401 mg of N

Sodium Chlorite

亜塩素酸ナトリウム

$NaClO_2$ Mol. Wt. 90.44

Sodium chlorite [7758-19-2]

Content Sodium Chlorite contains not less than 70.0% of sodium chlorite ($NaClO_2$).

Description Sodium Chlorite occurs as a white powder. It is odorless or has a slight odor.

Identification

(1) Sodium Chlorite responds to all tests for Sodium Salt and for Chlorite in the Qualitative Tests.

(2) To 2 ml of a solution of Sodium Chlorite (1 in 100), add 100 ml of phosphate buffer (pH8), and measure the absorbance. The solution exhibits its absorption maximum at a wavelength of 258–262 nm.

Purity

(1) Heavy metals Not more than 10 μg/g as Pb.

Sample Solution Weigh 4.0 g of Sodium Chlorite, dissolve it in 20 ml of water, and add 1 ml of nitric acid and 20 ml of hydrochloric acid. Evaporate to dryness on a water bath, and to the residue, add water to make 50 ml.

Test Solution Measure 25 ml of the sample solution, neu-

tralize with diluted ammonia solution (1 in 6), add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(2) Arsenic Not more than 1.0 μg/g as As_2O_3.

Test Solution 25 ml of the sample solution prepared in test (1).

Apparatus Use Apparatus B.

Assay Weigh accurately about 1 g of Sodium Chlorite, and dissolve it in water to make exactly 250 ml. Measure exactly 20 ml of this solution, transfer into an iodine bottle, and add 12 ml of diluted sulfuric acid (3 in 100), 20 ml of water, and 4 g of potassium iodide. Immediately stopper tightly, allow to stand in a dark place for 15 minutes, and titrate with 0.1 mol/L sodium thiosulfate (indicator: starch TS). Perform a blank test in the same manner, and make any necessary correction.

Each ml of 0.1 mol/L sodium thiosulfate = 2.261 mg of $NaClO_2$

Sodium Chlorite Solution

亜塩素酸ナトリウム液

Content Sodium Chlorite Solution contains 4.0–25.0 % of sodium chlorite ($NaClO_2$ = 90.44) and the equivalent of 95–100% of the labeled content.

Description Sodium Chlorite Solution is a clear, colorless to light yellow liquid. It is odorless or has a slight odor.

Identification

(1) Sodium Chlorite Solution responds to all tests for Sodium Salt and for Chlorite in the Qualitative Tests.

(2) Sodium Chlorite Solution is alkaline.

(3) Measure an appropriate quantity of diluted Sodium Chlorite Solution (1 in 100) so that the absorbance of the resulting solution is between 0.2 and 0.7, and add a phosphate buffer solution (pH 8). The solution exhibits its absorption maximum at a wavelength of 258–262 nm.

Purity

(1) Heavy metals Not more than 10 μg/g of $NaClO_2$ as Pb.

Test Solution Weigh an amount of Sodium Chlorite Solution equivalent to 4.0 g of $NaClO_2$, and add 2 ml of nitric acid and 20 ml of hydrochloric acid. Concentrate on a water bath, add water to the residue to make 50 ml, and use this solution as the sample solution. Measure 25 ml of the sample solution, neutralize with diluted ammonia solution (1 in 6), and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(2) Arsenic Not more than 1.0 μg/g of $NaClO_2$ as As_2O_3.

Test Solution 25 ml of the sample solution prepared in test (1).

Apparatus Use Apparatus B.

Assay Weigh accurately about 10 g of Sodium Chlorite Solution, add water to make exactly 100 ml, and use this solution as the sample solution. Measure exactly a quantity of this solution equivalent to about 0.06 g of $NaClO_2$, transfer into an iodine bottle, add 12 ml of diluted sulfuric acid (3 in 100), and add water to make about 55 ml. Add 4 g of potassium iodide, immediately stopper tightly, allow to stand in a dark place for 15 minutes, and titrate with 0.1 mol/L sodium thiosulfate (indicator: starch TS). Perform a blank test in the same manner as the sample solution, and make any necessary correction.

Each ml of 0.1 mol/L sodium thiosulfate = 2.261 mg of $NaClO_2$

Sodium Chondroitin Sulfate

コンドロイチン硫酸ナトリウム

Content Sodium Chondroitin Sulfate, when dried, contains 2.5–3.8% of nitrogen (N = 14.01) and 5.5 – 7.0% of sulfur (S = 32.07).

Description Sodium Chondroitin Sulfate occurs as a white to whitish powder.

Identification

(1) To 5 ml of a solution of Sodium Chondroitin Sulfate (1 in 100), add 1 ml of acriflavine hydrochloride solution (1 in 200). A yellow-brown precipitate is formed.

(2) To 5 ml of a solution of Sodium Chondroitin Sulfate solution (1 in 100), add 1 ml of hydrochloric acid, heat in a water bath for 10 minutes, and cool. Add 1 ml of barium chloride solution (3 in 25). A white precipitate is formed.

(3) The residue on ignition of Sodium Chondroitin Sulfate responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Clarity of solution Almost clear.

Test Solution Weigh 0.10 g of Sodium Chondroitin Sulfate, add 20 ml of water, and dissolve while shaking well.

(2) pH 5.5–7.5 (1.0 g, water 100 ml).

(3) Chloride Not more than 0.14% as Cl.

Test Solution Weigh 0.050 g of Sodium Chondroitin Sulfate, dissolve it in 10 ml of water, add 15 ml of ethanol and 6 ml of diluted nitric acid (1 in 10), shake, and filter. Wash the residue with 50% (vol) ethanol, combine the filtrate and the washings, and add 50% (vol) ethanol to make 50 ml.

Control Solution To 0.20 ml of 0.01 mol/L hydrochloric acid, add 6 ml of diluted nitric acid (1 in 10) and 50% (vol) ethanol to make 50 ml.

(4) Inorganic sulfate Not more than 0.24% as SO_4.

Sample Solution Weigh 0.10 g of Sodium Chondroitin Sulfate, dissolve it in 15 ml of water, add 1 ml of hydrochloric acid, and shake well. Add 2 ml of aluminum chloride solution (1 in 5), shake well again, and add 5 ml of ammonia TS little by little while shaking. Centrifuge, and collect the supernatant. Add 5 ml of water to the residue, shake, centrifuge, and combine the supernatant with the washings. Repeat this procedure using 5 ml of water, combine the supernatant and the washings, and neutralize with diluted hydrochloric acid (1 in 4).

Control Solution Use 0.50 ml of 0.005 mol/L sulfuric acid.

Procedure Proceed as directed in the Sulfur Limit Test.

(5) Heavy metals Not more than 40 μg/g as Pb (0.50 g,

previously dried, Method 2, Control solution Lead Standard Solution 2.0 ml).

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Loss on Drying Not more than 10.0% (105°C, 4 hours).

Residue on Ignition 23.0–31.0% (dried sample).

Assay

(1) Nitrogen Weigh accurately about 1 g of Sodium Chondroitin Sulfate, previously dried, and proceed as directed in the Kjeldahl Method under Nitrogen Determination.

1 ml of 0.05 mol/L sulfuric acid = 1.401 mg of N

(2) Sulfur Weigh accurately about 0.5 g of Sodium Chondroitin Sulfate, previously dried, into a Kjeldahl flask, dissolve it in 30 ml of water, and add 5 g of potassium chlorate. Then add 30 ml of nitric acid in small portions, heat until the solution becomes about 5 ml, and cool. Transfer quantitatively to a beaker, using 25 ml of hydrochloric acid, and concentrate on a water bath to about 5 ml. Add 100 ml of water to the solution, neutralize with ammonia TS, add 5 ml of diluted hydrochloric acid (1 in 10), and add 5 ml of barium chloride solution (3 in 25) while boiling. Cover the beaker with a watch glass, and heat on a water bath for 2 hours while replenishing the water. Cool, filter through a filter paper for quantitative analysis (5C), and wash the beaker and the residue on the filter paper with warm water until the washings do not respond to the tests for Chloride. Dry the residue together with the filter paper, ignite at 450–550°C to constant weight, and weigh accurately. Calculate the content of sulfur by the formula:

$$\text{Content (\%) of sulfur (S)} = \frac{\text{Weight (g) of the residue} \times 0.1374}{\text{Weight (g) of the sample}} \times 100$$

Sodium Copper Chlorophyllin

銅クロロフィリンナトリウム

Description Sodium Copper Chlorophyllin occurs as a blue-black to green-black powder. It is odorless or has a slight, characteristic odor.

Identification

(1) Place 1 g of Sodium Copper Chlorophyllin into a porcelain crucible, moisten with a small amount of sulfuric acid, and heat gradually. After it is almost completely incinerated at the lowest possible temperature, allow to cool. Add 1 ml of sulfuric acid, heat gradually until fumes of sulfuric acid have practically ceased to be evolved, and allow to cool. Add 10 ml of diluted hydrochloric acid (1 in 4) to the residue, dissolve by heating on a water bath, filter if necessary, and add water to make 10 ml. Perform the tests, given below, using this solution as the test solution.

(i) Perform the Flame Coloration Test on the test solution. A green color is imparted to the flame, and then changes to yellow color.

(ii) To 5 ml of the test solution, add 0.5 ml of sodium diethyldithiocarbamate solution (1 in 1,000). A brown precipitate is formed.

(2) To 1 ml of a solution of Sodium Copper Chlorophyllin (1 in 1,000), add phosphate buffer (pH 7.5) to make 100 ml, and measure the absorbance. The solution exhibits absorption maxima at wavelengths of 403–407 nm and 627–633 nm. When the absorbances at these absorption maxima are expressed as A_1 and A_2, respectively, A_1/A_2 is not more than 4.0.

Purity

(1) Specific absorbance $E^{1\%}_{1cm}$ (maximum absorption wavelength near 405 nm): Not less than 508 (on the dried basis).

This test should be protected from direct light, and the apparatus used in the test should be light-resistant.

Weigh accurately about 0.1 g of Sodium Copper Chlorophyllin, and dissolve it in water to make exactly 100 ml. Measure exactly 1 ml of this solution, add phosphate buffer (pH 7.5) to make exactly 100 ml, and promptly measure the absorbance.

(2) pH 9.5–11.0 (1.0 g, water 100 ml).

(3) Inorganic copper salt Not more than 0.03% as Cu.

Test Solution Weigh 1.0 g of Sodium Copper Chlorophyllin, and dissolve it in 60 ml of water.

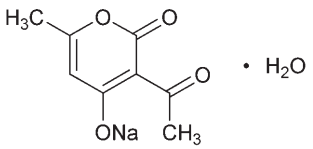
Procedure Analyze a 2-μl portion of the test solution by thin-layer chromatography using a 4:2:1 mixture of 1-butanol/water/acetic acid as the developing solvent. No control solution is used. Use a thin-layer plate coated with silica gel for thin-layer chromatography and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm, and air-dry the plate. Spray with sodium diethyldithiocarbamate solution (1 in 1,000). No light brown spot is observed.

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Loss on Drying Not more than 5.0% (105°C, 2 hours).

Sodium Dehydroacetate

デヒドロ酢酸ナトリウム



$C_8H_7NaO_4 \cdot H_2O$ Mol. Wt. 208.14

Monosodium 3-acetyl-4-oxido-6-methyl-2*H*-pyran-2-one monohydrate [4418-26-2]

Content Sodium Dehydroacetate, calculated on the anhydrous basis, contains 98.0–102.0% of sodium dehydroacetate ($C_8H_7NaO_4$ = 190.13).

Description Sodium Dehydroacetate occurs as a white crystalline powder. It is odorless or has a slight odor.

Identification

(1) To 0.1 g of Sodium Dehydroacetate, add 1 ml of water, 3 to 5 drops of a solution of salicylaldehyde in ethanol (1 in 5), and 0.5 ml of sodium hydroxide solution (1 in 3), and heat in a water bath. A red color develops.

(2) To 2 ml of a solution of Sodium Dehydroacetate (1 in 100), add 3 drops of potassium sodium tartrate solution (7

in 50) and 2 drops of strong cupric acetate TS, and shake. A whitish purple precipitate is formed.

(3) Sodium Dehydroacetate responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Color of solution Colorless (0.50 g, water 10 ml).

(2) Dehydroacetic acid Weigh 0.5 g of Sodium Dehydroacetate, dissolve it in 10 ml of water, add 1 ml of diluted hydrochloric acid (1 in 4), filter the resulting precipitate, and wash thoroughly with water. The melting point is 109–112°C.

(3) Free alkali Weigh 1.0 g of Sodium Dehydroacetate, and dissolve it in 20 ml of freshly boiled and cooled water. When 2 drops of phenolphthalein TS is added a pink color develops, but it disappears on the addition of 0.30 ml of 0.05 mol/L sulfuric acid.

(4) Chloride Not more than 0.011% as Cl.

Test Solution Weigh 1.0 g of Sodium Dehydroacetate, dissolve it in 30 ml of water, and add 9.5 ml of diluted nitric acid (1 in 10) dropwise while shaking well. Filter, wash with water, and combine the filtrate and the washings. Add water to make 50 ml.

Control Solution To 0.30 ml of 0.01 mol/L hydrochloric acid, add 6 ml of diluted nitric acid (1 in 10) and water to make 50 ml.

(5) Sulfate Not more than 0.014% as SO_4.

Test Solution Weigh 1.0 g of Sodium Dehydroacetate, dissolve it in 30 ml of water, and add 3 ml of diluted hydrochloric acid (1 in 4) dropwise while shaking well. Filter, wash with water, and combine the filtrate and the washings. Add water to make 50 ml.

Control Solution To 0.30 ml of 0.005 mol/L sulfuric acid, add 1 ml of diluted hydrochloric acid (1 in 4) and water to make 50 ml.

(6) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(7) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

(8) Readily carbonizable substances Perform the test, using 0.30 g of Sodium Dehydroacetate as the test sample and Matching Fluid C.

Water Content 8.3–10.0% (0.3 g, Back Titration).

Assay Weigh accurately about 0.4 g of Sodium Dehydroacetate, add 50 ml of acetic acid for nonaqueous titration, and titrate with 0.1 mol/L perchloric acid (indicator: 10 drops of α-naphtholbenzein TS) until the brown color of the solution changes to green. Calculate on the anhydrous basis.

Each ml of 0.1 mol/L perchloric acid = 19.01 mg of $C_8H_7NaO_4$

Sodium Dihydrogen Phosphate

Monosodium Phosphate
Sodium Phosphate, Monobasic

リン酸二水素ナトリウム

$NaH_2PO_4 \cdot nH_2O$ (n = 2 or 0) Mol. Wt. dihydrate 156.01
anhydrous 119.98

Sodium dihydrogenphosphate dihydrate [13472-35-0]
Sodium dihydrogenphosphate [7558-80-7]

Definition Sodium Dihydrogen Phosphate occurs in two forms: the crystalline form (dihydrate) called Sodium Dihydrogen Phosphate (crystal) and the anhydrous form called Sodium Dihydrogen Phosphate (anhydrous).

Content Sodium Dihydrogen Phosphate, when dried, contains 98.0–103.0% of sodium dihydrogen phosphate (NaH_2PO_4).

Description Sodium Dihydrogen Phosphate (crystal) occurs as colorless to white crystals or as a white crystalline powder. Sodium Dihydrogen Phosphate (anhydrous) occurs as a white powder or granules.

Identification A solution of Sodium Dihydrogen Phosphate (1 in 20) responds to all tests for Sodium Salt and for Phosphate in the Qualitative Tests.

Purity For Sodium Dihydrogen Phosphate (crystal), dry the sample before performing the tests.

(1) Clarity and color of solution Colorless and very slightly turbid (2.0 g, water 20 ml).

(2) pH 4.3–4.9 (1.0 g, water 100 ml).

(3) Chloride Not more than 0.11% as Cl (0.20 g, Control Solution 0.01 mol/L hydrochloric acid 0.60 ml).

(4) Sulfate Not more than 0.048% as SO_4 (0.50 g, Control Solution 0.005 mol/L sulfuric acid 0.50 ml).

(5) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of Sodium Dihydrogen Phosphate, dissolve by adding 2 ml of diluted acetic acid (1 in 20) and 30 ml of water, and add water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Drying

Crystal: 22.0–24.0% (40°C, 16 hours, then 120°C, 4 hours).

Anhydrous: Not more than 2.0% (120°C, 4 hours).

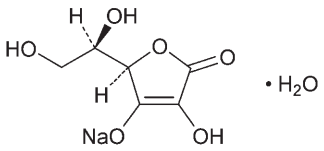
Assay Weigh accurately about 3 g of Sodium Dihydrogen Phosphate, previously dried, dissolve it in 30 ml of water, add 5 g of sodium chloride, and dissolve by shaking well. While keeping at about 15°C, titrate with 1 mol/L sodium hydroxide (indicator: 3–4 drops of thymol blue TS).

Each ml of 1 mol/L sodium hydroxide = 120.0 mg of NaH_2PO_4

Sodium Erythorbate

Sodium Isoascorbate

エリソルビン酸ナトリウム



$C_6H_7NaO_6 \cdot H_2O$ Mol. Wt. 216.12
Monosodium (2*R*)-2[(1*R*)-1,2-dihydroxyethyl]-4-hydroxy-5-oxo-2,5-dihydrofuran-3-olate monohydrate
[anhydrous 6381-77-7]

Content Sodium Erythorbate, when dried, contains not less than 98.0% of sodium erythorbate ($C_6H_7NaO_6 \cdot H_2O$).

Description Sodium Erythorbate occurs as a white to yellowish white crystalline powder, granules, or fine granules. It is odorless and has a slightly salty taste.

Identification

(1) Proceed as directed in Identification (1) and (2) for Erythorbic Acid.

(2) Sodium Erythorbate responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +95.5 to +98.0° (previously dried, 1 g, water 10 ml).

(2) Clarity and color of solution Weigh 1.0 g of Sodium Erythorbate, and dissolve it in 10 ml of water. The solution is clear, and its color is not darker than that of Matching Fluid J.

(3) pH 6.0–8.0 (1.0 g, water 20 ml).

(4) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Drying Not more than 0.25% (reduced pressure, 24 hours).

Assay Weigh accurately about 1 g of Sodium Erythorbate, previously dried, and dissolve it in metaphosphoric acid solution (1 in 50) to make exactly 250 ml. Measure exactly 50 ml of this solution, and titrate with 0.05 mol/L iodine (indicator: starch TS).

Each ml of 0.05 mol/L iodine = 10.81 mg of $C_6H_7NaO_6 \cdot H_2O$

Sodium Ferrocyanide

Sodium Hexacyanoferrate(II)

フェロシアン化ナトリウム

$Na_4[Fe(CN)_6] \cdot 10H_2O$ Mol. Wt. 484.06
Sodium hexacyanoferrate(II) decahydrate [13601-19-9]

Content Sodium Ferrocyanide includes not less than 99.0% of sodium ferrocyanide ($Na_4[Fe(CN)_6] \cdot 10H_2O$).

Description Sodium Ferrocyanide occurs yellow crystals or crystalline powder.

Identification

(1) Proceed as directed in Identification (1) for Potassium Ferrocyanide.

(2) Sodium Ferrocyanide responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Cyanide Proceed as directed in Purity (1) for Potassium Ferrocyanide.

(2) Ferricyanide Proceed as directed in Purity (2) for Potassium Ferrocyanide.

Assay Weigh accurately about 1 g of Sodium Ferrocyanide, and dissolve it in 200 ml of water. To this solution, add 10 ml of sulfuric acid, and titrate with 0.02 mol/L potassium permanganate. The endpoint is when the pink color of the solution persists for 30 seconds.

Each ml of 0.02 mol/L potassium permanganate = 48.41 mg of $Na_4[Fe(CN)_6] \cdot 10H_2O$

Sodium Ferrous Citrate

Sodium Iron Citrate

クエン酸第一鉄ナトリウム

Iron(II) sodium salt of 2-hydroxypropane-1,2,3-tricarboxylic acid

Content Sodium Ferrous Citrate contains 10.0–11.0% of Fe (=55.85).

Description Sodium Ferrous Citrate occurs as a green-white to greenish yellow powder. It is odorless and has a weak iron taste.

Identification

(1) To 5 ml of a solution of Sodium Ferrous Citrate (1 in 100), add 1 ml of diluted hydrochloric acid (1 in 4) and 0.5 ml of freshly prepared potassium ferricyanide solution (1 in 10). A blue color develops.

(2) To 5 ml of a solution of Sodium Ferrous Citrate (1 in 100), add 2 ml of ammonia solution. A red-brown color develops, but no precipitate is formed.

(3) Ignite 3 g of Sodium Ferrous Citrate at 500–600°C for 3 hours. The resulting residue responds to all tests for Sodium Salt in the Qualitative Tests.

(4) To 0.5 g of Sodium Ferrous Citrate, add 5 ml of water and 10 ml of potassium hydroxide solution (1 in 25), heat in a water bath for 10 minutes while stirring well, cool, and

filter. Take a portion of the filtrate, neutralize with diluted acetic acid (1 in 2), add an excessive amount of calcium chloride solution (3 in 40), and boil. A white, crystalline precipitate is formed. The precipitate does not dissolve in sodium hydroxide solution (1 in 25), but dissolves in diluted hydrochloric acid (1 in 4).

Purity

(1) Sulfate Not more than 0.48% as SO_4.

Test Solution Weigh 0.40 g of Sodium Ferrous Citrate, dissolve it in 50 ml of water, and add water to make 100 ml. Measure 10 ml of this solution, add 1 ml of diluted hydrochloric acid (1 in 4) and 0.1 g of hydroxylamine hydrochloride, and boil for 1 minute. Cool, and add water to make 50 ml.

Control Solution To 0.40 ml of 0.005 mol/L of sulfuric acid, add 1 ml of diluted hydrochloric acid (1 in 4) and water to make 50 ml.

(2) Ferric salt Weigh 2.0 g of Sodium Ferrous Citrate into a flask with a ground-glass stopper, dissolve it in 5 ml of hydrochloric acid and 30 ml of water, add 4 g of potassium iodide, stopper, and allow to stand in a dark place for 15 minutes. Add 2 ml of starch TS, and shake well. A color develops, but it disappears on the addition of 1.0 ml of 0.1 mol/L sodium thiosulfate to the solution.

(3) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of Sodium Ferrous Citrate in a porcelain dish, and add 3 ml of aqua regia to dissolve. Evaporate in a water bath to dryness. Dissolve the residue by adding 5 ml of diluted hydrochloric acid (1 in 2), and transfer to a separating funnel. Rinse the dish twice with two 5-ml portions of diluted hydrochloric acid (1 in 2), and add the rinses to the separating funnel. Wash the aqueous layer twice with two 40-ml portions of diethyl ether, and then once with a 20-ml diethyl ether. Discard the washings, and add 0.05 g of hydroxylamine hydrochloric acid to the aqueous layer to dissolve. Heat in a water bath for 10 minutes, add 1 drop of phenolphthalein TS, and add ammonia solution until the solution turns pink. Cool, and add dilute hydrochloric acid (1 in 2) dropwise until the color almost disappears. Add 4 ml of diluted acetic acid (1 in 20), shake well, add 50 ml of water, and filter if necessary.

Control Solution Place 2.0 ml of Lead Standard Solution into a porcelain dish, add 1 ml of sulfuric acid, and then proceed as directed for the test solution.

(4) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 1.0 g of Sodium Ferrous Citrate, add 10 ml of water, 1 ml of sulfuric acid, and 10 ml of sulfurous acid, evaporate to about 2 ml, and add water to make 10 ml. Use 5 ml of this solution as the test solution.

Apparatus Use Apparatus B.

Standard Color To 4.0 ml of Arsenic Standard Solution, add 10 ml of water, 1 ml of sulfuric acid, and 10 ml of sulfurous acid. Then proceed in the same manner as the preparation of the test solution.

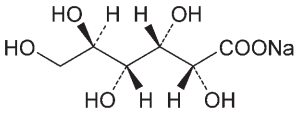
(5) Tartrate Weigh 1.0 g of Sodium Ferrous Citrate, add 5 ml of water and 10 ml of potassium hydroxide solution (1 in 15), heat in a water bath for 10 minutes while stirring well, cool, and filter. Measure 5 ml of the filtrate, add diluted acetic acid (1 in 4) to make it weakly acidic, then add 2 ml of acetic acid, and allow to stand for 24 hours. No white, crystalline precipitate is formed.

Assay Weigh accurately about 1 g of Sodium Ferrous Citrate, transfer into a flask with a ground-glass stopper, add 25 ml of diluted sulfuric acid (1 in 20) and 2 ml of nitric acid, and boil for 10 minutes. After cooling, add 20 ml of water and 4 g of potassium iodide, immediately stopper tightly, allow to stand in a dark place for 15 minutes, and add 100 ml of water. Titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: starch TS). Perform a blank test in the same manner.

Each ml of 0.1 mol/L sodium thiosulfate = 5.585 mg of Fe

Sodium Gluconate

グルコン酸ナトリウム



$C_6H_{11}NaO_7$ Mol. Wt. 218.14

Monosodium D-gluconate [527-07-1]

Content Sodium Gluconate, when dried, contains 98.0–102.0% of sodium gluconate ($C_6H_{11}NaO_7$).

Description Sodium Gluconate occurs as a white to yellowish white crystalline powder or granules. It has a slight, characteristic odor.

Identification

(1) Sodium Gluconate responds to all tests for Sodium Salt in the Qualitative Tests.

(2) Measure 5 ml of a solution of Sodium Gluconate (1 in 10), and proceed as directed in Identification (2) for Glucono-δ-Lactone.

Purity

(1) Clarity and color of solution Colorless and almost clear (1.0 g, water 10 ml).

(2) pH 6.2–7.8 (1.0 g, water 10 ml).

(3) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Lead Not more than 10 μg/g as Pb (1.0 g, Method 1).

(5) Arsenic Not more than 4.0 μg/g As_2O_3 (0.50 g, Method 1, Apparatus B).

(6) Reducing sugars Not more than 0.50% as D-glucose.

Weigh 1.0 g of Sodium Gluconate, and proceed as directed in Purity (3) for Zinc Gluconate. Titrate excess iodine with 0.1 mol/L sodium thiosulfate. The volume of the sodium thiosulfate solution consumed is not less than 8.15 ml.

Loss on Drying Not more than 0.30% (105°C, 2 hours).

Assay Weigh accurately about 0.15 g of Sodium Gluconate, previously dried, and dissolve it in 75 ml of acetic acid, and titrate with 0.1 mol/L perchloric acid solution until the red color of the solution disappears (indicator: 10 drops of quinaldine red TS). Perform a blank test in the same manner.

Each ml of 0.1 mol/L perchloric acid = 21.81 mg of $C_6H_{11}NaO_7$

Sodium Hydrogen Sulfite Solution

亜硫酸水素ナトリウム液

Content Sodium Hydrogen Sulfite Solution contains not less than 34.0% of sodium hydrogen sulfite ($NaHSO_3$ = 104.06).

Description Sodium Hydrogen Sulfite Solution is a light yellow liquid having an odor of sulfur dioxide.

Identification Diluted Sodium Hydrogen Sulfite Solution (1 in 5) responds to all tests for Sodium Salt and for Sulfite in the Qualitative Tests.

Purity

(1) Clarity of solution Very slightly turbid (3.0 g, water 20 ml).

(2) Heavy metals Not more than 4.0 μg/g as Pb.

Test Solution Weigh 5.0 g of Sodium Hydrogen Sulfite Solution, add 15 ml of boiling water and 5 ml of hydrochloric acid, and evaporate to dryness on a water bath. To the residue, add 10 ml of hot water and 2 ml of hydrochloric acid, and evaporate to dryness on a water bath again. To this residue, add 2 ml of diluted acetic acid (1 in 20) and water to dissolve, and make 50 ml. Filter if necessary.

Control Solution Measure 2.0 ml of Lead Standard Solution, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(3) Arsenic Not more than 2.0 μg/g as As_2O_3.

Test Solution Weigh 10 g of Sodium Hydrogen Sulfite Solution, and add water to make 25 ml. Measure 5 ml of this solution, add 2 ml of sulfuric acid, and heat on a water bath until sulfur dioxide no longer evolves. Evaporate to about 2 ml, and add water to make 10 ml. Use 5 ml of this solution as the test solution.

Apparatus Use Apparatus B.

Assay Weigh accurately about 0.5 g of Sodium Hydrogen Sulfite Solution, and proceed as directed under Sulfite Determination.

Each ml of 0.05 mol/L iodine = 5.203 mg of $NaHSO_3$

Sodium Hydrosulfite

Hydrosulfite

次亜硫酸ナトリウム

$Na_2S_2O_4$ Mol. Wt. 174.11

Sodium dithionite [7775-14-6]

Content Sodium Hydrosulfite contains not less than 85.0% of sodium hydrosulfite ($Na_2S_2O_4$).

Description Sodium Hydrosulfite occurs as a white to bright grayish white crystalline powder. It is odorless or has a slight odor of sulfur dioxide.

Identification

(1) To 10 ml of a solution of Sodium Hydrosulfite (1 in 100), add 2 ml of cupric sulfate solution (1 in 20). A gray-black color develops.

(2) To 10 ml of a solution of Sodium Hydrosulfite (1 in 100), add 1 ml of potassium permanganate solution (1 in 300). The color of the solution disappears immediately.

(3) Sodium Hydrosulfite responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Clarity of solution Slightly turbid.

Test Solution To 10 ml of formalin, add 10 ml of water, and neutralize with sodium hydroxide solution (1 in 25). Dissolve 0.50 g of Sodium Hydrosulfite in 10 ml of the solution obtained, and allow to stand for 5 minutes.

(2) Heavy metals Not more than 10 μg/g as Pb.

Sample Solution Weigh 5.0 g of Sodium Hydrosulfite, dissolve it in 30 ml of boiling water, and add 5 ml of hydrochloric acid. Evaporate to dryness on a water bath, add 15 ml of boiling water and 5 ml of hydrochloric acid to the residue, and evaporate to dryness on a water bath again. Dissolve the residue with water to make about 20 ml, filter, and add water to make 25 ml.

Test Solution Measure 10 ml of the sample solution, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(3) Zinc Not more than 80 μg/g as Zn.

Procedure Measure 5 ml of the sample solution prepared in Purity (2) above, add 0.1 ml of ammonia TS, filter, and transfer the filtrate into a Nessler tube. Add water to make 20 ml, add 5 ml of diluted hydrochloric acid (1 in 4) and 0.1 ml of freshly prepared potassium ferrocyanide solution (1 in 10), and allow to stand for 15 minutes. The solution is not more turbid than a control solution prepared as follows: Place 8.0 ml of Zinc Standard Solution into a Nessler tube, add water to make 20 ml, add 5 ml of diluted hydrochloric acid (1 in 4) and 0.1 ml of freshly prepared potassium ferrocyanide solution (1 in 10), and allow to stand for 15 minutes.

(4) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 5.0 g of Sodium Hydrosulfite, and dissolve it in water to make 25 ml. Measure 5 ml of this solution, add 1 ml of sulfuric acid, evaporate to about 2 ml, and add water to make 10 ml. Use 5 ml of this solution as the test solution.

Apparatus Use Apparatus B.

(5) Disodium ethylenediaminetetraacetate Weigh 0.5 g of Sodium Hydrosulfite, dissolve it in 5 ml of water, add 2 ml of potassium chromate solution (1 in 200) and 2 ml of arsenic trioxide TS, and heat in a water bath for 2 minutes. No purple color develops.

(6) Formate Not more than 0.050% as HCHO.

Procedure Weigh 1.0 g of Sodium Hydrosulfite, and dissolve it in water to make 1,000 ml. Measure 10 ml of this solution, add 5 ml of diluted hydrochloric acid (1 in 2), and add about 0.3 g of magnesium dust in small portions. After effervescence is almost no longer evolved, cover with a watch glass, and allow to stand for 2 hours. Measure 1 ml of this solution, add 2 ml of sulfuric acid and 0.5 ml of chromotropic acid TS, and heat in a water bath for 10 minutes. The color of the solution is not darker than that of a control solution prepared as follows: To 1.0 ml of Dilute Formaldehyde Standard Solution, add 5 ml of diluted hydrochloric acid (1 in 2), and thereafter treat in the same manner as the sample.

Assay Add 10 ml of water to 10 ml of formalin, and neu-

tralize with sodium hydroxide solution (1 in 25). To the resulting solution, add about 2 g of Sodium Hydrosulfite, accurately weighed, add water to dissolve it, and make exactly 500 ml. Measure exactly 25 ml of this solution, adjust the pH to 1.1–1.5 with diluted hydrochloric acid (1 in 10), and titrate with 0.05 mol/L iodine for sodium hydrosulfite (indicator: starch TS).

Each ml of 0.05 mol/L iodine = 4.353 mg of $Na_2S_2O_4$

Sodium Hydroxide

Caustic Soda

水酸化ナトリウム

$NaOH \cdot nH_2O$ (n=1 or 0) Mol. Wt. monohydrate 58.01
anhydrous 40.00

Sodium hydroxide monohydrate [12200-64-5]
Sodium hydroxide [1310-73-2]

Definition Sodium Hydroxide occurs in two forms: the crystalline form called Sodium Hydroxide (crystal) and the anhydrous form called Sodium Hydroxide (anhydrous). Sodium Hydroxide (crystal) is a mixture of sodium hydroxide (NaOH) and sodium hydroxide monohydrate ($NaOH \cdot H_2O$).

Content Sodium Hydroxide (crystal) contains 70.0–75.0% of sodium hydroxide (NaOH). Sodium Hydroxide (anhydrous) contains not less than 95.0% of sodium hydroxide (NaOH).

Description Sodium Hydroxide (crystal) occurs as a white crystalline powder or granules. Sodium Hydroxide (anhydrous) occurs as white lumps having various shapes including pellets, flakes, and rods, or as a white powder.

Identification

(1) A solution of Sodium Hydroxide (1 in 50) is strongly alkaline.

(2) Sodium Hydroxide responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Clarity and color of solution Colorless and almost clear.

Test Solution Weigh 50 g of Sodium Hydroxide, and dissolve it in freshly boiled and cooled water to make 250 ml. Use this solution as the sample solution. Measure 5.0 ml of the sample solution, and mix with 20 ml of water.

(2) Sodium carbonate The content of sodium carbonate (Na_2CO_3) obtained in the Assay is not more than 2.0%.

(3) Heavy metals Not more than 30 μg/g as Pb.

Test Solution Measure exactly 5 ml of the sample solution prepared in Purity (1) above, neutralize by gradually adding diluted hydrochloric acid (1 in 4), and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution To 3.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(4) Mercury Not more than 0.10 μg/g as Hg.

Test Solution Measure exactly 10 ml of the sample solution prepared in Purity (1) above, add 1 ml of potassium permanganate solution (3 in 50) and about 30 ml of water, and shake. Neutralize by gradually adding purified hydrochloric acid, add 5 ml of diluted sulfuric acid (1 in 2), and cool.

Control Solution To 2.0 ml of Mercury Standard Solution, add 1 ml of potassium permanganate solution (3 in 50), 30 ml of water, and the same amount of purified hydrochloric acid as used for preparing the test solution. Then add 5 ml of diluted sulfuric acid (1 in 2), and cool.

Procedure To the test solution, add hydroxylamine hydrochloride solution (1 in 5) until the purple color of the potassium permanganate disappears and the precipitate of manganese dioxide dissolves, add water to make 100 ml, and transfer into a gas washing bottle for the atomic absorption spectrophotometer. Add 10 ml of stannous chloride TS, immediately connect with the atomic absorption spectrophotometer, and set off the diaphragm pump to circulate the air. When the reading on the recorder increases rapidly and then indicates a constant value, measure the absorbance. The absorbance is not greater than that of the control solution treated in the same manner as the test solution.

(5) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Measure 2.5 ml of the sample solution prepared in Purity (1), add 5 ml of water, and neutralize by gradually adding hydrochloric acid.

Apparatus Use Apparatus B.

Assay Weigh accurately about 50 g of Sodium Hydroxide, and add freshly boiled and cooled water to make exactly 1,000 ml. Use this solution as the sample solution. Measure exactly 25 ml of the sample solution, add 10 ml of freshly boiled and cooled water, and titrate with 1 mol/L hydrochloric acid (indicator: 1 ml of bromophenol blue TS). When the solution reaches neutral, add exactly 1 ml of 1 mol/L hydrochloric acid, boil for about 5 minutes, and cool. Titrate the excess acid with 0.1 mol/L sodium hydroxide, and determine the volume (a ml) of 1 mol/L hydrochloric acid consumed. Separately, measure exactly 25 ml of the sample solution, transfer into a flask with a ground-glass stopper, and add 25 ml of freshly boiled and cooled water. To the solution, add 10 ml of barium chloride solution (3 in 25), stopper, shake gently, and titrate with 1 mol/L hydrochloric acid (indicator: 1 ml of phenolphthalein TS). Record the volume of 1 mol/L hydrochloric acid consumed as b (ml).

$$\text{Content (\%) of sodium hydroxide (NaOH)} = \frac{0.04000 \times b \times 40}{\text{Weight (g) of the sample}} \times 100$$

$$\text{Content (\%) of sodium carbonate } (Na_2CO_3) = \frac{0.05299 \times (a - b) \times 40}{\text{Weight (g) of the sample}} \times 100$$

Sodium Hydroxide Solution

水酸化ナトリウム液

Content Sodium Hydroxide Solution contains 95–120% of the labeled content of sodium hydroxide (NaOH = 40.00).

Description Sodium Hydroxide Solution is a colorless or slightly colored liquid.

Identification

(1) Diluted Sodium Hydroxide Solution (1 in 50) is

strongly alkaline.

(2) Sodium Hydroxide Solution responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Clarity and color of solution Colorless and almost clear.

Sample Solution To Sodium Hydroxide Solution, add freshly boiled and cooled water to prepare the equivalent of 20% (w/v) solution of NaOH, calculated from the labeled content.

Test Solution Measure 5.0 ml of the sample solution, and mix with 20 ml of water.

(2) Sodium Carbonate Not more than 2.0% as Na_2CO_3 per NaOH.

Proceed as directed in Purity (2) for Sodium Hydroxide.

(3) Heavy metals Not more than 30 μg/g of NaOH as Pb.

Proceed as directed in Purity (3) for Sodium Hydroxide.

(4) Mercury Not more than 0.10 μg/g of NaOH as Hg.

Proceed as directed in Purity (4) for Sodium Hydroxide.

(5) Arsenic Not more than 4.0 μg/g of NaOH as As_2O_3.

Proceed as directed in Purity (5) for Sodium Hydroxide.

Assay Weigh accurately an amount of Sodium Hydroxide Solution equivalent to about 5 g of sodium hydroxide (NaOH). Add freshly boiled and cooled water to make exactly 100 ml, and use this solution as the sample solution. Measure exactly 25 ml of the sample solution, and proceed as directed in the Assay for Sodium Hydroxide.

$$\text{Content (\%) of sodium hydroxide (NaOH)} = \frac{0.04000 \times b \times 4}{\text{Weight (g) of the sample}} \times 100$$

$$\text{Content (\%) of sodium carbonate } (Na_2CO_3) \text{ per sodium hydroxide (NaOH)} = \frac{0.05299 \times (a - b) \times 4}{\text{Weight (g) of the sample}} \times \frac{100}{\text{Content (\%) of sodium hydroxide}}$$

Sodium Hypochlorite

Hypochlorite of Soda

次亜塩素酸ナトリウム

NaClO Mol. Wt. 74.44

Sodium hypochlorite

Content Sodium Hypochlorite contains not less than 4.0% of available chlorine.

Description Sodium Hypochlorite is a colorless to light green-yellow liquid having an odor of chlorine.

Identification

(1) Sodium Hypochlorite responds to all tests for Sodium Salt and for Chlorite in the Qualitative Tests.

(2) To 4 ml of a solution of Sodium Hypochlorite (1 in 25), add 100 ml of phosphate buffer (pH 8), and measure the absorbance. The solution exhibits an absorption maximum at a wavelength of 291–294 nm.

(3) Dip a red litmus paper in Sodium Hypochlorite. The color changes to blue, and then fades.

Assay Weigh accurately about 3 g of Sodium Hypochlorite, and add 50 ml of water. Add 2 g of potassium iodine and 10 ml of diluted acetic acid (1 in 4), immediately stopper tightly, and allow to stand in a dark place for 15 minutes. Titrate the liberated iodine with 0.1 mol/L sodium thiosulfate (indicator: starch TS). Perform a blank test in the same manner, and make any necessary correction.

Each ml of 0.1 mol/L sodium thiosulfate = 3.545 mg of Cl

Sodium Iron Chlorophyllin

鉄クロロフィリンナトリウム

Description Sodium Iron Chlorophyllin occurs as a green-black powder. It is odorless or has a slight, characteristic odor.

Identification

(1) Place 1 g of Sodium Iron Chlorophyllin in a ceramic crucible, and add a small amount of sulfuric acid to moisten. Heat the crucible gradually to almost incinerate the sample at as low temperature as possible, and cool. Again add 1 ml of sulfuric acid, gradually heat until sulfuric acid vapor no longer develops, and cool. To the residue, add 10 ml of diluted hydrochloric acid (1 in 4), and dissolve by heating on a water bath. Filter if necessary, and add water to make 10 ml. Make the resulting solution weakly alkaline with ammonia TS, add 10 ml of hydrogen sulfide TS, allow to stand for 30 minutes, and filter. Perform the following tests for the filtrate and the residue on the filter paper.

(i) To the filtrate, add 1 ml of diluted hydrochloric acid (1 in 4), and perform the Flame Coloration Test. A yellow color is imparted to the flame.

(ii) Dissolve the residue on the filter paper by adding 2 ml of diluted nitric acid (1 in 10), and add water to make 5 ml. To the resulting solution, add 2–3 drops of ammonium thiocyanate solution (2 in 25). A red color develops.

(2) To 1 ml of a solution of Sodium Iron Chlorophyllin (1 in 1,000), add phosphate buffer (pH 7.5) to make 100 ml, and measure the absorbance. The solution exhibits absorption maxima at wavelengths of 396–400 nm and 652–658 nm. When the absorbances at the absorption maxima are expressed as A_1 and A_2, respectively, A_1/A_2 is not more than 9.5.

Purity

(1) Specific absorbance $E^{1\%}_{1cm}$ (maximum absorbance wavelength near 398 nm): Not less than 400 (on the dried basis).

This test should be protected from direct light, and the apparatus used in the test should be light-resistant.

Weigh accurately about 0.1 g of Sodium Iron Chlorophyllin, and dissolve it in water to make exactly 100 ml. Measure exactly 1 ml of the this solution, add phosphate buffer (pH 7.5) to make exactly 100 ml, and promptly measure the absorbance.

(2) pH 9.5–11.0 (1.0 g, water 100 ml).

(3) Inorganic iron salt Not more than 0.09% as Fe.

Test Solution Weigh 1.0 g of Sodium Iron Chlorophyllin, and dissolve it in 60 ml of water.

Procedure Analyze a 2-μl portion of the test solution by thin-layer chromatography using a 4:2:1 mixture of 1-buta-

nol/water/acetic acid as the developing solvent. No control solution is used. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm, and air-dry the plate. Spray with sodium ferrocyanide solution (1 in 1,000). No blue spot is observed.

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Loss on Drying Not more than 5.0% (105°C, 2 hours).

Proceed as directed in Purity (9) for Lactic Acid, using 1.0 ml of this solution.

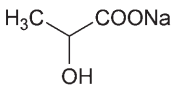
Assay Weigh accurately an amount of Sodium Lactate equivalent to about 0.3 g of sodium lactate, and evaporate to dryness on a water bath. Completely dissolve the residue in 60 ml of a 4:1 mixture of acetic acid/acetic anhydride, and titrate with 0.1 mol/L perchloric acid (indicator: 1 ml of crystal violet–acetic acid TS) until the color of the solution changes to blue. Perform a blank test in the same manner, and make any necessary correction.

Each ml of 0.1 mol/L perchloric acid = 11.21 mg of $C_3H_5NaO_3$

Sodium Lactate

Sodium Lactate Solution

乳酸ナトリウム



$C_3H_5NaO_3$ Mol. Wt. 112.06

Monosodium 2-hydroxypropanoate [72-17-3]

Content Sodium Lactate contains not less than 40.0% of sodium lactate ($C_3H_5NaO_3$) and the equivalent of 95–110% of the labeled content.

Description Sodium Lactate is a colorless, clear, syrupy liquid. It is odorless or has a slight, characteristic odor.

Identification Sodium Lactate responds to all tests for Sodium Salt and for Lactate in the Qualitative Tests.

Purity

(1) pH 6.5–7.5.

To 1.0 ml of Sodium Lactate, add 5 ml of water, and shake. Measure the pH of this solution.

(2) Sulfate Not more than 0.012% as SO_4 for 60% sodium lactate (an amount equivalent to 0.60 g of sodium lactate, Control solution 0.005 mol/L sulfuric acid 0.25 ml).

(3) Heavy metals Not more than 20 μg/g as Pb for 60% sodium lactate (an amount equivalent to 0.60 g of sodium lactate, Method 1, Control solution Lead Standard Solution 2.0 ml).

(4) Iron Not more than 10 μg/g as Fe for 60% sodium lactate (an amount equivalent to0.60 g of sodium lactate, Method 1, Control solution Iron Standard Solution 1.0 ml).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 for 60% sodium lactate.

Test Solution Weigh an amount of Sodium Lactate equivalent to 0.60 g of sodium lactate, and add water to make 10 ml. Use 5 ml of this solution.

Apparatus Use Apparatus B.

(6) Volatile fatty acids Weigh 5 g of Sodium Lactate, add 2 ml of diluted sulfuric acid (1 in 20), and heat on a water bath. No butyric acid-like odor is evolved.

(7) Methanol Not more than 0.20% v/w as CH_3OH for 60% sodium lactate.

Weigh an amount of Sodium Lactate equivalent to 3.0 g of sodium lactate, add 8 ml of water, distill the solution, collect 5 ml of the initial distillate, and add water to make 100 ml.

Sodium DL-Malate

Sodium *dl*-Malate
Sodium Malate

DL-リンゴ酸ナトリウム

n=3 or 1/2

$C_4H_4Na_2O_5 \cdot nH_2O$ (n=3 or 1/2) Mol. Wt. trihydrate 232.10
hemihydrate 187.06

Disodium (2*RS*)-2-hydroxybutanedioate trihydrate
Disodium (2*RS*)-2-hydroxybutanedioate hemihydrate
[anhydrous 676-46-0]

Definition Sodium DL-Malate occurs as trihydrate and hemihydrate.

Content Sodium DL-Malate, when dried, contains 98.0–102.0% of sodium DL-malate ($C_4H_4Na_2O_5$ = 178.05).

Description Sodium DL-Malate occurs as white crystalline powder or lumps. It is odorless and has a salty taste.

Identification

(1) Place 1 ml of a solution of Sodium DL-Malate (1 in 20) into a porcelain dish, add 0.010 g of sulfanilic acid, and proceed as directed in Identification (1) in DL-Malic Acid.

(2) Proceed as directed in Identification (2) for DL-Malic Acid.

(3) Sodium DL-Malate responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Clarity and color of solution Colorless and clear (1.0 g, water 10 ml).

(2) Free alkali Not more than 0.2% as Na_2CO_3.

Weigh 1.0 g of Sodium DL-Malate, dissolve it in 20 ml of freshly boiled and cooled water, and add 2 drops of phenolphthalein TS. A pink color develops, and it disappears on the addition of 0.40 ml of 0.05 mol/L sulfuric acid.

(3) Chloride Not more than 0.011% as Cl (1.0 g, Control solution 0.01 mol/L hydrochloric acid 0.30 ml).

(4) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of Sodium DL-Malate, dissolve it in 30 ml of water, neutralize with diluted hydrochloric acid (1 in 100), add 2 ml of diluted acetic acid (1 in 20) and

water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

(6) Readily oxidizable substances Weigh 0.10 g of Sodium DL-Malate, dissolve by adding 25 ml of water and 25 ml of diluted sulfuric acid (1 in 20), keep at 20°C, and add 1.0 ml of 0.02 mol/L potassium permanganate. The pink color of the solution does not disappear within 3 minutes.

Loss on Drying

Trihydrate: 20.5–23.5% (130°C, 4 hours).

Hemihydrate: Not more than 7.0% (130°C, 4 hours).

Assay Weigh accurately about 0.15 g of Sodium DL-Malate, previously dried, dissolve it in 30 ml of acetic acid for nonaqueous titration, and titrate with 0.1 mol/L perchloric acid. The endpoint is usually confirmed by using a potentiometer. When crystal violet–acetic acid TS (1 ml) is used as the indicator, the endpoint is when the color of the solution changes from purple through blue to green. Perform a blank test in the same manner, and make any necessary correction.

Each ml of 0.1 mol/L perchloric acid = 8.903 mg of $C_4H_4Na_2O_5$

Sodium Metaphosphate

メタリン酸ナトリウム

Content Sodium Metaphosphate, when dried, contains the equivalent of 60.0–83.0% phosphorus(V) oxide (P_2O_5 = 141.94).

Description Sodium Metaphosphate occurs as white fibrous crystals or powder or as colorless to white glassy flakes or lumps.

Identification

(1) To a solution of Sodium Metaphosphate (1 in 40), add diluted acetic acid (1 in 20) or sodium hydroxide solution (1 in 20) to make it weakly acidic, and add 5 ml of egg white TS. A white precipitate is formed.

(2) Sodium Metaphosphate responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Clarity and color of solution Colorless and very slightly turbid (powder 1.0 g, water 20 ml).

(2) Chloride Not more than 0.21% as Cl (powder 0.10 g, Control solution 0.01 mol/L hydrochloric acid 0.60 ml).

(3) Orthophosphate Weigh 1.0 g of powdered Sodium Metaphosphate, and add 2–3 drops of silver nitrate solution (1 in 50). No brilliant yellow color develops.

(4) Sulfate Not more than 0.048% as SO_4.

Test Solution Weigh 0.40 g of powdered Sodium Metaphosphate, add 30 ml of water and 2 ml of diluted hydrochloric acid (1 in 4), dissolve by boiling for 1 minute, cool, and add water to make 50 ml.

Control Solution To 0.40 ml of 0.005 mol/L sulfuric acid, add 1 ml of diluted hydrochloric acid (1 in 4) and water to make 50 ml.

(5) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of powdered Sodium Metaphosphate, dissolve it in 30 ml of water, neutralize with diluted acetic acid (1 in 20) or ammonia TS, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Measure exactly 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (powder 0.50 g, Method 1, Apparatus B).

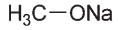
Loss on Drying Not more than 5.0% (110°C, 4 hours).

Assay Proceed as directed in the Assay for Potassium Polyphosphate.

Sodium Methoxide

Sodium Methylate

ナトリウムメトキシド



CH_3ONa Mol. Wt. 54.02

Sodium methoxide [124-41-4]

Content Sodium Methoxide contains not less than 95.0% of sodium methoxide (CH_3ONa).

Description Sodium Methoxide occurs as a white, hygroscopic, fine powder.

Identification

(1) A solution of Sodium Methoxide (1 in 100) is alkaline.

(2) To 1 drop of a solution of Sodium Methoxide (1 in 100), add 0.1 ml of diluted sulfuric acid (1 in 20) and 0.2 ml of potassium permanganate solution (1 in 300), and allow to stand for 5 minutes. Add 0.2 ml of anhydrous sodium sulfite solution (1 in 5) and 3 ml of sulfuric acid, and then add 0.2 ml of chromotropic acid TS. A red-purple to purple color develops.

(3) Sodium Methoxide responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Clarity of solution Very slightly turbid.

Test Solution Weigh 5.0 g of Sodium Methoxide, and dissolve it in freshly boiled and cooled water to make 100 ml. Use this solution as the sample solution. Measure 20 ml of the sample solution, add 30 ml of freshly boiled and cooled water.

(2) Sodium carbonate Not more than 0.5% as Na_2CO_3.

Proceed as directed in Assay (iii).

(3) Sodium hydroxide Not more than 2.0% as NaOH.

Proceed as directed in Assay (iv).

(4) Heavy metals Not more than 25 μg/g as Pb.

Test Solution Measure 16 ml of the sample solution prepared in Purity (1), neutralize by gradually adding diluted hydrochloric acid (1 in 4), and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution To exactly 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(5) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Measure 10 ml of the sample solution prepared in Purity (1) above, neutralize by gradually adding diluted hydrochloric acid (1 in 4), and evaporate to dryness on a water bath. Dissolve the residue by adding 5 ml of water.

Apparatus Use Apparatus B.

Assay

(i) Promptly and accurately weigh about 0.5 g of Sodium Methoxide, using a titration flask for the Karl Fischer method, immediately add 10 ml of salicylic acid–methanol TS, stopper tightly, dissolve, and cool. Proceed as directed under Direct Titration in Water Determination (Karl Fischer Method). Perform a blank test on 10 ml of salicylic acid–methanol TS in the same manner, and calculate the sum (A) of the contents of sodium hydroxide and sodium carbonate as sodium hydroxide (NaOH) by the formula:

$$\text{A (\%)} = \frac{(a - b) \times f \times 2.222}{\text{Weight (g) of the sample} \times 1{,}000} \times 100$$

a = volume (ml) of water determination TS consumed in the test,
b = volume (ml) of water determination TS consumed in the blank test,
f = number of mg of water equivalent to 1 ml of water determination TS.

(ii) Quickly and accurately weigh about 2 g of Sodium Methoxide in an Erlenmeyer flask with a ground-glass stopper, and immediately dissolve by gently adding about 50 ml of freshly boiled and cooled water. Add 10 ml of barium chloride solution (3 in 25), stopper, allow to stand for 5 minutes, and titrate with 1 mol/L hydrochloric acid (indicator: 2 drops of phenolphthalein TS). Calculate the sum (B) of the contents of sodium methoxide and sodium hydroxide as sodium methoxide (CH_3ONa) by the formula:

$$\text{B (\%)} = \frac{0.054 \times \left(\begin{array}{c}\text{Volume (ml) of 1 mol/L} \\ \text{hydrochloric acid consumed}\end{array}\right)}{\text{Weight (g) of the sample}} \times 100$$

(iii) Add 1 ml of 1 mol/L hydrochloric acid to the solution left after titration in (ii) above, boil gently for about 5 minutes, cool, and titrate the excess acid with 0.1 mol/L sodium hydroxide. Calculate the content (C) of sodium carbonate (Na_2CO_3) by the formula:

$$\text{C (\%)} = \frac{0.053 \times \left[1 - \left(\begin{array}{c}\text{Volume (ml) of 0.1 mol/L} \\ \text{sodium hydroxide consumed}\end{array}\right) \times 0.1\right]}{\text{Weight (g) of the sample}} \times 100$$

(iv) Calculate the content (D) of sodium hydroxide (NaOH) by the formula:

$$\text{D (\%)} = \text{A} - (\text{C} \times 0.377)$$

(v) Calculate the content (E) of sodium methoxide (CH_3ONa) by the formula:

$$\text{E (\%)} = \text{B} - (\text{D} \times 1.350)$$

Storage Standards Store in a hermetic container.

Sodium Nitrate

硝酸ナトリウム

$NaNO_3$ Mol. Wt. 84.99

Sodium nitrate [7631-99-4]

Content Sodium Nitrate, when dried, contains not less than 99.0% of sodium nitrate ($NaNO_3$).

Description Sodium Nitrate occurs as colorless crystals or as a white crystalline powder. It is odorless and has a slightly salty taste.

Identification Sodium Nitrate responds to all tests for Sodium Salt and for Nitrate in the Qualitative Tests.

Purity

(1) Clarity and color of solution Colorless and clear.

Proceed as directed in Purity (1) for Potassium Nitrate.

(2) Chloride Not more than 0.21% as Cl (0.10 g Control solution 0.01 mol/L hydrochloric acid 0.60 ml).

(3) Heavy metals Not more than 20 μg/g as Pb.

Proceed as directed in Purity (3) for Potassium Nitrate.

(4) Arsenic Not more than 4.0 μg/g as As_2O_3.

Proceed as directed in Purity (4) for Potassium Nitrate.

Loss on Drying Not more than 1.0% (105°C, 4 hours).

Assay Proceed as directed in the Assay for Potassium Nitrate.

Each ml of 0.05 mol/L sulfuric acid = 8.499 mg of $NaNO_3$

Sodium Nitrite

亜硝酸ナトリウム

$NaNO_2$ Mol. Wt. 69.00

Sodium Nitrite [7632-00-0]

Content Sodium Nitrite, when dried, contains not less than 97.0% of sodium nitrite ($NaNO_2$).

Description Sodium Nitrite occurs as a white to light yellow crystalline powder or granular or rod-shaped lumps.

Identification Sodium Nitrite responds to all tests for Sodium Salt and for Nitrite in the Qualitative Tests.

Purity

(1) Clarity of solution Almost clear (1.0 g, water 20 ml).

(2) Chloride Not more than 0.71% as Cl.

Test Solution Weigh 1.0 g of Sodium Nitrite, and dissolve it in water to make 500 ml. Measure 10 ml of this solution, add 3 ml of diluted acetic acid (1 in 4), and warm gradually. After the gas is no longer evolved, add 6 ml of diluted nitric acid (1 in 10), and add water to make 50 ml.

Control Solution To 0.40 ml of 0.01 mol/L hydrochloric acid, add 3 ml of diluted acetic acid (1 in 4), 6 ml of diluted nitric acid (1 in 10), and water to make 50 ml.

(3) Sulfate Not more than 0.24% as SO_4.

Test Solution Weigh 1.0 g of Sodium Nitrite and dissolve it in water to make 100 ml. Measure 10 ml of this solution,

add 1 ml of hydrochloric acid, evaporate to dryness in a water bath, dissolve the residue in 1 ml of diluted hydrochloric acid (1 in 4) and 20 ml of water, and add water to make 50 ml.

Control Solution Measure 0.50 ml of 0.005 mol/L sulfuric acid, add 1 ml of hydrochloric acid, evaporate to dryness in a water bath, and then proceed as directed for the test solution.

(4) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of Sodium Nitrite, dissolve it in 10 ml of water, add 1 ml of hydrochloric acid, evaporate to dryness in a water bath, and continue heating in a water bath until the odor of hydrochloric acid disappears. Dissolve the residue by adding 2 ml of diluted acetic acid (1 in 20) and 20 ml of water, and further add water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, add 1 ml of hydrochloric acid, evaporate to dryness in a water bath, and then proceed as directed for the test solution.

(5) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.50 g of Sodium Nitrite, dissolve it in 5 ml of water, add 2 ml of hydrochloric acid, evaporate to dryness in a water bath, and dissolve the residue in 5 ml of water.

Apparatus Use Apparatus B.

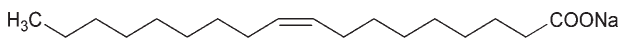
Loss on Drying Not more than 3.0% (100°C, 5 hours).

Assay Weigh accurately about 1 g of Sodium Nitrite, previously dried, dissolve it in water to make exactly 100 ml, and refer to this solution as solution A. Weigh exactly 40 ml of 0.02 mol/L potassium permanganate, transfer into an Erlenmeyer flask, and add 100 ml of water and 5 ml of sulfuric acid. To this solution, add exactly 10 ml of solution A while keeping the tip of the pipet below the surface of the liquid. Allow to stand for 5 minutes, add exactly 25 ml of 0.05 mol/L oxalic acid, warm to about 80°C, and titrate the excess oxalic acid with 0.02 mol/L potassium permanganate while hot.

Each ml of 0.02 mol/L potassium permanganate = 3.450 mg of $NaNO_2$

Sodium Oleate

オレイン酸ナトリウム



$C_{18}H_{33}NaO_2$ Mol. Wt. 304.44

Monosodium (9*Z*)-octadec-9-enoate [143-19-1]

Description Sodium Oleate occurs as a white to yellowish powder or as a light brown-yellow, coarse powder or lumps. It has a characteristic odor and taste.

Identification

(1) To 50 ml of a solution of Sodium Oleate (2 in 50), add 5 ml of diluted sulfuric acid (1 in 20) while stirring, filter through a filter paper moistened previously with water, and wash the residue with water until the washings no longer shows acidity to methyl orange TS. Filter the oily residue through a dry filter paper, transfer 2–3 drops of the oily solution into a small test tube, and superimpose about a 1-ml layer of sulfuric acid. A brown-red band develops at the junction. Take another 1–3 drops of the oily solution, dissolve it in 3–4 ml of diluted acetic acid (1 in 4), add 1 drop of a solution of chromium trioxide in acetic acid (1 in 10), and add 10–30 drops of sulfuric acid while shaking. A dark purple color develops.

(2) The residue on ignition of Sodium Oleate responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Clarity of solution Almost clear (0.50 g, water 20 ml).

(2) Free Alkali Not more than 0.5%.

Weigh accurately about 5 g of powdered Sodium Oleate, add 100 ml of neutralized ethanol, and dissolve while heating. Filter the insoluble residue while the liquid is hot, wash with neutralized ethanol at about 40°C until the washings become colorless, and combine the filtrate and the washings. Cool, titrate with 0.05 mol/L sulfuric acid, and determine the volume consumed(a ml). Wash the above residue 5 times with 10 ml of boiling water each time, combine all the washings, cool, add 3 drops of bromophenol blue TS, titrate with 0.05 mol/L sulfuric acid, and determine the volume consumed (b ml). Calculate the content of free alkali by the formula:

$$\text{Content (\%) of free alkali} = \frac{(0.0040 \times a) + (0.0053 \times b)}{\text{Weight (g) of the sample}} \times 100$$

(3) Heavy metals Not more than 40 μg/g as Pb (0.50 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 5.0 g of Sodium Oleate, add 30 ml of boiling water, and dissolve while stirring well. Add 6 ml of diluted sulfuric acid (1 in 20) dropwise, remove the deposited fatty acid by extracting with diethyl ether, and add water to make 50 ml. Use 5 ml of this solution for the test.

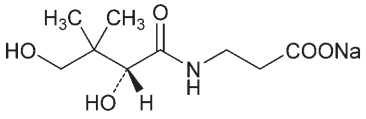
Apparatus Use Apparatus B.

Standard Color Measure 10.0 ml of Arsenic Standard Solution, add 30 ml of water and 6 ml of diluted sulfuric acid (1 in 20), and add water to make 50 ml. Measure 10.0 ml of this solution, and proceed in the same manner as the test solution.

Residue on Ignition 22.0–25.0%.

Sodium Pantothenate

パントテン酸ナトリウム



$C_9H_{16}NNaO_5$ Mol. Wt. 241.22

Monosodium 3-[(2*R*)-2,4-dihydroxy-3,3-dimethylbutanoylamino]propanoate [75033-16-8]

Content Sodium Pantothenate, when calculated on the

dried basis, contains 5.6–6.0% of nitrogen (N=14.01) and 9.3–9.7% of sodium (Na=22.99).

Description Sodium Pantothenate occurs as a white powder. It is odorless and has a slightly acid taste.

Identification

(1) Proceed as directed in Identification (1) and (2) for Calcium Pantothenate.

(2) A solution of Sodium Pantothenate (1 in 20) responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +25.0 to +28.5° (previously dried, 1.25 g, water, 25 ml).

(2) pH 9.0–10.0.

Weigh 2.0 g of Sodium Pantothenate, and add water to make 10 ml. Use this solution for the pH determination.

(3) Calcium Weigh 1.0 g of Sodium Pantothenate, dissolve it in 10 ml of water, and add 0.5 ml of diluted acetic acid (1 in 20) and 0.5 ml of ammonium oxalate solution (1 in 25). No precipitate is formed.

(4) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

(6) Alkaloid Proceed as directed in Purity (5) for Calcium Pantothenate.

Loss on Drying Not more than 5.0% (reduced pressure, 24 hours).

Assay

(1) Nitrogen Weigh accurately about 0.05 g of Sodium Pantothenate, proceed as directed in the Semi-micro Kjeldahl Method under Nitrogen Determination, and calculate on the dried basis.

(2) Sodium Weigh accurately about 0.6 g of Sodium Pantothenate, dissolve it in 50 ml of acetic acid, and titrate with 0.1 mol/L perchloric acid (indicator: 1 ml of crystal violet–acetic acid TS) until the color of the solution changes from purple through blue to green. Perform a blank test in the same manner, make any necessary correction, and calculate on the dried basis.

Each ml of 0.1 mol/L perchloric acid = 2.299 mg of Na

Sodium *o*-Phenylphenate

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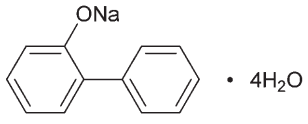

$C_{12}H_9NaO \cdot 4H_2O$ Mol. Wt. 264.25

Monosodium 2-phenylphenolate tetrahydrate

[anhydrous 132-27-4]

Content Sodium *o*-Phenylphenate, when calculated on the anhydrous basis, contains not less than 95.0% of sodium *o*-phenylphenate ($C_{12}H_9NaO$ = 192.19).

Description Sodium *o*-Phenylphenate occurs as a white or light pink to pink powder, flakes, or lumps having a characteristic odor.

Identification

(1) Proceed as directed in Identification (1) and (2) for *o*-Phenylphenol.

(2) Sodium *o*-Phenylphenate responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) pH 11.1–12.2 (1.0 g, water 50 ml).

(2) *o*-Phenylphenol Weigh 1.0 g of powdered Sodium o-Phenylphenate, dissolve it in 50 ml of water, add diluted hydrochloric acid (1 in 4) until the solution is weakly acidic, and allow to stand for 1 hour. Filter the formed precipitate, wash with a small amount of water, and dry in a desiccator (sulfuric acid) for 24 hours. The melting point is 55–58°C.

(3) Sodium hydroxide Not more than 1.0%.

Weigh accurately about 5 g of powdered Sodium *o*-Phenylphenate, dissolve it in 50 ml of 50% (vol) ethanol, and titrate with 1 mol/L hydrochloric acid (indicator: 1 ml of bromophenol blue TS). Calculate the content by the formula:

$$\text{Content (\%) of sodium hydroxide (NaOH)} = \left[\left(\begin{array}{c}\text{Volume (ml) of 1 mol/L}\\ \text{hydrochloric acid consumed}\end{array}\right) - \frac{\text{Weight (g) of the sample}}{0.264}\right] \times \frac{0.04}{\text{Weight (g) of the sample}} \times 100$$

(4) Heavy metals Not more than 20 μg/g as Pb (powdered sample 1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 5.0 g of powdered Sodium *o*-Phenylphenate, transfer into a Kjeldahl flask, add 20 ml of nitric acid, and heat weakly until the contents become fluid. After cooling, add 5 ml of sulfuric acid, and heat until white fumes are evolved. If the solution is still brown in color, cool, add 5 ml of nitric acid, and heat. Repeat this procedure until the color of the solution becomes colorless to light yellow. After cooling, add 15 ml of ammonium oxalate solution (1 in 25), and heat until white fumes are evolved again. After cooling, add water to make 25 ml, and use 5 ml of this solution as the test solution.

Apparatus Use Apparatus B.

Standard Color Place 10 ml of Arsenic Standard Solution into a Kjeldahl flask, add 20 ml of nitric acid, and then proceed in the same manner as the preparation of the test solution.

(6) *p*-Phenylphenol and other organic impurities Not more than 0.1%, as *p*-phenylphenol, of *o*-phenylphenol.

Test Solution Weigh 2.0 g of Sodium *o*-Phenylphenate, dissolve it in 100 ml of water, add diluted hydrochloric acid (1 in 4) until the solution is weakly acidic, and allow to stand for 1 hour. Filter the formed precipitate, wash with a small amount of water, and dry in a desiccator (sulfuric acid) for 24 hours. Weigh 1.0 g of the prepared sample, and add 5 ml of ethanol and 5 ml of a solution of caffeine in ethanol (1 in 1,000) to dissolve.

Procedure Proceed as directed in Purity (3) for *o*-Phenylphenol.

Water Content 25.0–28.0% (0.1 g, Direct Titration).

Use 20 ml of methanol for water determination and 10 ml of acetic acid instead of 25 ml of methanol for water determination.

Assay Weigh accurately about 3 g of powdered Sodium *o*-Phenylphenate, and dissolve it in several drops of sodium hydroxide solution (1 in 25) and water to make exactly 500 ml. Proceed as directed in the Assay for *o*-Phenylphenol, using this solution as the test solution.

$$\text{Content (\%) of sodium } o\text{-phenylphenate } (C_{12}H_9NaO) = \frac{4.805 \times (a - b)}{\text{Anhydrous basis weight (g) of the sample} \times 50} \times 100$$

a = volume (ml) of 0.1 mol/L sodium thiosulfate consumed in the blank test,
b = volume (ml) of 0.1 mol/L sodium thiosulfate consumed in this test.

Sodium Polyacrylate

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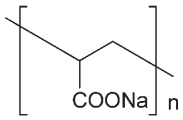


$(C_3H_3NaO_2)_n$
Poly(sodium 1-carboxylatoethylene)
Description Sodium Polyacrylate occurs as a white powder. It is odorless.
Identification
(1) To 10 ml of a solution of Sodium Polyacrylate (1 in 500), add 1 ml of magnesium sulfate TS, and shake. A white precipitate is formed.
(2) The residue on ignition of Sodium Polyacrylate responds to all tests for Sodium Salt in the Qualitative Tests.
Purity
(1) Free alkali Weigh 0.20 g of Sodium Polyacrylate, add 60 ml of water, and dissolve while shaking well. Add 3 ml of calcium chloride solution (3 in 40), heat on a water bath for about 20 minutes, cool, and filter. Wash the residue on the filter paper with water, combine the filtrate and the washings, and add water to make 100 ml. Refer to this solution as solution A. Measure 50 ml of solution A, and add 2 drops of phenolphthalein TS. No pink color develops.
(2) Sulfate Not more than 0.48% as SO_4.
Sample Solution 20 ml of solution A prepared in Purity (1).
Control Solution 0.40 ml of 0.005 mol/L sulfuric acid.
(3) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).
(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).
(5) Residual monomers No more than 1.0%.
Weigh accurately about 1 g of Sodium Polyacrylate, transfer into a 300-ml iodine bottle, add 100 ml of water, and dissolve by allowing to stand for about 24 hours with occasional shaking. Add exactly 10 ml of potassium bromate–potassium bromide TS, shake well, add quickly about 10 ml of hydrochloric acid, and immediately stopper tightly. Shake well, place about 20 ml of potassium iodide TS in the iodine bottle, and allow to stand in a dark place for 20 minutes. Loosen the stopper to allow the potassium iodide TS to flow into the solution, immediately stopper tightly, shake well, and titrate with 0.1 mol/L sodium thiosulfate (indicator: starch TS). Perform a blank test in the same manner, and calculate the content by the formula:

$$\text{Content (\%) of residual monomer} = \frac{0.0047 \times (a - b)}{\text{Weight (g) of the sample}} \times 100$$

a = volume (ml) of 0.1 mol/L sodium thiosulfate consumed in the blank test,
b = volume (ml) of 0.1 mol/L sodium thiosulfate consumed in this test.

(6) Low molecular weight polymers Not more than 5.0%.
Dry a glass filter (1G4) at 105°C for 30 minutes, allow to cool in a desiccator, and weigh accurately. Weigh accurately about 2 g of Sodium Polyacrylate, add 200 ml of water, and dissolve with occasional shaking. Add 50 ml of hydrochloric acid while stirring, warm in a water bath at 40°C for 30 minutes while stirring, and allow to stand for 24 hours. Filter the solution, add 1 drop of phenolphthalein TS to the filtrate, add sodium hydroxide solution (2 in 5) until the color of the filtrate changes to a slightly pink color, and then add diluted hydrochloric acid (1 in 30) dropwise until the pink color disappears. Add 200 ml of water, add 25 ml of calcium chloride solution (3 in 40) dropwise while stirring, and warm in a water bath at about 40°C for 30 minutes while stirring. Filter this solution with suction through the glass filter, previously prepared, wash the residue three times with about 10 ml of water each time, dry at 105°C for 3 hours, and allow to cool in a desiccator. Weigh accurately, and calculate the content by the formula:

$$\text{Content (\%) of low molecular weight polymers} = \frac{\text{Weight (g) of the residue} \times 1.032}{\text{Weight (g) of the sample}} \times 100$$

Loss on Drying Not more than 10.0% (105°C, 4 hours).
Residue on Ignition Not more than 76.0% (calculated on the dried basis).

Sodium Polyphosphate

ポリリン酸ナトリウム

Content Sodium Polyphosphate, when dried, contains the equivalent of 53.0–80.0% of phosphorus(V) oxide (P_2O_5 = 141.94).
Description Sodium Polyphosphate occurs as a white powder or as colorless to white glassy fragments or lumps.
Identification
(1) To 10 ml of a solution of Sodium Polyphosphate (1 in 100), add diluted acetic acid (1 in 20) to make weakly acidic, and add 1 ml of silver nitrate solution (1 in 50). A white precipitate is formed.

(2) Sodium Polyphosphate responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Clarity and color of solution Colorless and very slightly turbid.

Test Solution Weigh 1.0 g of powdered Sodium Polyphosphate, add 20 ml of water, and dissolve by heating.

(2) Chloride Not more than 0.21% as Cl (powder 0.10 g, Control solution 0.01 mol/L hydrochloric acid 0.60 ml).

(3) Orthophosphate Weigh 1.0 g of powdered Sodium Polyphosphate, and add 2–3 drops of silver nitrate solution (1 in 50). No brilliant yellow color develops.

(4) Sulfate Not more than 0.048% as SO_4.

Test Solution Weigh 0.40 g of powdered Sodium Polyphosphate, add 30 ml of water and 2 ml of diluted hydrochloric acid (1 in 4), dissolve by boiling for 1 minute, cool, and add water to make 50 ml.

Control Solution To 0.40 ml of 0.005 mol/L sulfuric acid, add 1 ml of diluted hydrochloric acid (1 in 4) and water to make 50 ml.

(5) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of powdered Sodium Polyphosphate, dissolve it in 20 ml of water, neutralize with diluted acetic acid (1 in 20) or ammonia TS, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

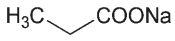
(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (powder 0.50 g, Method 1, Apparatus B).

Loss on Drying Not more than 5.0% (110°C, 4 hours).

Assay Proceed as directed in the Assay for Potassium Polyphosphate.

Sodium Propionate

プロピオン酸ナトリウム



$C_3H_5NaO_2$ Mol. Wt. 96.06

Monosodium propanoate [137-40-6]

Content Sodium Propionate, when dried, contains not less than 99.0% of sodium propionate ($C_3H_5NaO_2$).

Description Sodium Propionate occurs as white crystals, crystalline powder, or granules. It is odorless or has a slight, characteristic odor.

Identification

(1) Proceed as directed in Identification (1) for Calcium Propionate.

(2) Sodium Propionate responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Clarity and color of solution Colorless and slightly turbid (1.0 g, water 20 ml).

(2) Free acid and free alkali Proceed as directed in Purity (2) for Calcium Propionate.

(3) Heavy metals Not more than 10 μg/g as Pb.

Proceed as directed in Purity (3) for Calcium Propionate.

(4) Arsenic Not more than 4.0 μg/g as As_2O_3.

Proceed as directed in Purity (4) for Calcium Propionate.

Loss on Drying Not more than 5.0% (105°C, 1 hour).

Assay Weigh accurately about 0.25 g of Sodium Propionate, previously dried, dissolve it in 40 ml of acetic acid for nonaqueous titration, and warm if necessary. Titrate with 0.1 mol/L perchloric acid (indicator: 2 drops of crystal violet–acetic acid TS). Perform a blank test in the same manner, and make any necessary correction.

Each ml of 0.1 mol/L perchloric acid = 9.606 mg of $C_3H_5NaO_2$

Sodium Pyrophosphate

Tetrasodium Pyrophosphate
Tetrasodium Diphosphate

ピロリン酸四ナトリウム

$Na_4P_2O_7 \cdot nH_2O$ (n=10 or 0) Mol. Wt. decahydrate 446.06
anhydrous 265.90

Sodium diphosphate decahydrate [13472-36-1]

Sodium diphosphate [7722-88-5]

Definition Sodium Pyrophosphate occurs in two forms: the crystalline form (decahydrate) called Sodium Pyrophosphate (crystal) and the anhydrous form called Sodium Pyrophosphate (anhydrous).

Content Sodium Pyrophosphate, when dried, contains not less than 97.0% of sodium pyrophosphate ($Na_4P_2O_7$).

Description Sodium Pyrophosphate (crystal) occurs as colorless to white crystals or as a white crystalline powder. Sodium Pyrophosphate (anhydrous) occurs as a white powder or lumps.

Identification

(1) To 10 ml of a solution of Sodium Pyrophosphate (1 in 100), add diluted acetic acid (1 in 20) to make it slightly acidic, and add 1 ml of silver nitrate solution (1 in 50). A white precipitate is formed.

(2) Sodium Pyrophosphate responds to all tests for Sodium Salt in the Qualitative Tests.

Purity Dry Sodium Pyrophosphate before performing the following tests.

(1) Clarity and color of solution Colorless and slightly turbid (1.0 g, water 20 ml).

(2) pH 9.9–10.7 (1.0 g, water 100 ml).

(3) Chloride Not more than 0.21% as Cl (0.10 g, Control solution 0.01 mol/L hydrochloric acid 0.60 ml).

(4) Orthophosphate Weigh 1.0 g of Sodium Pyrophosphate, and add 2–3 drops of silver nitrate solution (1 in 50). No brilliant yellow color develops.

(5) Sulfate Not more than 0.038% as SO_4 (0.50 g, Control solution 0.005 mol/L sulfuric acid 0.40 ml).

(6) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of Sodium Pyrophosphate, dissolve it in 20 ml of water, neutralize with diluted acetic acid (1 in 20), and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solu-

tion, and add 2.0 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(7) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Drying

Crystal: Not more than 42.0% (110°C, 4 hours).

Anhydrous: Not more than 5.0% (110°C, 4 hours).

Assay Weigh accurately about 3 g of Sodium Pyrophosphate, previously dried, dissolve it in 75 ml of water, keep at about 15°C, and titrate with 1 mol/L hydrochloric acid (indicator: 3–4 drops of methyl orange–xylene cyanol FF TS).

Each ml of 1 mol/L hydrochloric acid = 133.0 mg of $Na_4P_2O_7$

Sodium Pyrosulfite

Sodium Acid Sulfite
Sodium Metabisulfite

ピロ亜硫酸ナトリウム

$Na_2S_2O_5$ Mol. Wt. 190.11

Sodium disulfite [7681-57-4]

Content Sodium Pyrosulfite contains not less than 93.0% of sodium pyrosulfite ($Na_2S_2O_5$).

Description Sodium Pyrosulfite occurs as a white powder having an odor of sulfur dioxide.

Identification Sodium Pyrosulfite responds to all tests for Sodium Salt and for Sulfite in the Qualitative Tests .

Purity

(1) Clarity of solution Very slightly turbid (0.50 g, water 10 ml).

(2) Heavy metals Not more than 10 μg/g as Pb.

Test Solution Weigh 2.0 g of Sodium Pyrosulfite, dissolve it in 15 ml of hot water, add 5 ml of hydrochloric acid, and evaporate to dryness on a water bath. To the residue, add 10 ml of hot water and 2 ml of hydrochloric acid, and evaporate to dryness on a water bath again. Dissolve this residue by adding 2 ml of diluted acetic acid (1 in 20) and 20 ml of water, add water to make 50 ml, and filter if necessary.

Control Solution To 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(3) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.50 g of Sodium Pyrosulfite, and dissolve it in 10 ml of water. Add 1 ml of sulfuric acid, heat on a hot plate until white fumes are evolved, and add water to make 5 ml.

Apparatus Use Apparatus B.

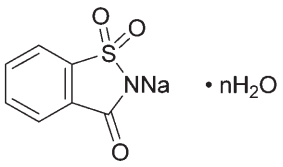
Assay Weigh accurately about 0.2 g of Sodium Pyrosulfite, and proceed as directed under Sulfite Determination.

Each ml of 0.05 mol/L iodine = 4.753 mg of $Na_2S_2O_5$

Sodium Saccharin

Soluble Saccharin

サッカリンナトリウム



n=2 or 0

$C_7H_4NNaO_3S{\cdot}nH_2O$ (n=2 or 0) Mol. Wt. dihydrate 241.20
anhydrous 205.17

2-Sodio-1,2-benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide dihydrate [6155-57-3]

2-Sodio-1,2-benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide [128-44-9]

Content Sodium Saccharin, when dried, contains 99.0–101.0% of sodium saccharin ($C_7H_4NNaO_3S$).

Description Sodium Saccharin occurs as colorless to white crystals or powder having an extremely sweet taste.

Identification

(1) To 10 ml of a solution of Sodium Saccharin (1 in 10), add 1 ml of diluted hydrochloric acid (1 in 4), allow to stand for 1 hour, and filter the white crystalline precipitate formed. Wash the residue on the filter paper thoroughly with water, dry at 105°C for 2 hours, and measure the melting point. It is 226–230°C.

(2) Proceed as directed in Identification (1) for Saccharin.

(3) Proceed as directed in Identification (2) for Saccharin.

(4) A solution of Sodium Saccharin (1 in 10) responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) Clarity and color of solution
Colorless and clear (powder sample 1.0 g, water 1.5 ml).
Colorless and clear (powder sample 1.0 g, ethanol 70 ml).

(2) Free acid and free alkali Weigh 1.0 g of Sodium Saccharin, dissolve it in 10 ml of freshly boiled and cooled water, and add 1 drop of phenolphthalein TS. No pink color develops. Add 1 drop of 0.1 mol/L sodium hydroxide. A pink color develops.

(3) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

(5) Benzoate and salicylate Weigh 0.5 g of Sodium Saccharin, dissolve it in 10 ml of water, and add 5 drops of acetic acid and 3 drops of iron(III) chloride solution (1 in 10). No precipitate is formed, and purple to red-purple color does not develop.

(6) *o*-Toluenesulfonamide Not more than 25 μg/g as *o*-toluenesulfonamide.

Test Solution Weigh 10 g of Sodium Saccharin, dissolve it in 50 ml of water, and proceed as directed in Purity (6) for Saccharin.

Loss on Drying Not more than 15.0% (120°C, 4 hours).

Assay Weigh accurately about 0.3 g of Sodium Saccharin,

previously dried, dissolve it in 20 ml of acetic acid for non-aqueous titration, and titrate with 0.1 mol/L perchloric acid (indicator: 2 drops of crystal violet–acetic acid TS) until the color of the solution changes from purple through blue to green. Perform a blank test in the same manner, and make any necessary correction.

Each ml of 0.1 mol/L perchloric acid = 20.52 mg of $C_7H_4NNaO_3S$

Sodium Starch Phosphate

デンプンリン酸エステルナトリウム

Description Sodium Starch Phosphate occurs as a white to whitish powder. It is almost odorless.

Identification

(1) To 0.1 g of Sodium Starch Phosphate, add 10 ml of water to make a homogeneous paste by heating while shaking if necessary, and cool. To 5 drops of the resulting solution, add 10 ml of water, shake, and add 1 drop of iodine TS. A blue to red-purple color develops.

(2) Weigh accurately about 4 g of Sodium Starch Phosphate, previously dried, add 70 ml of water, make a homogeneous paste by heating while stirring, and allow to stand at 40°C for 30 minutes. Add 20 ml of amylase TS, allow to stand at 40°C for another 30 minutes, and cool. Pour the solution into a column (with an internal diameter of 1 cm) packed with about 20 ml of strongly acidic cation-exchange resin, and allow it to flow through. Adjust the flow rate to about 2 ml per minute. After the solution flows through completely, wash the resin column with 150 ml of water, combine the solution and the washings, and then add water to make 250 ml. Refer to this solution as solution A.

Measure 100 ml of solution A, pour into a column (with an internal diameter of 1 cm) packed with about 15 ml of weakly basic anion-exchange resin, and allow it to flow through. Adjust the flow rate to about 2 ml per minute. After the solution flows through completely, wash the resin column with 80 ml of water, combine the solution and the washings, and then add water to make 200 ml. Refer to this solution as solution B.

Measure 20 ml of solution B, transfer into a Kjeldahl flask, concentrate to about 2 ml by gently heating, and cool. Add 5 ml of sulfuric acid and 3 ml of hydrogen peroxide, heat gently until white fumes are evolved, and cool. Add 50 ml of water, and boil gently for 15 minutes. Cool, neutralize with ammonia solution or ammonia TS while cooling, and add water to make 100 ml. Refer to this solution as solution C.

To 10 ml of solution C, add 1 ml of diluted sulfuric acid (3 in 10), 2 ml of ammonium molybdate TS, and 1 ml of 1-amino-2-naphthol-4-sulfonic acid TS. The solution evolves a green-blue to blue color within 5 minutes.

(3) Ignite 1 g of Sodium Starch Phosphate at 450–550°C for 3 hours. The resulting residue responds to all tests for Sodium Salt in the Qualitative Tests.

Purity

(1) pH 6.0–7.5.

Weigh 0.50 g of Sodium Starch Phosphate, add 50 ml of water, and make uniformly pasty by heating while shaking if necessary. Allow to cool, and measure.

(2) Heavy metals Not more than 30 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 3.0 ml).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(4) Combined phosphorus 0.2–3.0%.

Test Solution Measure 10 ml of solution C prepared in Identification (2), add 1 ml of diluted sulfuric acid (3 in 10), 2 ml of ammonium molybdate TS, 1 ml of 1-amino-2-naphthol-4-sulfonic acid TS, and water to make 25 ml, and allow to stand for 30 minutes.

Control Solution Place 1 ml of diluted sulfuric acid (3 in 10), 2 ml of ammonium molybdate TS, and 1 ml of 1-amino-2-naphthol-4-sulfonic acid TS in a volumetric flask, and add water to make 25 ml.

Standard Curve Measure 5.0 ml of Monopotassium Phosphate Standard Solution, and add water to make 1,000 ml. Measure 5.0-ml, 10-ml and 20-ml portions of this solution, and to each, add 1 ml of diluted sulfuric acid (3 in 10), 2 ml of ammonium molybdate TS, 1 ml of 1-amino-2-naphthol-4-sulfonic acid TS, and water to make 25 ml. Allow to stand for 30 minutes, measure the absorbance of each solution at a wavelength of 740 nm, and prepare a calibration curve.

Procedure Measure the absorbance of the test solution at a wavelength of 740 nm. If necessary, adjust the volume of solution C so that the absorbance is 0.2–0.7. Calculate the content (mg) of combined phosphorus from absorbance of the test solution using the calibration curve, and determine the percentage of combined phosphorus in the weighed amount of the sample.

(5) Inorganic phosphorus Not more than 20%.

Measure 10 ml of solution A prepared in Identification (2), transfer into a Kjeldahl flask, and proceed as directed for the preparation of solution C using solution B in (2). Refer to the solution as solution D. Measure 10 ml of solution D, add 1 ml of diluted sulfuric acid (3 in 10), 2 ml of ammonium molybdate TS, 1 ml of 1-amino-2-naphthol-4-sulfonic acid TS, and water to make 25 ml, and allow to stand for 30 minutes. Measure the absorbance at a wavelength of 740 nm. If necessary, adjust the volume of solution D so that the absorbance is 0.2–0.7. Then proceed as directed in Purity (4), and determine the total amount (mg) of phosphorus. From this value and the amount (mg) of combined phosphorus obtained in Purity (4), calculate the ratio of inorganic phosphorus to the total phosphorus by the formula:

$$\text{Ratio (\%) of inorganic phosphorus of total phosphorous} = \frac{\left(\begin{array}{c}\text{Total amount (mg)}\\ \text{of phosphorus}\end{array}\right) - \left(\begin{array}{c}\text{Amount (mg) of}\\ \text{combined phosphorus}\end{array}\right)}{\text{Total amount (mg) of phosphorus}} \times 100$$

Loss on Drying Not more than 15.0% (105°C, 4 hours).

Sodium Sulfate

硫酸ナトリウム

$Na_2SO_4 \cdot nH_2O$ (n=10 or 0) Mol. Wt. decahydrate 322.20
anhydrous 142.04

Sodium sulfate decahydrate [7727-73-3]
Sodium sulfate [7757-82-6]

Definition Sodium Sulfate occurs in two forms: the crystalline form (decahydrate) called Sodium Sulfate (crystal) and the anhydrous form called Sodium Sulfate (anhydrous).

Content Sodium Sulfate, when dried, contains not less than 99.0% of sodium sulfate (Na_2SO_4).

Description Sodium Sulfate (crystal) occurs as colorless crystals or as a white crystalline powder. Sodium Sulfate (anhydrous) occurs as a white powder.

Identification Sodium Sulfate responds to all tests for Sodium Salt and for Sulfate in the Qualitative Tests.

Purity For Sodium Sulfate (crystal), dry the sample before performing the test.

(1) Clarity and color of solution Colorless and almost clear (1.0 g, water 10 ml.)

(2) Chloride Not more than 0.11% as Cl (0.10 g, Control solution 0.01 mol/L hydrochloric acid 0.30 ml).

(3) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Drying

Crystal: 51.0–57.0% (105°C, 4 hours).

Anhydrous: Not more than 5.0% (105°C, 4 hours).

Assay Weigh accurately about 0.4 g of Sodium Sulfate, previously dried, dissolve it in 200 ml of water, add 1 ml of hydrochloric acid, boil, and add gradually 30 ml of barium chloride solution (1 in 6). Heat this solution in a water bath for 1 hour, cool, and filter through a filter paper for quantitative analysis (5C). Wash the residue on the filter paper with warm water until the washings are free of chloride. Dry the residue with the filter paper, ignite to constant weight, and accurately weigh as Barium Sulfate ($BaSO_4$).

$$\text{Content (\%) of sodium sulfate } (Na_2SO_4) = \frac{\text{Weight (g) of } BaSO_4 \times 0.6086}{\text{Weight (g) of the sample}} \times 100$$

Sodium Sulfite

Soda Sulfite

亜硫酸ナトリウム

$Na_2SO_3 \cdot nH_2O$ (n=7 or 0) Mol. Wt. heptahydrate 252.15
anhydrous 126.04

Disodium sulfite heptahydrate [10102-15-5]
Disodium sulfite [7757-83-7]

Definition Sodium Sulfite occurs in two forms: the crystalline form (heptahydrate) called Sodium Sulfite (crystal) and the anhydrous form called Sodium Sulfite (anhydrous).

Content Sodium Sulfite, when calculated on the anhydrous basis, contains not less than 95.0% of sodium sulfite (Na_2SO_3).

Description Sodium Sulfite occurs as colorless to white crystals or as a white powder.

Identification Sodium Sulfite responds to all tests for Sodium Salt and for Sulfite in the Qualitative Tests.

Purity In the case of Sodium Sulfite (crystal), use two times the quantity of the sample specified for each of the purity tests below.

(1) Clarity and color of solution Colorless and almost clear (0.50 g, water 10 ml).

(2) Heavy metals Not more than 10 μg/g as Pb (on the anhydrous basis).

Test Solution Weigh 2.0 g of Sodium Sulfite, dissolve it in 15 ml of boiling water, add 5 ml of hydrochloric acid, and evaporate to dryness on a water bath. To the residue, add 10 ml of boiling water and 2 ml of hydrochloric acid, and evaporate to dryness on a water bath again. Dissolve this residue in 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml, and filter if necessary.

Control Solution Measure exactly 2.0 ml of Lead Standard Solution, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (on the anhydrous basis).

Test Solution Weigh 0.50 g of Sodium Sulfite, and dissolve it in 5 ml of water. Add 1 ml of sulfuric acid, heat on a hot plate until white fumes are evolved, and add water to make 5 ml.

Apparatus Use Apparatus B.

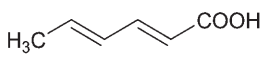
Assay Weigh accurately a quantity of Sodium Sulfite equivalent to about 0.25 g of Sodium Sulfite (anhydrous), proceed as directed under Sulfite Determination, and calculate the content by the formula:

$$\text{Content (\%) of sodium sulfate } (Na_2SO_3) = \frac{a \times (50 - b)}{\text{Weight (g) of the sample} \times 10} \times 100$$

a = 12.61 (crystal) or 6.302 (anhydrous),
b = volume (ml) of 0.1 mol/L sodium thiosulfate consumed.

Sorbic Acid

ソルビン酸



$C_6H_8O_2$ Mol. Wt. 112.13

(2*E*,4*E*)-Hexa-2,4-dienoic acid [110-44-1]

Content Sorbic Acid, when calculated on the anhydrous basis, contains not less than 99.0% of sorbic acid ($C_6H_8O_2$).

Description Sorbic Acid occurs as colorless needles or as a white crystalline powder. It is odorless or has a slight, characteristic odor.

Identification

(1) To 1 ml of a solution of Sorbic Acid in acetone (1 in 100), add 1 ml of water and 2 drops of bromine TS, and shake. The color of the solution immediately disappears.

(2) A solution of Sorbic Acid in 2-propanol (1 in 400,000) exhibits an absorption maximum at a wavelength of 252–256 nm.

Purity

(1) Melting point 132–135°C.

(2) Color of solution Weigh 0.20 g of Sorbic Acid, and dissolve it in 5.0 ml of acetone. The color of the solution is not darker than that of Matching Fluid C.

(3) Chloride Not more than 0.014% as Cl.

Sample Solution Weigh 1.50 g of Sorbic Acid, add 120 ml of water, and dissolve while boiling, and cool. Add water to make 120 ml, and filter. Use 40 ml of the filtrate as the sample solution.

Control Solution 0.20 ml of 0.01 mol/L hydrochloric acid.

(4) Sulfate Not more than 0.048% as SO_4.

Sample Solution 40 ml of the filtrate prepared in Purity (3).

Control Solution 0.50 ml of 0.005 mol/L sulfuric acid.

(5) Heavy metals Not more than 10 μg/g as Pb.

Test Solution To the residue obtained by ignition of Sorbic Acid, add 1 ml of hydrochloric acid and 0.2 ml of nitric acid, and evaporate to dryness on a water bath. To the residue, add 1 ml of diluted hydrochloric acid (1 in 4) and 15 ml of water, dissolve by heating, and cool. Add 1 drop of phenolphthalein TS, and then add ammonia TS dropwise until the solution turns to pale pink. Add 2 ml of diluted acetic acid (1 in 20), filter if necessary, and add water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Water Content Not more than 0.50% (2.0 g, Direct Titration).

Residue on Ignition Not more than 0.20%.

Assay Weigh accurately about 1 g of Sorbic Acid, and dissolve it in neutralized ethanol to make exactly 100 ml. Measure exactly 25 ml of this solution, and titrate with 0.1 mol/L sodium hydroxide (indicator: 2–3 drops of phenolphthalein TS). Calculate on the anhydrous basis.

Each ml of 0.1 mol/L sodium hydroxide = 11.21 mg of $C_6H_8O_2$.

Sorbitan Esters of Fatty Acids

ソルビタン脂肪酸エステル

Definition Sorbitan Esters of Fatty Acids are esters of fatty acids and sorbitan.

Description Sorbitan Esters of Fatty Acids occur as a white to yellow-brown powder, flakes, granules, or waxy lumps or as a white to yellow-brown liquid.

Identification

(1) Dissolve 0.5 g of the sample in 5 ml of anhydrous ethanol by heating, add 5 ml of diluted sulfuric acid (1 in 20), heat in a water bath for 30 minutes, and cool. Oil drops or a white to yellowish white solid is deposited. Add 5 ml of diethyl ether to the separated oil drops or solid, and shake. It dissolves.

(2) Measure 2 ml of the liquid residue obtained when the oil drops or solid were removed in Identification (1), add 2 ml of freshly prepared catechol solution (1 in 10), shake, add 5 ml of sulfuric acid, and shake. A pink to red-brown color develops.

Purity

(1) Acid value Not more than 15 (Fats and Related Substances Tests).

(2) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

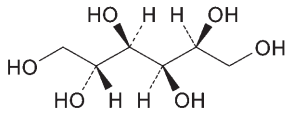
(4) Polyoxyethylene Weigh 1.0 g of the sample, and dissolve it in 10 ml of isooctane, and add 20 ml of water. Warm, shake well, and cool. Add 10 ml of ammonium thiocyanate –cobalt nitrate TS, shake well, and allow to stand. The color of the isooctane layer does not change to blue.

Residue on Ignition Not more than 1.5%.

D-Sorbitol

Sorbitol
D-Sorbit

D-ソルビトール



$C_6H_{14}O_6$ Mol. Wt. 182.17

D-Glucitol [50-70-4]

Content D-Sorbitol, when dried, contains not less than 90.0% of D-sorbitol ($C_6H_{14}O_6$).

Description D-Sorbitol occurs as a white powder or granules. It is odorless and has a cool, sweet taste.

Identification

(1) To 1 ml of a solution of D-Sorbitol (7 in 10), add 2 ml of ferrous sulfate TS and 1 ml of sodium hydroxide solution (1 in 5). A blue-green color develops, but no turbidity appears.

(2) To 1 ml a solution of D-Sorbitol (1 in 100), add 1 ml of freshly prepared catechol solution (1 in 10), shake well, add 2 ml of sulfuric acid, and shake. A red color develops immediately.

Purity

(1) Free acid Weigh 5 g of D-Sorbitol, dissolve it in 50 ml of freshly boiled and cooled water, add 1 drop of phenolphthalein TS and 0.5 ml of 0.01 mol/L sodium hydroxide, and shake. The color of the solution changes to pink, and it persists for not less than 30 seconds.

(2) Heavy metals Not more than 10 μg/g as Pb (2.0 g,

Method 1, Control solution Lead Standard Solution 2.0 ml).

(3) Nickel Weigh 0.50 g of D-Sorbitol, dissolve it in 5 ml of water, add 3 drops of a solution of dimethylglyoxime in ethanol (1 in 100) and 3 drops of ammonia TS, and allow to stand for 5 minutes. The color of the solution does not change to pink.

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

(5) Reducing sugars Not more than 0.68% as D-glucose.

Weigh 1.0 g of D-Sorbitol into a flask, and dissolve it in 25 ml of water. Add 40 ml of Fehling's TS, boil gently for 3 minutes, and allow to stand to precipitate cuprous oxide. After cooling, carefully filter the supernatant through a glass filter (1G4), leaving as much precipitate as possible in the flask. Discard the filtrate. Immediately add warm water to the precipitate in the flask, wash, and carefully filter the washings through the glass filter, and discard the filtrate. Repeat the washing and filtering process until the washings are no longer alkaline. To the precipitate in the flask, immediately add 20 ml of ferric sulfate TS to dissolve, and filter through the glass filter. Wash the flask and the glass filter with water, and combine the filtrate and the washings. Heat the solution obtained to 80°C, and add 2.0 ml of 0.02 mol/L potassium permanganate. The pink color of the solution does not disappear immediately.

(6) Saccharide Not more than 4.4% as D-glucose.

Weigh 10 g of D-Sorbitol, dissolve it in 25 ml of water, add 8 ml of diluted hydrochloric acid (1 in 4), and heat under a reflux condenser in a water bath for 3 hours. Cool, and neutralize with sodium hydroxide solution (1 in 25), using methyl orange TS as the indicator. Add water to make 100 ml. Measure 10 ml of this solution, add 10 ml of water and 40 ml of Fehling's TS, boil gently for 3 minutes, and proceed as directed under Purity (5), using 13 ml of 0.02 mol/L potassium permanganate.

Loss on Drying Not more than 3.0% (not more than 0.7 kPa, 80°C, 3 hours).

Residue on Ignition Not more than 0.02% (5 g).

Assay Weigh accurately about 1 g each of D-Sorbitol and D-sorbitol for assay, previously dried, separately dissolve them in water to make exactly 50 ml of each. Use these solutions as the test solution and standard solution, respectively. Analyze 10 μl portions of the test solution and the standard solution by liquid chromatography using the conditions given below. Measure the peak areas (A_T and A_S) of the D-sorbitol for the test solution and the standard solution, and determine the content by the formula:

$$\text{Content (\%) of D-sorbitol } (C_6H_4O_6) = \frac{\text{Weight (g) of D-sorbitol for assay}}{\text{Weight (g) of the sample}} \times \frac{A_T}{A_S} \times 100$$

Operating Conditions

Detector: Differential refractometer.

Column: A stainless steel tube of 4–8 mm internal diameter and 20–50 cm length.

Column packing material: 5- to-12-μm strongly acidic cation exchange resin for liquid chromatography.

Column temperature: A constant temperature of 40–85°C.

Mobile phase: Water.

Flow rate: A constant rate of 0.5–1.0 ml/min.

D-Sorbitol Syrup

D-Sorbit Syrup

D-ソルビトール液

Content D-Sorbitol Syrup contains not less than 50.0–75.0% of D-sorbitol ($C_6H_{14}O_6$ = 182.17).

Description D-Sorbitol Syrup is a colorless, clear, syrupy liquid. Colorless crystals may be deposited while cool. It is odorless and has a sweet taste.

Identification Proceed as directed in Identification (1) and (2) for D-Sorbitol.

Purity

(1) Specific gravity d_{25}^{25}: 1.285–1.315.

(2) Free Acid Proceed as directed in Purity (1) for D-Sorbitol.

(3) Heavy metals Not more than 10 μg/g as Pb.

Proceed as directed in Purity (2) for D-Sorbitol.

(4) Nickel Proceed as directed in Purity (3) for D-Sorbitol.

(5) Arsenic Not more than 4.0 μg/g As_2O_3.

Proceed as directed in Purity (4) for D-Sorbitol.

(6) Reducing sugars Not more than 0.68% as D-glucose.

Proceed as directed in Purity (5) for D-Sorbitol.

(7) Saccharide Not more than 6.8% as D-glucose.

Proceed as directed in Purity (6) for D-Sorbitol, using 20 ml of 0.02 mol/L potassium permanganate.

Residue on Ignition Not more than 0.02%.

Weigh accurately about 5 g of D-Sorbitol Syrup, add 2–3 drops of sulfuric acid, and heat gently to boil. Set it fire, incinerate, and cool. Using the substance obtained, proceed as directed under Residue on Ignition.

Assay Weigh accurately about 1 g of D-Sorbitol Syrup, and proceed as directed in the Assay for D-Sorbitol.

Spirulina Color

スピルリナ色素

Definition Spirulina Color is obtained from the entire part of the alga *Spirulina platensis* Geitler and consists mainly of phycocyanin. It may contain dextrin or lactose.

Color Value The Color Value ($E_{1cm}^{10\%}$) of Spirulina Color is not less than 25 and in the range of 90–110% of the labeled value.

Description Spirulina is a blue powder or liquid having a slight characteristic odor.

Identification

(1) Weigh the equivalent of 0.4 g of Spirulina Color with a Color Value 25, and dissolve it in 100 ml of citrate buffer (pH 6.0). The solution is blue and emits red fluorescence.

(2) Heat a small amount of the solution obtained in Identi-

fication (1) at 90°C for 30 minutes. The fluorescence disappears.

(3) To 5 ml of the solution obtained in Identification (1), add 3.3 g of powdered ammonium sulfate in small portions to dissolve, and allow to stand. A blue precipitate is produced.

(4) To 5 ml of the solution obtained in Identification (1), add 1 ml of iron (III) chloride TS, and allow to stand for 20 minutes. The solution turns blue-green to dark purple.

(5) To 5 ml of the solution obtained in Identification (1), add 0.1 ml of sodium hypochlorite TS. The solution turns light yellow.

(6) A solution of Spirulina Color in citrate buffer (pH 6.0) exhibits an absorption maximum at a wavelength of 610–630 nm.

Purity

(1) Heavy metals Not more than 40 μg/g as Pb (0.50 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 8.0 μg/g as Pb (1.25 g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50g, Method 3, Apparatus B).

Color Value Test Proceed as directed in the Color Value Test, using the following operating conditions:

Operating Conditions

Solvent: Citrate buffer (pH 6.0).

Wavelength: Maximum absorption wavelength of 610–630 nm.

Stevia Extract

ステビア抽出物

Definition Stevia Extract is obtained by extraction from the leaves of *Stevia rebaudiana* Bertoni and consists mainly of steviol glycosides.

Content Stevia Extract, when calculated on the dried basis, contains not less than 80.0% of steviol glycosides.

Description Stevia Extract occurs as a white to light yellow powder, flakes, or granules. It is odorless or has a slight characteristic odor. It has a strong sweet taste.

Identification Prepare a test solution by dissolving 0.5 g of Stevia Extract in 100 ml of water. Separately, prepare a standard solution by dissolving 5 mg each of stevioside for assay and rebaudioside A for assay in 10 ml of water. Analyze 10 μl portions of the test solution and the standard solution by liquid chromatography using the operating conditions given in the Assay. Two main peaks of the test solution correspond to the retention times of stevioside and rebaudioside A in the standard solution, or at least one peak corresponds to either of the two substances in the standard solution.

Purity

(1) Heavy metals Not more than 2.0 μg/g as Pb (5.0 g, Method 1).

(2) Arsenic Not more than 2.0 μg/g as As_2O_3 (1.0 g, Method 3, Apparatus B).

Loss on Drying Not more than 6.0% (105°C, 2 hours).

Residue on Ignition Not more than 1.0%.

Assay

Test Solution Dissolve 0.06–0.12 g of Stevia Extract, weighed accurately, in a 4:1 mixture of acetonitrile/water to make exactly 100 ml.

Standard Solution Weigh accurately about 0.05 g of stevioside for assay, previously dried, and dissolve it in a 4:1 mixture of acetonitrile/water to make exactly 100 ml.

Procedure Analyze 10 μl portions of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Measure the peak areas of stevioside, dulcoside A, rebaudioside A, and rebaudioside C for the test solution and stevioside for the standard solution, and express as A_a, A_b, A_c, A_d, and A_s, respectively. The peaks of dulcoside A and rebaudioside C elute at relative retention times of 0.25–0.40 and 0.63–080, respectively, when assuming that the retention time of rebaudioside A is 1.0. Determine the content of steviol glycosides using the following formulas. For A_b and A_d, if two peaks are observed within the specified retention time, use the peak area of the preceding peak.

$$\text{Content (\%) of stevioside} = \frac{\text{Weight (g) of stevioside for assay}}{\text{Dry basis weight (g) of the sample}} \times \frac{A_a}{A_s} \times 100$$

$$\text{Content (\%) of dulcoside A} = \frac{\text{Weight (g) of stevioside for assay}}{\text{Dry basis weight (g) of the sample}} \times \frac{A_b \times 0.98}{A_s} \times 100$$

$$\text{Content (\%) of rebaudioside A} = \frac{\text{Weight (g) of stevioside for assay}}{\text{Dry basis weight (g) of the sample}} \times \frac{A_c \times 1.20}{A_s} \times 100$$

$$\text{Content (\%) of rebaudioside C} = \frac{\text{Weight (g) of stevioside for assay}}{\text{Dry basis weight (g) of the sample}} \times \frac{A_d \times 1.18}{A_s} \times 100$$

Content (%) of steviol glycosides
= Contents (%) of stevioside + dulcoside A + rebaudioside A + rebaudioside C

Operating Conditions

Detector: Ultraviolet absorption spectrophotometer (determination wavelength: 210 nm).

Column: A stainless steel tube of 4.6 mm internal diameter and 15 cm length.

Column packing material: 5-μm amino-bonded silica gel for liquid chromatography.

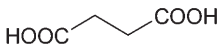
Column temperature: 40°C.

Mobile phase: A 4:1 mixture of acetonitrile/water.

Flow rate: Adjust so that the retention time of rebaudioside A is about 21 minutes.

Succinic Acid

コハク酸



$C_4H_6O_4$ Mol. Wt. 118.09

Butanedioic acid [110-15-6]

Content Succinic Acid contains not less than 99.0% of succinic acid ($C_4H_6O_4$).

Description Succinic Acid occurs as colorless to white crystals or as a white crystalline powder. It is odorless and has a characteristic acid taste.

Identification Adjust the pH of a solution of 5 ml of Succinic Acid (1 in 20) to about 7 with ammonia TS. Add 2–3 drops of iron(III) chloride solution (1 in 10). A brown precipitate is formed.

Purity

(1) Melting point 185–190°C.

(2) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of Succinic Acid, dissolve it in 20 ml of water, add 1 drop of phenolphthalein TS, and add ammonia TS dropwise until the color of the solution changes to a slightly pink color. Add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

(4) Readily oxidizable substances Weigh 1.0 g of Succinic Acid, dissolve it in 25 ml of water and 25 ml of diluted sulfuric acid (1 in 20), and add 4.0 ml of 0.02 mol/L potassium permanganate. The pink color of the solution does not disappear within 3 minutes.

Residue on Ignition Not more than 0.025% (5 g).

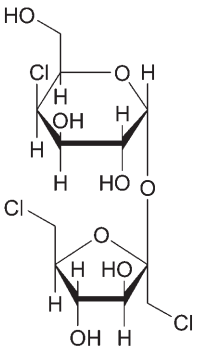
Assay Weigh accurately about 1 g of Succinic Acid, and dissolve it in water to make exactly 250 ml. Measure exactly 25 ml of this solution, and titrate with 0.1 mol/L sodium hydroxide (indicator: 2–3 drops of phenolphthalein TS).

Each ml of 0.1 mol/L sodium hydroxide = 5.904 mg of $C_4H_6O_4$

Sucralose

Trichlorogalactosucrose

スクラロース



$C_{12}H_{19}Cl_3O_8$ Mol. Wt. 397.64

1,6-Dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-α-D-galactopyranoside [56038-13-2]

Content Sucralose, when calculated on the anhydrous basis, contains 98.0–102.0% of Sucralose ($C_{12}H_{19}Cl_3O_8$).

Description Sucralose occurs as a white to off-white crystalline powder. It is odorless and has a sweet taste.

Identification Determine the absorption spectrum of Sucralose as directed in the Potassium Bromide Disk Method under Infrared Spectrophotometry, and compare with the Reference Spectrum of Sucralose. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: + 84.0 to +87.5° (1.0 g, water 10 ml, on the anhydrous basis).

(2) Lead Not more than 1.0 μg/g as Pb (10.0 g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50g, Method 4, Apparatus C).

(4) Other chlorinated disaccharides Not more than 0.5%.

Test Solution Dissolve 1.0 g of Sucralose in 10 ml of methanol.

Control Solution Measure 0.5 ml of the test solution, and add methanol to make 100 ml.

Procedure Analyze a 5-μl portion each of the test solution and the control solution by thin-layer chromatography using a 7:3 mixture of sodium chloride solution (1 in 20)/acetonitrile as the developing solvent. Use a thin-layer plate that has been coated with octadecylsilanized silica gel for thin-layer chromatography as the solid support and then dried at 110°C for one hour. Stop the development when the developing solvent has ascended to a point 15 cm above the original line, and air-dry to remove the solvent. Spray with 15% sulfuric acid–methanol TS, and heat at 125°C for 10 minutes. The main spot from the test solution corresponds to the spot from the control solution. Even if any other spot is observed, it does not have a darker color than the spot from the control solution.

(5) Chlorinated monosaccharides Not more than 0.16% as fructose.

Test solution Weigh 2.5 g of Sucralose, and add methanol to make exactly 10 ml.

Control Solution A Weigh exactly 10.0 g of D-mannitol, and add water to make exactly 100 ml.

Control Solution B Weigh exactly 10.0 g of D-mannitol and 0.040 g of fructose, and add water to make exactly 100 ml.

Procedure Apply 1 µl each of the test solution and control solutions A and B onto a thin-layer chromatographic plate coated with a 0.25-mm thick layer of silica gel, and air-dry. Repeat this procedure four more times. Spray the plate with *p*-anisidine–phthalic acid TS, and heat at 98–102°C for about 10 minutes to fix the color. The spot from the test solution does not have a darker color than the spot from control solution B. If any spot is observed for control solution A, prepare a second plate, and repeat the procedure once more.

(6) Triphenylphosphine oxide Not more than 0.015%.

Test Solution Weigh accurately about 0.1 g of Sucralose, dissolve it in a 67:33 mixture of acetonitrile/water to make exactly 10 ml.

Standard Solution Weigh exactly 0.100 g of triphenylphosphine oxide, dissolve it in a 67:33 mixture of acetonitrile/water to make exactly 10 ml. Measure exactly 1 ml of the resulting solution, and add a 67:33 mixture of acetonitrile/water to make exactly 100 ml. Then measure exactly 1 ml of the resulting solution, and add a 67:33 mixture of acetonitrile/water to make exactly 100 ml.

Procedure Analyze 25 µl portions of the test solution and the standard solution by liquid chromatography using the conditions given below. Measure the peak areas (A_S and A_T) of triphenylphosphine oxide (TPPO) for the test solution and the standard solution, and calculate the content of TPPO in Sucralose from the formula:

$$\text{Content (\%) of TPPO } (C_{18}H_{15}OP) = \frac{1}{\text{Weight (g) of the sample (g)} \times 1{,}000} \times \frac{A_T}{A_S}$$

Operating Conditions

Detector: Ultraviolet spectrophotometer (determination wavelength: 220 nm).

Column: A stainless steel tube of 4.6 mm internal diameter and 15 cm length.

Column packing material: 5-µm octadecylsilanized silica gel for liquid chromatography.

Column temperature: 40ºC.

Mobile phase: A 67:33 mixture of acetonitrile/water.

Flow rate: 1.5 ml/min.

(7) Methanol Not more than 0.10%.

Test solution Weigh accurately about 2.0 g of Sucralose, add water to make exactly 10 ml, and mix.

Control Solution Measure exactly 2.0 g of methanol, add water to make exactly 100 ml, and mix. Measure exactly 1 ml of this solution, add water to make exactly 100 ml, and mix.

Procedure Analyze 1 µl portions of the test solution and the control solution by gas chromatography using the operating conditions given below. Measure the peak areas (A_T and A_S) of methanol for the test solution and the control solution, and calculate the content of methanol by the formula:

$$\text{Methanol (\%)} = \frac{2.0}{\text{Weight of the sample (g)} \times 1{,}000} \times \frac{A_T}{A_S} \times 100$$

Operating Conditions

Detector: Flame ionization detector.

Column: A glass tube about of 2 m length and 2–4 mm internal diameter.

Column packing material: 150- to 180-µm styrene-divinylbenzene porous polymer for gas chromatography.

Column temperature: A constant temperature of 140–160°C.

Inlet temperature: 200°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust so that the peak of methanol appears about 4 minutes after injection.

Residue on Ignition Not more than 0.7%.

Water Content Not more than 2.0% (1 g, Direct Titration).

Assay Weigh accurately about 1g of Sucralose, and dissolve it in water to make exactly 100 ml. Measure exactly 10 ml of this solution, add 10 ml of sodium hydroxide solution (1 in 10), and gently heat under reflex condenser for 30 minutes. Cool, neutralize with diluted nitric acid, and titrate with 0.1 mol/L silver nitrate. The endpoint is confirmed using a silver electrode as the indicator electrode and a silver–silver chloride electrode as the reference electrode. Perform a blank test in the same manner, and make any necessary correction and calculate on the dried basis.

Each ml of 0.1 mol/L silver nitrate = 13.25 mg of $C_{12}H_{19}Cl_3O_8$

Sucrose Esters of Fatty Acids

ショ糖脂肪酸エステル

Definition Sucrose Esters of Fatty Acids are categorized into two types: esters of sucrose with fatty acids and sucrose acetate isobutyrate.

Description Sucrose Esters of Fatty Acids occur as white to yellow-brown powdery or massive substances or as colorless to red-brown, viscous resinous or liquid substances. They are odorless or have a slight, characteristic odor.

Identification

(1) To 1 g of the sample, add 25 ml of ethanolic potassium hydroxide TS, and heat under a reflux condenser on a water bath for 1 hour. Add 50 ml of water to the solution, and distill until about 30 ml of liquid remains in the flask. After cooling, add 10 ml of diluted hydrochloric acid (1 in 4) to the residual liquid, shake well, add sodium chloride to make a saturated solution, and extract twice with 30 ml of diethyl ether each time. Collect and combine the diethyl ether layers. Wash the diethyl layer with 20 ml of sodium chloride saturated solution, dehydrate with 2 g of anhydrous sodium sulfate, and evaporate the diethyl ether. Completely remove the diethyl ether by letting in air, and cool the residue to 10°C. In the case of esters of sucrose with fatty acids, an oil drop or colorless to light yellow-brown solid is formed. In the case of sucrose acetate isobutyrate, a liquid with odors of acetic acid and isobutyric acid remains.

(2) In a test tube, place 2 ml of the aqueous layer that has remained after the diethyl ether layer is removed in Identification (1), warm in a water bath until the odor of diethyl ether disappears, and cool. Add 1 ml of anthrone TS gently

down the inside of the test tube. The boundary surface of the two layers turns blue to green.

Purity

(1) Acid value Not more than 6.0.

Test Solution Weigh accurately about 3 g of the sample, and dissolve it in 60 ml of a 2:1 mixture of 2-propanol/water.

Procedure Proceed as directed in the Acid Value Test in the Fats and Related Substances Tests.

(2) Lead Not more than 2.0 μg/g as Pb (5.0 g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50g, Method 3, Apparatus B).

(4) Free sucrose Not more than 5.0%.

Sample Solution Weigh accurately about 2 g of the sample, add 40 ml of 1-butanol, and dissolve by warming on a water bath. Extract free sucrose twice with 20 ml of sodium chloride solution (1 in 20) each time, and combine the extracts. Add 2 ml of diluted hydrochloric acid (1 in 4), and heat in a water bath for 30 minutes. After cooling, add 2–3 drops of phenolphthalein TS, neutralize with sodium hydroxide solution (1 in 25), and add water to make exactly 100 ml.

Procedure Measure exactly 20 ml of the sample solution, add 20 ml of Bertrand's TS A and 20 ml of Bertrand's TS B, boil gently for 3 minutes, and allow to stand to precipitate cuprous oxide (the color of the supernatant is purple-blue). Filter the supernatant through a glass filter (1G4), wash the precipitate in the flask with hot water, and filter the washings through the glass filter (care should be taken not to expose cuprous oxide to air). Repeat the washing and filtration procedure until the washings are no longer alkaline. Dissolve the precipitate in the flask in 20 ml of Bertrand's TS C, filter the solution through the glass filter, and wash the inside of flask and the glass filter with water. Combine the filtrate and the washings, and titrate with Bertrand's TS D. Calculate the amount of copper from the consumption of Bertrand's TS D, determine the amount of invert sugar from the Bertrand Table, and calculate the content of free sucrose by the formula:

$$\text{Content (\%) of free sucrose} = \frac{\text{Amount (g) of the invert sugar} \times 0.95 \times 5}{\text{Weight (g) of the sample}} \times 100$$

(5) Dimethyl sulfoxide Not more than 2.0 μg/g as dimethyl sulfoxide.

This specification does not apply to sucrose acetate isobutyrate.

Test Solution Weigh accurately about 5 g of the sample, dissolve it in tetrahydrofuran to make exactly 25 ml.

Standard Solutions Weigh accurately about 0.1 g of dimethyl sulfoxide, and dissolve it in tetrahydrofuran to make exactly 100 ml. Measure exactly 1 ml of this solution, and add tetrahydrofuran to prepare a standard stock solution of exactly 100 ml. Transfer exactly 0.5 ml, 1 ml, 2 ml, and 5 ml of the standard stock solution into separate 50-ml volumetric flasks, and dilute each with tetrahydrofuran to volume.

Procedure Analyze 3 μl portions of the test solution and the standard solutions by gas chromatography using the conditions below. Measure the peak heights or peak areas of dimethyl sulfoxide for the standard solutions, and prepare a calibration curve on a logarithmic paper. Measure the peak height or peak area of dimethylformamide for the test solution, and determine its amount from the calibration curve.

Operating Conditions

Detector: Flame photometric detector (with sulfur filter).

Column: A glass tube of 3 mm internal diameter and 2 m length.

Column packing material

Liquid phase: 10% Polyethylene glycol 20M of the amount of support and 3% potassium hydroxide of the amount of support.

Support: 180- to 250-μm diatomaceous earth for gas chromatography.

Column temperature: A constant temperature of 150–170ºC.

Injection port temperature: 210ºC.

Carrier gas: Nitrogen.

Flow rate: Adjust so that the peak of dimethyl sulfoxide appears about 3 minutes after injection.

(6) Dimethyl formamide Not more than 1.0 μg/g as dimethyl formamide.

Test Solution Weigh accurately about 2 g of the sample, and dissolve it in tetrahydrofuran to make exactly 20 ml.

Standard Solutions Weigh accurately about 0.1 g of dimethyl formamide, and dissolve it in tetrahydrofuran to make exactly 100 ml. Measure exactly 1 ml of this solution, and add tetrahydrofuran to prepare a standard stock solution of exactly 100 ml. Next, transfer exactly 0.5 ml, 1 ml, and 2 ml of the standard stock solution into separate 100-ml volumetric flasks, and dilute each with tetrahydrofuran to volume.

Procedure Analyze 1μl portions of the test solution and the standard solutions by gas chromatography using the conditions below. Measure the peak areas for the standard solutions, and prepare a calibration curve. Measure the peak area of dimethyl formamide for the test solution, and determine its amount from the calibration curve.

Operating Conditions

Detector: Nitrogen phosphorous detector.

Column: A silicate glass capillary tube (0.32 mm internal diameter and 30 m length) coated with a 0.5-μm thick layer of polyethylene glycol.

Column temperature: Maintain the temperature at 40ºC for 2 minutes, raise to 160ºC at a rate of 20ºC/minute, and then maintain at 160ºC for 2 minutes.

Injection port temperature: 180ºC.

Injection: Splitless.

Carrier gas: Helium.

Flow rate: Adjust so that the peak of dimethyl formamide appears about 6 minutes after injection.

(7) Other solvents

The specifications given below do not apply to sucrose acetate isobutyrate

Ethyl methyl ketone Not more than 10 μg/g.

Ethyl acetate, 2-propanol, and propylene glycol Not more than 0.035% as the sum of these three solvents.

Methanol Not more than 10 μg/g.

2-Methyl-1-propanol Not more than 10 μg/g.

(i) Ethyl methyl ketone, ethyl acetate, 2-propanol, methanol, and 2-methyl-1-propanol

Standard Solutions Weigh accurately about 0.2 g each of ethyl methyl ketone, ethyl acetate, 2-propanol, methanol, and 2-methyl-1-propanol in a volumetric flask, mix them, and dissolve the mixture in water to make exactly 50 ml. Refer to this solution as standard solution A. Transfer exactly

5 ml and 10 ml of standard solution A into separate 20-ml volumetric flasks, and dilute each with water to volume. Refer to the solutions obtained as standard solutions B and C, respectively.

Test Solution Weigh 1.00 g of the sample into a vial, and add exactly 5 µl of water.

Standard Additions Test Solutions Prepare three vials containing 1.00 g each of the sample. To each vial, add a 5 µl-potion of solutions A, B, and C, respectively, to prepare standard additions test solutions.

Procedure Determine the solvent contents by headspace gas chromatography for the test solution and the standard additions test solutions under the conditions below.

Measure the peak area of each solvent for each solution. Prepare a linear regression by plotting the values obtained on a graph, with the added amount of each solvent on the abscissa and the peak area on the ordinate. Determine the amount of each solvent in the sample from the distance between the coordinate origin and the intersection point of the regression line and the abscissa.

Operating Conditions

Detector: Flame ionization detector.

Column: A silicate glass capillary tube (0.53 mm internal diameter and 30 m length) coated with a 1.5-µm thick layer of dimethyl polysiloxane.

Column temperature: 40ºC.

Injection port temperature: 110ºC.

Injection: Splitless

Carrier gas: Nitrogen.

Flow rate: Adjust so that the peak of 2-methyl-1-propanol appears about 5 minutes after equilibrium injection.

Headspace sampler

Equilibrium temperature in the vial: 80ºC.

Equilibrium time in the vital: 40 minutes.

Amount of injection: 1.0 ml.

(ii) Propylene glycol

Test Solution Weigh accurately about 1 g of the sample, add 0.1 ml of the internal standard, and dissolve the mixture in pyridine to make exactly 10 ml. Measure exactly 0.5 ml of this solution, add 0.25 ml of hexamethyldisilazane and 0.1 ml of trimethylchlorosilane, and shake vigorously. Allow it to stand for 30 minutes at room temperature, and centrifuge. Use the supernatant as the test solution.

Internal standard To 0.025 g of ethylene glycol, add pyridine to make exactly 50 ml.

Standard Solutions Weigh accurately about 0.025 g of propylene glycol, and add pyridine to make exactly 50 ml. Transfer exactly 40 µl, 200 µl, 500 µl, and 1,000 µl of this solution into separate 10-ml volumetric flasks, add 0.1 ml of the internal standard to each, and dilute with pyridine to volume. Using these solutions, proceed in the same manner as the preparation of the test solution.

Procedure Analyze 1 µl portions of the test solution and the standards solutions by gas chromatography using the following operating conditions. Prepare a calibration curve, and determine the amount of propylene glycol by the internal standard method.

Operating Conditions

Detector: Flame ionization detector.

Column: A silicate glass capillary tube (0.32 mm internal diameter and 30 m length) coated with a 0.25-µm thick layer of dimethyl polysiloxane.

Column temperature: Maintain the temperature at 60ºC for 5 minutes, raise to 250ºC at a rate of 20ºC/minute, and maintain at 250ºC for 5 minutes.

Injection port temperature: 230ºC.

Injection: Splitless.

Carrier gas: Helium.

Flow rate: Adjust so that the peak of a propylene glycol derivative appears about 8 minutes after injection.

Water Content Not more than 4.0% (Back Titration).

Residue on Ignition Not more than 2.0%.

Sulfuric Acid

硫酸

H_2SO_4 Mol. Wt. 98.08

Sulfuric acid [7664-93-9]

Content Sulfuric Acid contains not less than 94.0% of sulfuric acid (H_2SO_4).

Description Sulfuric Acid is a colorless or slightly brownish, transparent or almost transparent, viscous liquid.

Identification

(1) A diluted solution of Sulfuric Acid (1 in 100) is strongly acidic.

(2) A diluted solution of Sulfuric Acid (1 in 100) responds to all tests for Sulfate in the Qualitative Tests.

Purity

(1) Chloride Not more than 0.005% as Cl (2.0 g, Control solution 0.01 mol/L hydrochloric acid 0.30 ml).

(2) Nitrate Not more than 10 µg/g as NO_3.

Add 5 g of Sulfuric Acid gradually to 8 ml of water, add 1 ml of a solution of brucine in sulfuric acid (1 in 500) and sulfuric acid to make 25 ml, shake well, and warm at about 80°C for 10 minutes. The color of the solution is not darker than that of the solution prepared as follows: To 0.50 ml of Nitrate Standard Solution, add 8 ml of water, and then add 5 ml of sulfuric acid gradually. Add 1 ml of a solution of brucine in sulfuric acid (1 in 500) and sulfuric acid to make 25 ml. Shake well, and warm at about 80°C for 10 minutes.

(3) Heavy metals Not more than 20 µg/g as Pb.

Test Solution Add 1.0 g of Sulfuric Acid to 10 ml of water, neutralize with ammonia TS, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Measure exactly 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(4) Iron Not more than 0.010% as Fe (0.10 g, Method 2, Control solution Iron Standard Solution 1.0 ml).

(5) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

(6) Readily oxidizable substances Not more than 40 µg/g as SO_3.

Add 8 g of Sulfuric Acid to 10 ml of cold water while cooling, and add 0.10 ml of 0.02 mol/L potassium permanganate. The pink color of the solution does not disappear within 5 minutes.

Residue on Ignition Not more than 0.02% (10 g).

Assay To 50 ml of water, add about 2 g of Sulfuric Acid, weighed accurately, cool, and add water to make exactly 100 ml. Measure exactly 25 ml of this solution, and titrate with

0.5 mol/L sodium hydroxide (indicator: 1–2 drops of bromothymol blue TS).

Each ml of 0.5 mol/L sodium hydroxide = 24.52 mg of H_2SO_4

Talc

タルク

Definition Talc is natural, hydrated magnesium silicate that is carefully selected. It occasionally contains a small amount of aluminum silicate.

Description Talc occurs as a white to gray-white, fine crystalline powder. It has a smooth feel and is odorless.

Identification Mix 0.2 g of Talc, 0.9 g of anhydrous sodium carbonate, and 1.3 g of anhydrous potassium carbonate, transfer the mixture into a platinum or nickel crucible, and fuse completely by heating. After cooling, transfer the contents into a beaker with about 5 ml of hot water, add hydrochloric acid until effervescence no longer occurs, add another 10 ml of hydrochloric acid, and evaporate to dryness on a water bath. After cooling, add 20 ml of water, boil, and filter. A gelatinous substance remains, and the filtrate responds to the test for Magnesium Salt.

Purity

(1) pH 7.5–9.5.

Weigh 10.0 g of Talc, add 100 ml of water, heat for 2 hours on a water bath with occasional shaking while replenishing the lost water, and cool. Filter with suction using a filter holder (47 mm in diameter) equipped with a membrane filter (0.45 μm in pore size). If the filtrate is turbid, repeat the filtration with suction through the same filter. Wash the container and the residue on the filter with water, combine the filtrate and the washings, and add water to make 100 ml. Refer to this solution as solution A. Measure the pH of solution A.

(2) Water-soluble substances Not more than 0.20%.

Measure 50 ml of solution A prepared in Purity (1), evaporate to dryness, dry the residue at 105°C for 2 hours, and weigh.

(3) Hydrochloric acid-soluble substances Not more than 2.0%.

Weigh 1.0 g of Talc, add 20 ml of diluted hydrochloric acid (1 in 4), warm for 15 minutes at 50°C while shaking, cool, and filter. Wash the container and the residue on the filter paper with a small amount of water, combine the filtrate and the washings, and add water to make 20 ml. Measure 10 ml of this solution, add 1 ml of diluted sulfuric acid (1 in 20), evaporate to dryness, and ignite at 550°C to constant weight and weigh the residue.

(4) Heavy metals Not more than 40 μg/g as Pb.

Test Solution Weigh 2.0 g of Talc, add 16 ml of diluted hydrochloric acid (1 in 4) and 20 ml of water, shake well, boil gently, cool, and filter. Wash the residue with water, combine the filtrate and the washings, and add water to make 100 ml. Refer to this solution as solution B. Measure 25 ml of solution B, evaporate to dryness on a water bath, add 2 ml of diluted acetic acid (1 in 20) and 20 ml of water the residue to dissolve, filter if necessary, and add water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(5) Water-soluble iron Measure 20 ml of solution A prepared in Purity (1), make it weakly acidic with hydrochloric acid, and add 1 drop of freshly prepared potassium ferrocyanide solution (1 in 10). No blue color develops.

(6) Lead Not more than 10 μg/g as Pb.

Test Solution Measure 25 ml of solution B prepared in Purity (4), evaporate to dryness on a water bath, and dissolve the residue in diluted hydrochloric acid (1 in 10) to make 10 ml.

Control Solution To 1.0 ml of Lead Standard Solution, add diluted hydrochloric acid (1 in 10) to make 20 ml.

Procedure Proceed as directed in Method 1 in the Lead Limit Test, using the test solution and the control solution.

(7) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.50 g of Talc, add 5 ml of diluted sulfuric acid (3 in 50), heat gradually to boiling while shaking well, cool rapidly, and filter. Wash the residue with 5 ml of diluted sulfuric acid (3 in 50), then with 10 ml of water, and combine the filtrate and the washings, and evaporate on a water bath to make 5 ml.

Apparatus Use Apparatus B.

Loss on Ignition Not more than 6.0% (550°C, constant weight).

Tamarind Seed Gum

タマリンドシードガム

Definition Tamarind Seed Gum is obtained from the seeds of the tamarind tree *Tamarindus indica* Linné and consists mainly of polysaccharides. It may contain sucrose, glucose, lactose, dextrin, or maltose.

Description Tamarind Seed Gum occurs as a white to light brown powder. It is odorless or has a slight, characteristic odor.

Identification

(1) Add gradually 2 g of Tamarind Seed Gum to 100 ml of sodium hydroxide solution (1 in 125), and dissolve by vigorous stirring. To 5 ml of this solution, add 3 ml of saturated sodium sulfuric acid. White lumps are produced.

(2) To the solution obtained in Identification (1), add gently a few drops of iodine–potassium iodide TS. Dark blue-green lumps are produced on the solution surface, and the color disappears on stirring.

Purity

(1) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 10 μg/g as Pb (1.0 g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50g, Method 3, Apparatus B).

(4) Protein Not more than 3.0 %.

Weigh accurately about 0.5 g of Tamarind Seed Gum, and proceed as directed in the Semi-micro Kjeldahl Method under Nitrogen Determination.

Each ml of 0.005 mol/L sulfuric acid = 0.8754 mg of protein

Loss on Drying Not more than 14.0 % (105°C, 5 hours).

Ash Not more than 5.0 % (on the dried basis).
Microbial Limits Proceed as directed in the Microbial Limit Tests. The total bacterial count is not more than 10,000/g, and *Escherichia coli* is negative.

Tara Gum

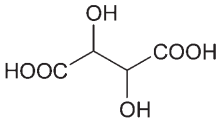
タラガム

Definition Tara Gum is obtained from the seeds of the tara tree *Caesalpinia spinosa* Kuntze and consists mainly of polysaccharides. It may contain sucrose, glucose, lactose, dextrin, or maltose.
Description Tara Gum occurs as a white to light yellow powder having almost no odor.
Identification
(1) Proceed as directed in Identification (1) for Carob Been Gum. A viscous liquid is formed. Heat 100 ml of this liquid on a water bath for 10 minutes, and cool to room temperature. The liquid is more viscous than before heating.
(2) Proceed as directed in Identification (2) for Carob Been Gum.
Purity
(1) Acid-insoluble substances Not more than 5.0 %.
Proceed as directed in Purity (5) for Processed Eucheuma Algae.
(2) Lead Not more than 2.0 μg/g as Pb (5.0 g, Method 1).
(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50g, Method 3, Apparatus B).
(4) Protein Not more than 3.5%.
Weigh accurately about 0.2 g of Tara Gum, and proceed as directed in the Semi-micro Kjeldahl Method under Nitrogen Determination.
Each ml of 0.005 mol/L sulfuric acid = 0.7984 mg of protein
(5) Starch Dissolve 0.10 g of Tara Gum in 10 ml of water by heating with stirring. After cooling, add 2 drops of iodine TS. No blue color develops.
Loss on Drying Not more than 15.0 % (105°C, 5 hours).
Ash Not more than 1.5 % (550°C, 1 hour).
Microbial Limits Proceed as directed in the Microbial Limit Tests. The total bacterial count is not more than 10,000/g, and *Escherichia coli* is negative.

DL-Tartaric Acid

***dl*-Tartaric Acid**

DL-酒石酸

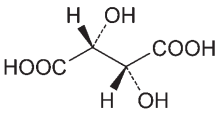


$C_4H_6O_6$ Mol. Wt. 150.09
2,3-Dihydroxybutanedioic acid [133-37-9]
Content DL-Tartaric Acid, when dried, contains not less than 99.5% of DL-tartaric acid ($C_4H_6O_6$).
Description DL-Tartaric Acid occurs as colorless crystals or as a white crystalline powder. It is odorless and has an acid taste.
Identification
(1) A solution of DL-Tartaric Acid (1 in 10) has no optical rotation.
(2) A solution of DL-Tartaric Acid (1 in 10) is acidic.
(3) DL-Tartaric Acid responds to all tests for Tartrate in the Qualitative Tests.
Purity
(1) Melting point 200–206°C (decomposition).
(2) Sulfate Not more than 0.048% as SO_4 (0.50 g, Control solution 0.005 mol/L sulfuric acid 0.50 ml).
(3) Heavy metals Not more than 10 μg/g as Pb.
Test Solution To the residue on ignition of DL-Tartaric Acid, add 1 ml of hydrochloric acid and 0.2 ml of nitric acid, and evaporate to dryness on a water bath. To the residue, add 1 ml of diluted hydrochloric acid (1 in 4) and 30 ml of water, filter if necessary, add 1 drop of phenolphthalein TS, and then add ammonia TS dropwise until the color of the solution changes to a slightly pink color. Add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.
Control Solution Measure 2.0 ml of Lead Standard Solution, and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.
(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).
(5) Readily oxidizable substances Dissolve 1.0 g of DL-Tartaric Acid in 25 ml of water and 25 ml of diluted sulfuric acid (1 in 20). Add 4.0 ml of 0.02 mol/L potassium permanganate, keeping the solution at 20°C. The pink color of the solution does not disappear within 3 minutes.
Loss on Drying Not more than 0.50% (3 hours).
Residue on Ignition Not more than 0.10% (2.0 g).
Assay Weigh accurately about 1.5 g of DL-Tartaric Acid, previously dried, and dissolve it in water to make exactly 250 ml. Measure exactly 25 ml of this solution, and titrate with 0.1 mol/L sodium hydroxide (indicator: 2–3 drops of phenolphthalein TS).
Each ml of 0.1 mol/L sodium hydroxide = 7.504 mg of $C_4H_6O_6$

L-Tartaric Acid

Tartaric Acid
***d*-Tartaric Acid**
L(+)-Tartaric Acid

L-酒石酸



$C_4H_6O_6$ Mol. Wt. 150.09
(2*R*,3*R*)-2,3-Dihydroxybutanedioic acid [87-69-4]

Content L-Tartaric Acid, when dried, contains not less than 99.5% of L-tartaric acid ($C_4H_6O_6$).

Description L-Tartaric Acid occurs as colorless crystals or as a white, fine crystalline powder. It is odorless and has an acid taste.

Identification

(1) A solution of L-Tartaric Acid (1 in 10) is dextrorotatory.

(2) Proceed as directed in Identification (2) and (3) for DL-Tartaric Acid.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: +11.5 to +13.5°.

Weigh accurately about 10 g of L-Tartaric Acid, previously dried, dissolve it in water to make exactly 50 ml, and measure the angular rotation.

(2) Sulfate Not more than 0.048% as SO_4.

Proceed as directed in Purity (2) for DL-Tartaric Acid.

(3) Heavy metals Not more than 10 μg/g as Pb.

Proceed as directed in Purity (3) for DL-Tartaric Acid.

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

(5) Oxalate Dissolve 1.0 g of L-Tartaric Acid in 10 ml of water, and add 2 ml of calcium chloride solution (2 in 25). No turbidity appears.

Loss on Drying Not more than 0.50% (3 hours).

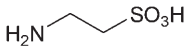
Residue on Ignition Not more than 0.10% (2.0 g).

Assay Proceed as directed in the Assay for DL-Tartaric Acid.

Each ml of 0.1 mol/L sodium hydroxide = 7.504 mg of $C_4H_6O_6$

Taurine (Extract)

タウリン(抽出物)



$C_2H_7NO_3S$ Mol. Wt. 125.15
2-Aminoethanesulfonic acid [107-35-7]

Definition Taurine (Extract) is obtained from the visceral organs or meat of fish, shellfish, or mammals and consists mainly of taurine.

Content Taurine (Extract), when dried, contains not less than 98.5% of taurine ($C_2H_7NO_3S$).

Description Taurine (Extract) occurs as a white crystalline powder having no odor.

Identification

(1) To 5 ml of a solution of Taurine (Extract) (1 in 20), add 5 drops of dilute hydrochloric acid and 5 drops of sodium nitrite solution (1 in 10). The solution effervesces, emitting a colorless gas.

(2) To 0.5 g of Taurine (Extract), add 7.5 ml of sodium hydroxide TS, heat gradually, and evaporate to dryness. Next, heat at 500°C for 2 hours to decompose. To the residue, add 5 ml of water, shake, and filter. To this mixture, add 1 drop of sodium nitroprusside TS. A red-purple color develops.

Purity

(1) Clarity and color of solution Colorless and clear (0.5 g, water 20 ml).

(2) Chloride Not more than 0.011% as Cl (1.0 g, Control solution 0.01 mol/L hydrochloric acid 0.30 ml).

(3) Sulfate Not more than 0.014% as SO_4 (1.5 g, Control solution 0.005 mol/L sulfuric acid 0.45 ml).

(4) Ammonium Not more than 0.020% as NH_4.

Weigh 0.10 g of Taurine (Extract) into a flask, dissolve it in 70 ml of water, and add 1 g of magnesium oxide. Connect the flask to distillation equipment, and place a receiver containing 10 ml of boric acid solution (1 in 200) so that the lower end of the condenser is immersed in the solution in the receiver. Distill at a rate of 5–7 ml/minute until 30 ml of distillate is obtained. To the distillate obtained, add water to make 50 ml. Measure 30 ml of this solution in a Nessler tube, add 6.0 ml of phenol–sodium pentacyanonitrosylferrate(III) TS, and mix. Add 4 ml of sodium hypochlorite–sodium hydroxide TS and water to make 50 ml, mix, and allow to stand for 60 minutes. The color of the resulting solution is not darker than that of a control solution prepared in the same manner as the sample, using 2.0 ml of Ammonium Standard Solution in place of the sample.

(5) Readily carbonizable substances Dissolve 0.10 g of Taurine (Extract) in 1 ml of 94.5–95.5% sulfuric acid. No color develops.

(6) Heavy metal Not more than 20 μg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(7) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 2, Apparatus B).

Loss on Drying Not more than 0.20% (105°C, 2 hours).

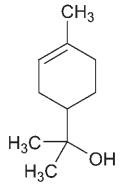
Residue on Ignition Not more than 0.50% (1.0 g).

Assay Weigh accurately about 0.2 g of Taurine (Extract), previously dried, dissolve it in 50 ml of water, and add 5 ml of formalin. Titrate with 0.1 mol/L sodium hydroxide (indicator: 3 drops of phenolphthalein TS). Separately, perform a blank test in the same manner, and make any necessary correction.

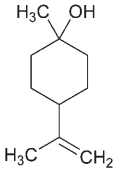
Each ml of 0.1 mol/L sodium hydroxide solution = 12.52 mg of $C_2H_7NO_3S$

Terpineol

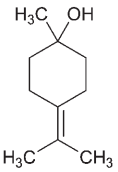
テルピネオール



α-Terpineol



β-Terpineol



γ-Terpineol

$C_{10}H_{18}O$ Mol. Wt. 154.25

Mixture of 2-(4-methylcyclohex-3-en-1-yl)propan-2-ol (α-terpineol), 1-methyl-4-(1-methylethenyl)cyclohexan-1-ol (β-terpineol), and 1-methyl-4-(1-methylethylidene)cyclohexan-1-ol (γ-terpineol)

Content Terpineol contains not less than 97.0% of terpineol ($C_{10}H_{18}O$).

Description Terpineol is a colorless or slightly yellowish, transparent liquid having a characteristic odor.

Identification To 1 ml of Terpineol, add 1 ml of acetic anhydride and 1 drop of phosphoric acid, allow to stand at 30°C for 10 minutes, and add 1 ml of water. Heat in hot water for 5 minutes while shaking, and cool. Add 8 ml of anhydrous sodium carbonate solution (1 in 8). An odor of terpinyl acetate is evolved.

Purity

(1) Refractive index n_D^{20}: 1.482–1.484.

(2) Specific gravity 0.932–0.938.

(3) Clarity of solution Clear (1.0 ml, 70% (vol) ethanol 2.0 ml).

Assay Weigh exactly 5.0 g of Terpineol and 20.0 g of xylene, transfer into a flask, add 10 ml of acetic anhydride and 1 g of anhydrous sodium acetate. Boil gently under a reflux condenser for 6 hours, and cool. Add 10 ml of water, heat in a water bath for 15 minutes with occasional shaking, and cool. Transfer the contents into a separating funnel, and separate the aqueous layer. Wash the oil layer with anhydrous sodium carbonate solution (1 in 8) until the washings is alkaline, and wash with sodium chloride solution (1 in 10) until the washings is neutral. Transfer into a dry container, add about 2 g of anhydrous sodium sulfate, shake, allow to stand for about 30 minutes, and filter. Weigh accurately about 5 g of the filtrate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests. In the test, boil the solution for 4 hours before titrating. Separately, perform a blank test in the same manner, and calculate the content by the formula:

$$\text{Content (\%) of terpineol } (C_{10}H_{18}O) = \frac{154.2 \times (a - b) \times 0.5}{[S - (a - b) \times 0.02102] \times 5/25 \times 1{,}000} \times 100$$

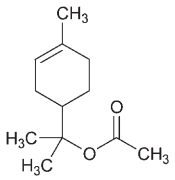
a = volume (ml) of 0.5 mol/L hydrochloric acid consumed in the blank test,

b = volume (ml) of 0.5 mol/L hydrochloric acid consumed in this test, and

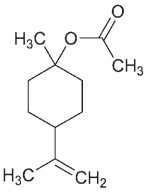
S = weight (g) of the filtrate.

Terpinyl Acetate

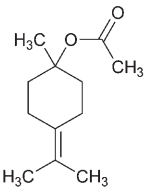
酢酸テルピニル



α-Terpinyl Acetate



β-Terpinyl Acetate



γ-Terpinyl Acetate

$C_{12}H_{20}O_2$ Mol. Wt. 196.29

Mixture of 2-(4-methylcyclohex-3-en-1-yl)propan-2-yl acetate (α-terpinyl acetate), 1-methyl-4-(1-methylethenyl)cyclohexyl acetate (β-terpinyl acetate) and 1-methyl-4-(1-methylethylidene)cyclohexyl acetate (γ-terpinyl acetate) [8007-35-0]

Content Terpinyl Acetate contains not less than 97.0% of terpinyl acetate ($C_{12}H_{20}O_2$).

Description Terpinyl Acetate is a colorless or slightly yellowish, transparent liquid having a characteristic odor.

Identification To 0.5 ml of Terpinyl Acetate, add 5 ml of ethanolic 10% potassium hydroxide TS. Heat under a reflux condenser in a water bath for 1 hour. The characteristic odor disappears, and an odor of terpineol is evolved. After cooling, add 6 ml of water and 2 ml of diluted hydrochloric acid (1 in 4). The solution responds to test (3) for Acetate in the Qualitative Tests.

Purity

(1) Refractive index n_D^{20}: 1.464–1.467.

(2) Specific gravity 0.956–0.965.

(3) Clarity of solution Clear (1.0 ml, 70% (vol) ethanol 5.0 ml).

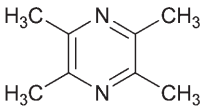
(4) Acid value Not more than 1.0 (Flavoring Substances Tests).

Assay Weigh accurately about 0.7 g of Terpinyl Acetate, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests. In the test, use 20 ml of 0.5 mol/L ethanolic potassium hydroxide, and boil the mixture for 2 hours before titrating.

Each ml of 0.5 mol/L ethanolic potassium hydroxide = 98.14 mg of $C_{12}H_{20}O_2$

2,3,5,6-Tetramethylpyrazine

2,3,5,6-テトラメチルピラジン



$C_8H_{12}N_2$ Mol. Wt. 136.20
2,3,5,6-Tetramethylpyrazine [1124-11-4]
Content 2,3,5,6-Tetramethylpyrazine contains not less than 95.0% of 2,3,5,6-tetramethylpyrazine ($C_8H_{12}N_2$).
Description 2,3,5,6-Tetramethylpyrazine occurs as white crystals or powder having a characteristic odor.
Identification Determine the absorption spectrum of 2,3,5,6-Tetramethylpyrazine as directed in the Paste Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.
Purity Melting point 85–90°C.
Assay Weigh accurately about 0.2 g of 2,3,5,6-Tetramethylpyrazine, and dissolve it in ethanol to make exactly 20 ml. Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay in the Flavoring Substances Tests. Use operating conditions (1).

Thaumatin

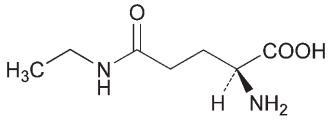
タウマチン

Definition Thaumatin is obtained from the seeds of *Thaumatococcus daniellii* Bentham and consists mainly of thaumatin.
Content Thaumatin, when dried, contains not less than 94% of thaumatin.
Description Thaumatin occurs as a light yellow-brown to gray-brown odorless powder or flakes having an intensely sweet taste.
Identification
(1) To 2 ml of a solution of Thaumatin (1 in 100), add 2 ml of ninhydrin–acetic acid TS and 2 ml of hydrazine sulfate solution (13 in 25,000), and heat in a water bath. A blue-purple color develops.
(2) A solution of Thaumatin (1 in 100,000) is sweet.
Purity
(1) Specific absorbance $E_{1cm}^{1\%}$ (maximum absorption wavelength near 278 nm): 11. 5–13.0.
Weigh accurately about 0.1 g of Thaumatin, and dissolve it in water to make exactly 200 ml. Measure the absorbance of this solution.
(2) Aluminum Not more than 100 µg/g as Al.
Test Solution Weigh accurately about 2 g of Thaumatin, and gently heat to carbonize. After cooling, add a small quantity of sulfuric acid, carefully heat until a white smoke no longer appears, and intensely heat at 450–550°C to incinerate. Add 0.2 mol/L hydrochloric acid to make exactly 25 ml.
Standard Solutions Measure exactly a certain volume of Aluminum Standard Stock Solution, and add water to prepare plural solutions with stepwise concentrations ranging 2.0–10.0 µg/ml of aluminum (Al = 26.98).
Procedure Measure the absorbances of the test solution and the standard solutions as directed under Flame Atomic Absorption Spectrophotometry using the operating conditions given below. Determine the aluminum content in the test solution, using a calibration curve obtained from the absorbances of the standard solutions.
Operating Conditions
Light source: Aluminum hollow cathode lamp.
Wavelength of analytical line: 309.3 nm.
Supporting gas: Nitrous oxide.
Combustible gas: Acetylene.
(3) Carbohydrate Not more than 3.0%.
Test Solution Weigh accurately about 0.5 g of Thaumatin, dissolve it in water, previously adjusted to pH 3 with hydrochloric acid, and make exactly 50 ml. To 0.10 ml of this solution, add exactly 6 ml of cysteine–sulfuric acid TS, heat in a water bath for 3 minutes, and cool with cold water for 5 minutes.
Standard Solutions Prepare plural glucose solutions with different concentrations ranging from 10 to 100µg/ml. Using 0.10 ml each of these solutions, prepare standard solutions as directed for the test solution.
Procedure Measure the absorbances of the test solution and the standard solutions at a wavelength of 400 nm, determine the content of carbohydrate (as D-glucose) in the test solution, using a calibration curve obtained from the absorbances of the standard solutions. As the control solution, use a solution prepared without sample in the same manner as for the test solution.
(4) Lead Not more than 10µg/g as Pb (1.0 g, Method 1).
(5) Arsenic Not more than 4.0µg/g as As_2O_3 (1.0 g, Method 3, Apparatus C, Control solution Arsenic Standard Solution 4.0 ml).
Loss on Drying Not more than 9.0% (105°C, 3 hours).
Residue on Ignition Not more than 2.0%.
Assay Weigh accurately about 0.15 g of Thaumatin, previously dried, and proceed as directed in the Kjeldahl Method under Nitrogen Determination. Calculate the thaumatin content by the formula:

$$\text{Content (\%) of thaumatin} = \frac{\left(\begin{array}{c}\text{Volume (ml) of 0.1 mol/L}\\ \text{sodium hydroxide consumed}\end{array}\right) \times 1.401 \times 6.25}{\text{Weight (g) of the sample} \times 1{,}000} \times 100$$

L-Theanine

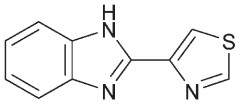
L-テアニン



$C_7H_{14}N_2O_3$ Mol. Wt. 174.20
(2*S*)-2-Amino-4-(*N*-ethylcarbamoyl)butanoic acid [3081-61-6]
Content L-Theanine, when calculated on the dried basis, contains 98.0–102.0% of L-theanine ($C_7H_{14}N_2O_3$).
Description L-Theanine occurs as a white crystalline powder. It is odorless and has a slightly characteristic and sweet taste.
Identification
(1) To 5 ml of a solution of L-Theanine (1 in 1,000), add 1 ml of ninhydrin solution (1 in 1,000), and heat for 3 minutes. A purple color develops.
(2) Dissolve about 1 g of L-Theanine in 10 ml of diluted hydrochloric acid (1 in 2), heat on a water bath under a reflux condenser for 6 hours, and add water to make 20 ml. Transfer 5 ml of this solution into a test tube, and add 2 g of sodium hydroxide. Suspend a red litmus paper moistened with water in the test tube, cover the mouth of the test tube, and heat in a water bath for 5 minutes. The litmus paper turns blue.
Purity
(1) Specific rotation $[\alpha]_D^{20}$: +7.7 to +8.5° (2.5 g, water, 50 ml, on the dried basis).
(2) Clarity and color of solution Colorless and almost clear (1.0 g, water 20 ml).
(3) pH 5.0–6.0 (1.0 g, water 100 ml).
(4) Chloride Not more than 0.021% as Cl (0.50 g, Control solution 0.01 mol/L hydrochloric acid 0.30 ml).
(5) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).
(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).
Loss on Drying Not more than 0.50% (105°C, 3 hours).
Residue on Ignition Not more than 0.20%.
Assay Weigh accurately about 0.35 g of L-Theanine, and proceed as directed in the Assay for DL-Alanine.
Each ml of 0.1 mol/L perchloric acid = 17.42 mg of $C_7H_{14}N_2O_3$

Thiabendazole

チアベンダゾール

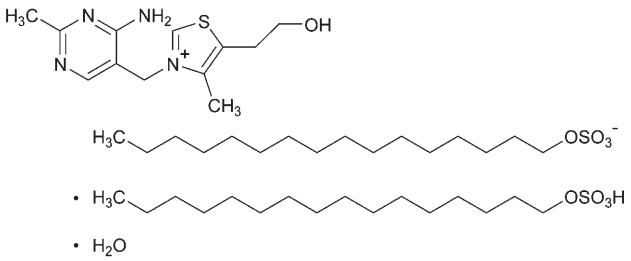


$C_{10}H_7N_3S$ Mol. Wt. 201.25
2-(1,3-Thiazol-4-yl)-1*H*-benzo[*d*]imidazole [148-79-8]
Content Thiabendazole, when dried, contains 98.0–101.0% of thiabendazole ($C_{10}H_7N_3S$).
Description Thiabendazole occurs as a white to whitish powder. It is odorless.
Identification
(1) Dissolve 5 mg of Thiabendazole in 5 ml of diluted hydrochloric acid (1 in 100), add 3 mg of *p*-phenylenediamine hydrochloride and about 0.1 g of zinc dust, and allow to stand for 2 minutes. An odor of hydrogen sulfide is evolved. To this solution, add 0.5 ml of ferric ammonium sulfate–sulfuric acid TS. A blue to blue-purple color develops.
(2) Dissolve 5 mg of Thiabendazole in 1,000 ml of diluted hydrochloric acid (1 in 100). The solution exhibits absorption maxima at wavelengths of 298–306 nm and 239–247 nm, and an absorption minimum at a wavelength of 254–262 nm.
Purity
(1) Melting point 296–303°C (decomposition).
(2) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).
Loss on Drying Not more than 0.50% (reduced pressure, 24 hours).
Residue on Ignition Not more than 0.20%.
Assay Weigh accurately about 0.2 g of Thiabendazole, previously dried, add 10 ml of acetic acid for nonaqueous titration, dissolve by warming, and cool. Add 50 ml of acetic anhydride and 1 ml of mercuric acetate TS for nonaqueous titration, and titrate with 0.1 mol/L perchloric acid (indicator: 1 ml of crystal violet–acetic acid TS) until the color of the solution changes from purple through blue to green. Separately, perform a blank test in the same manner, and make any necessary correction.
Each ml of 0.1 mol/L perchloric acid = 20.12 mg of $C_{10}H_7N_3S$

Thiamine Dicetylsulfate

Vitamin B_1 Dicetylsulfate

チアミンセチル硫酸塩



$C_{44}H_{84}N_4O_9S_3 \cdot H_2O$ Mol. Wt. 927.37

3-(4-Amino-2-methylpyrimidin-5-ylmethyl)-5-(2-hydroxyethyl)-4-methylthiazolium dihexadecylsulfate monohydrate

Content Thiamine Dicetylsulfate, when dried, contains 96.0–102.0% of thiamine dicetylsulfate ($C_{44}H_{84}N_4O_9S_3 \cdot H_2O$).

Description Thiamine Dicetylsulfate occurs as colorless to white crystals or as a white crystalline powder. It is odorless or has a slight, characteristic odor.

Identification

(1) To 0.1 g of Thiamine Dicetylsulfate, add 20 ml of potassium chloride–hydrochloric acid TS, boil gently for about 30 minutes, cool, and filter. To 1 ml of the filtrate, add 1 ml of lead acetate TS and 1 ml of sodium hydroxide solution (1 in 10). A yellow color develops. The solution turns brown when warmed on a water bath, and then a black-brown precipitate is formed on standing.

(2) To 1 ml of the filtrate obtained in Identification (1), add 5 ml of sodium hydroxide solution (1 in 50) and 0.5 ml of freshly prepared potassium ferricyanide solution (1 in 10), then add 5 ml of 2-methyl-1-propanol, and shake vigorously for 2 minutes. Allow to stand, and examine under ultraviolet light. The 2-methyl-1-propanol layer emits a blue-purple fluorescence, which disappears when the solution is acidic but reappears when it is made alkaline.

(3) To 1 g of Thiamine Dicetylsulfate, add 30 ml of water and 15 ml of hydrochloric acid, boil under a reflux condenser for about 4 hours, and cool. Extract twice with 15 ml of diethyl ether each time, combine the diethyl ether extracts, wash with water, and remove the diethyl ether by evaporating on a water bath. Dry the residue at 100°C for 15 minutes, and cool. Measure the melting point of the residue. It is 46–56°C.

Purity

(1) Chloride Not more than 0.057% as Cl.

Test Solution Weigh 0.25 g of Thiamine Dicetylsulfate, add 30 ml of water, shake well, and allow to stand for 10 minutes. Dissolve it by adding 6 ml of diluted nitric acid (1 in 10), filter, and wash with water. Combine the filtrate and the washings, and add water to make 50 ml.

Control Solution To 0.40 ml of 0.01 mol/L hydrochloric acid, add 6 ml of diluted nitric acid (1 in 10) and water to make 50 ml.

(2) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

Loss on Drying Not more than 2.0% (24 hours).

Residue on Ignition Not more than 0.30%.

Assay

Test Solution Weigh accurately about 0.14 g of Thiamine Dicetylsulfate, previously dried, add 40 ml of potassium chloride–hydrochloric acid TS, and heat on a water bath for 30 minutes with occasional shaking. After cooling, filter, wash with 50 ml of water, combine the filtrate and the washings, and add water to make exactly 100 ml. Measure exactly 2 ml of this solution, add exactly 5 ml of a solution of methyl benzoate in methanol (1 in 1,000), and add the mobile phase (the same solution used in the Assay for Thiamine Hydrochloride) to make exactly 100 ml.

Standard Solution Weigh accurately about 0.05 g of Thiamine Hydrochloride Reference Standard (the water content should be measured previously in the same manner as for Thiamine Hydrochloride), dissolve it in 40 ml of potassium chloride–hydrochloric acid TS, and add water to make exactly 100 ml. Measure exactly 2 ml of this solution, add exactly 5 ml of a solution of methyl benzoate in methanol (1 in 1,000), and add the mobile phase to make exactly 100 ml.

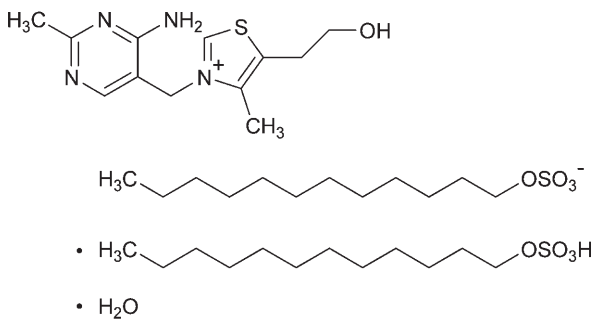
Procedure Proceed as directed in the Assay for Thiamine Hydrochloride, using the test solution and the standard solution. Calculate the content by the formula:

$$\text{Content (\%) of thiamine dicetylsulfate } (C_{44}H_{84}N_4O_9S_3 \cdot H_2O) = \frac{\left(\begin{array}{c}\text{Anhydrous basis weight (g) of Thiamine}\\ \text{Hydrochloride Reference Standard}\end{array}\right)}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 2.750 \times 100$$

Thiamine Dilaurylsulfate

Vitamin B_1 Dilaurylsulfate

チアミンラウリル硫酸塩



$C_{36}H_{68}N_4O_9S_3 \cdot H_2O$ Mol. Wt. 815.16

3-(4-Amino-2-methylpyrimidin-5-ylmethyl)-5-(2-hydoxyethyl)-4-methylthiazolium didodecylsulfate monohydrate

Content Thiamine Dilaurylsulfate, when dried, contains 98.0–102.0% of thiamine dilaurylsulfate ($C_{36}H_{68}N_4O_9S_3 \cdot H_2O$).

Description Thiamine Dilaurylsulfate occurs as colorless to white crystals or as a white crystalline powder. It is odorless or has a slight, characteristic odor.

Identification

(1) Proceed as directed in Identification (1) and (2) for Thiamine Dicetylsulfate.

(2) Proceed as directed in Identification (3) for Thiamine Dicetylsulfate. The melting point is 20–28°C.

Purity

(1) Chloride Not more than 0.057% as Cl.

Proceed as directed in Purity (1) for Thiamine Dicetylsulfate.

(2) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

Loss on Drying Not more than 2.0% (24 hours).

Residue on Ignition Not more than 0.30%.

Assay

Test Solution Weigh accurately about 0.12 g of Thiamine Dilaurylsulfate, previously dried, add 40 ml of potassium chloride–hydrochloric acid TS, and heat on a water bath for 30 minutes with occasional shaking. After cooling, filter, wash with 50 ml of water, combine the filtrate and the washings, and add water to make exactly 100 ml. Measure exactly 2 ml of this solution, add exactly 5 ml of a solution of methyl benzoate in methanol (1 in 1,000), and add the mobile phase (the same solution used in the Assay for Thiamine Hydrochloride) to make exactly 100 ml.

Standard Solution Weigh accurately about 0.05 g of Thiamine Hydrochloride Reference Standard (the water content should be measured previously in the same manner as for Thiamine Hydrochloride), dissolve it in 40 ml of potassium chloride–hydrochloric acid TS, and add water to make exactly 100 ml. Measure exactly 2 ml of this solution, add exactly 5 ml of a solution of methyl benzoate in methanol (1 in 1,000), and add the mobile phase to make exactly 100 ml.

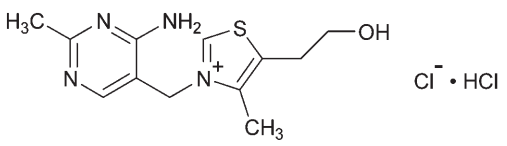
Procedure Proceed as directed in the Assay for Thiamine Hydrochloride, using the test solution and the standard solution. Calculate the content by the formula:

$$\text{Content (\%) of thiamine dilaurylsulfate } (C_{36}H_{68}N_4O_9S_3\cdot H_2O) = \frac{\left(\begin{array}{c}\text{Anhydrous basis weight (g) of Thiamine}\\ \text{Hydrochloride Reference Standard}\end{array}\right)}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 2.417 \times 100$$

Thiamine Hydrochloride

Vitamin B_1 Hydrochloride

チアミン塩酸塩



$C_{12}H_{17}ClN_4OS\cdot HCl$ Mol. Wt. 337.27

3-(4-Amino-2-methylpyrimidin-5-ylmethyl)-5-(2-hydoxyethyl)-4-methylthiazolium chloride monohydrochloride [67-03-8]

Content Thiamine Hydrochloride, calculated on the anhydrous basis, contains 98.0–102.0% of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS\cdot HCl$).

Description Thiamine Hydrochloride occurs as white to yellowish-white, fine crystals or crystalline powder. It is odorless or has a slight, characteristic odor.

Identification

(1) To 1 ml of a solution of Thiamine Hydrochloride (1 in 500), add 1 ml of lead acetate TS and 1 ml of sodium hydroxide solution (1 in 10). A yellow color develops. The solution turns brown when warmed on a water bath, and then a black-brown precipitate is formed on standing.

(2) To 5 ml of a solution of Thiamine Hydrochloride (1 in 500), add 2.5 ml of sodium hydroxide solution (1 in 25) and 0.5 ml of freshly prepared potassium ferricyanide solution (1 in 10), then add 5 ml of 2-methyl-1-propanol, shake vigorously for 2 minutes, and allow to stand. Examine under ultraviolet light. The 2-methyl-1-propanol layer emits a blue-purple fluorescence. The fluorescence disappears when the solution is acidic but reappears when the solution is made alkaline.

(3) Thiamine Hydrochloride responds to all tests for Chloride in the Qualitative Tests .

Purity

(1) Clarity and color of solution Weigh 1.0 g of Thiamine Hydrochloride, and dissolve it in water to make 10 ml. The solution is clear and not darker in color than a solution prepared by adding water to 1.5 ml of 1/60 mol/L potassium dichromate solution to make 1,000 ml.

(2) pH 2.7–3.4 (1.0 g, water 100 ml).

(3) Sulfate Not more than 0.011% as SO_4 (1.5 g Control solution 0.005 mol/L sulfuric acid 0.35 ml).

(4) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

Water Content Not more than 5.0% (0.50 g, Direct Titration).

Residue on Ignition Not more than 0.20%.

Assay

Test Solution and Standard Solution Weigh accurately about 0.1 g each of Thiamine Hydrochloride and Thiamine Hydrochloride Reference Standard (the water content should be previously measured in the same manner as for Thiamine Hydrochloride), dissolve each in the mobile phase prepared as directed in the Operating Conditions to make exactly 50 ml. To exactly 10 ml each of the solutions, add exactly 5 ml

of a solution of methyl benzoate in methanol (1 in 50), and then add the mobile phase to make two solutions of exactly 50 ml each. Use these solutions as the test solution and the standard solution, respectively.

Procedure Analyze 10 µl portions of these solutions by liquid chromatography using the conditions given below. Calculate the peak area ratio of thiamine to methyl benzoate for the test solution and the standard solution, and express as Q_T and Q_S, respectively. Calculate the content by the formula:

$$\text{Content (\%) of thiamine hydrochloride}(C_{12}H_{17}ClN_4OS\cdot HCl) = \frac{\left(\begin{array}{c}\text{Anhydrous basis weight (g) of Thiamine}\\ \text{Hydrochloride Reference Standard}\end{array}\right)}{\text{Anhydrous basis weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 100$$

Operating Conditions

Detector: Ultraviolet spectrophotometer (determination wavelength: 254 nm).

Column: A stainless steel tube of about 4 mm internal diameter and 15–30 cm length.

Column packing material: 5- to 10-µm octadecylsilanized silica gel for liquid chromatography.

Column temperature: A constant temperature at about 25°C.

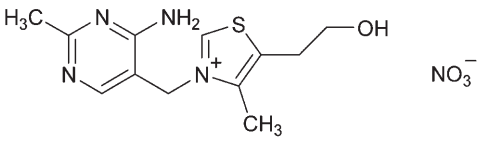
Mobile phase: Dissolve 1.1 g of sodium 1-octanesulfonate in 1,000 ml of diluted acetic acid (1 in 100). To 600 ml of this solution, add 400 ml of a 3:2 mixture of methanol/acetonitrile.

Flow rate: Adjust so that the retention time of thiamine is about 12 minutes.

Thiamine Mononitrate

Vitamin B_1 Mononitrate

チアミン硝酸塩



$C_{12}H_{17}N_5O_4S$ Mol. Wt. 327.36

3-(4-Amino-2-methylpyrimidin-5-ylmethyl)-5-(2-hydoxyethyl)-4-methylthiazolium nitrate [532-43-4]

Content Thiamine Mononitrate, when dried, contains 98.0–102.0% of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$).

Description Thiamine Mononitrate occurs as white to yellowish white crystals or crystalline powder. It is odorless or has a slight, characteristic odor.

Identification

(1) Proceed as directed in Identification (1) and (2) for Thiamine Hydrochloride.

(2) Thiamine Mononitrate responds to all tests for Nitrate in the Qualitative Tests.

Purity

(1) pH 6.5–8.0 (1.0 g, water 50 ml).

(2) Chloride Not more than 0.057% as Cl (0.25 g, Control solution 0.01 mol/L hydrochloric acid 0.40 ml).

(3) Heavy metals Not more than 20 µg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

Loss on Drying Not more than 1.0% (105°C, 2 hours).

Residue on Ignition Not more than 0.20%.

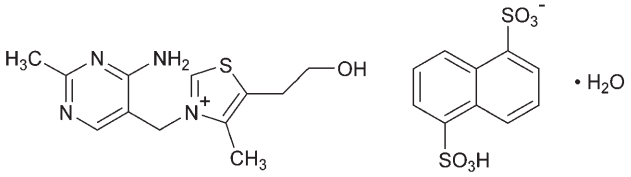
Assay Weigh accurately about 0.1 g each of Thiamine Mononitrate, previously dried, and Thiamine Hydrochloride Reference Standard (the water content should be measured in the same manner as for Thiamine Hydrochloride). Proceed as directed in the Assay for Thiamine Hydrochloride, and calculate the content by the formula:

$$\text{Content (\%) of thiamine mononitrate } (C_{12}H_{17}N_5O_4S) = \frac{\left(\begin{array}{c}\text{Anhydrous basis weight (g) of Thiamine}\\ \text{Hydrochloride Reference Standard}\end{array}\right)}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 0.9706 \times 100$$

Thiamine Naphthalene-1,5-disulfonate

Vitamin B_1 Naphthalene-1,5-disulfonate

チアミンナフタレン-1,5-ジスルホン酸塩



$C_{22}H_{24}N_4O_7S_3\cdot H_2O$ Mol. Wt. 570.66

3-(4-Amino-2-methylpyrimidin-5-ylmethyl)-5-(2-hydoxyethyl)-4-methylthiazolium naphthalene-1,5-disulfonate monohydrate

Content Thiamine Naphthalene-1,5-disulfonate, when dried, contains 98.0–102.0% of thiamine naphthalene-1,5-disulfonate ($C_{22}H_{24}N_4O_7S_3$ = 552.65).

Description Thiamine Naphthalene-1,5-disulfonate occurs as a white, fine crystalline powder. It is odorless or has a slight, characteristic odor.

Identification

(1) Proceed as directed in Identification (1) and (2) for Thiamine Hydrochloride.

(2) Dissolve 0.01 g of Thiamine Naphthalene-1,5-disulfonate in 100 ml of diluted hydrochloric acid (1 in 10,000). To 5 ml of the solution, add diluted hydrochloric acid (1 in 10,000) to make 100 ml. The solution exhibits an absorption maximum at a wavelength of 225–227 nm.

Purity

(1) Chloride Not more than 0.057% as Cl.

Proceed as directed in Purity (1) for Thiamine Dicetylsulfate.

(2) Heavy metals Not more than 20 µg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

Loss on Drying Not more than 5.0% (105°C, 2 hours).

Residue on Ignition Not more than 0.20%.

Assay

Test Solution Weigh accurately about 0.16 g of Thiamine Naphthalene-1,5-disulfonate, previously dried, add 30 ml of diluted hydrochloric acid (1 in 1,000), and dissolve by heating on a water bath. After cooling, add diluted hydrochloric acid (1 in 1,000) to make exactly 50 ml. Measure exactly 10 ml of this solution, add 50 ml of diluted hydrochloric acid (1 in 1,000), and add methanol to make exactly 100 ml. Measure exactly 25 ml of the second solution, add exactly 5 ml of a solution of methyl benzoate in methanol (1 in 200), and add water to make exactly 50 ml.

Standard Solution Weigh accurately about 0.1 g of Thiamine Hydrochloride Reference Standard (the water content should be measured previously in the same manner as for Thiamine Hydrochloride), dissolve it in diluted hydrochloric acid (1 in 1,000) to make exactly 50 ml. Then proceed in the same manner as the preparation of the test solution.

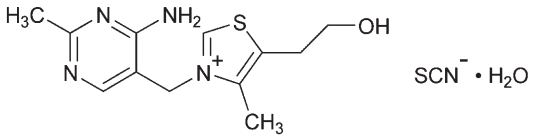
Procedure Proceed as directed in the Assay for Thiamine Hydrochloride, using the test solution and the standard solution. Calculate the content by the formula:

$$\text{Content (\%) of thiamine naphthalene-1,5-disulfonate } (C_{22}H_{24}N_4O_7S_3) = \frac{\left(\begin{array}{c}\text{Anhydrous basis weight (g) of Thiamine}\\ \text{Hydrochloride Reference Standard}\end{array}\right)}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 1.639 \times 100$$

Thiamine Thiocyanate

Vitamin B_1 Rhodanate

チアミンチオシアン酸塩



$C_{13}H_{17}N_5OS_2 \cdot H_2O$ Mol. Wt. 341.45

3-(4-Amino-2-methylpyrimidin-5-ylmethyl)-5-(2-hydoxyethyl)-4-methylthiazolium thiocyanate monohydrate [130131-60-1]

Content Thiamine Thiocyanate, when dried, contains 98.0–102.0% of thiamine thiocyanate ($C_{13}H_{17}N_5OS_2$ = 323.44).

Description Thiamine Thiocyanate occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor.

Identification

(1) Proceed as directed in Identification (1) and (2) for Thiamine Hydrochloride.

(2) Thiamine Thiocyanate saturated solution responds to all tests for Thiocyanate in the Qualitative Tests.

Purity

(1) Chloride Not more than 0.057% as Cl.

Weigh 0.25 g of Thiamine Thiocyanate, add 1.5 ml of water, 0.3 g of ammonium nitrate, and 0.9 ml of sodium hydroxide solution (2 in 5), and then add 3 ml of hydrogen peroxide gradually dropwise while shaking. Heat on a water bath for 30 minutes with occasional shaking, cool, and add 3 ml of diluted nitric acid (2 in 3) and water to make 50 ml. Add 0.1 ml of a dextrin solution (1 in 50) and 0.5 ml of silver nitrate solution (1 in 50), and allow to stand for 5 minutes. The solution is not more turbid than the control solution prepared, using 0.40 ml of 0.01 mol/L hydrochloric acid in the same manner as for the test solution.

(2) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

Loss on Drying Not more than 6.0% (105°C, 2 hours).

Residue on Ignition Not more than 0.20%.

Assay

Test Solution Weigh accurately about 0.1 g of Thiamine Thiocyanate, previously dried, and dissolve it in diluted hydrochloric acid (1 in 10,000) to make exactly 200 ml. Measure exactly 2 ml of this solution, add exactly 5 ml of a solution of methyl benzoate in methanol (1 in 50), and add the mobile phase (the same solution used in the Assay for Thiamine Hydrochloride) to make exactly 50 ml.

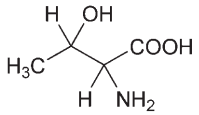
Standard Solution Weigh accurately about 0.1 g of Thiamine Hydrochloride Reference Standard (the water content should be measured previously as directed for Thiamine Hydrochloride), and proceed in the same manner as the preparation of the test solution.

Procedure Proceed as directed in the Assay for Thiamine Hydrochloride, using the test solution and the standard solution. Calculate the content by the formula:

$$\text{Content (\%) of thiamine thiocyanate } (C_{13}H_{17}N_5OS_2) = \frac{\left(\begin{array}{c}\text{Anhydrous basis weight (g) of Thiamine}\\ \text{Hydrochloride Reference Standard}\end{array}\right)}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 0.9590 \times 100$$

DL-Threonine

DL-トレオニン



$C_4H_9NO_3$ Mol. Wt. 119.12

2-Amino-3-hydroxybutanoic acid [80-68-2]

Content DL-Threonine, when calculated on the dried basis, contains 98.0–102.0% of DL-threonine ($C_4H_9NO_3$).

Description DL-Threonine occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor, and has a slightly sweet taste.

Identification

(1) To 5 ml of a solution of DL-Threonine (1 in 1,000), add 1 ml of ninhydrin solution (1 in 1,000), and heat for 3 minutes. A purple color develops.

(2) To 5 ml of a solution of DL-Threonine (1 in 10), add 0.5 g of potassium periodate, and heat in a water bath. An evolved gas changes the color of a red litmus paper moistened with water to blue.

(3) A solution of DL-Threonine (1 in 25) has no optical rotation.

Purity

(1) Clarity and color of solution Colorless and clear (1.0 g, water 20 ml).

(2) pH 5.0–6.5 (1.0 g, water 20 ml).

(3) Chloride Not more than 0.021% as Cl (0.50 g, Control solution 0.01 mol/L hydrochloric acid 0.30 ml).

(4) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

(6) Allothreonine

Test Solution Weigh 0.10 g of DL-Threonine, and dissolve it in water to make 50 ml.

Procedure Analyze 5 μl of the test solution by paper chromatography using a 5:3:1:1 mixture of 1-butanol/methyl ethyl ketone/water/ammonia TS as the developing solvent. No control solution is used. For the filter paper, use a No. 2 filter paper for chromatography. Stop the development when the developing solvent has ascended to a point about 30 cm above the original line. Air-dry the filter paper, then dry at 100°C for 20 minutes, spray with a solution of ninhydrin in acetone (1 in 50), and dry at 100°C for 5 minutes. Examine the chromatogram in daylight. Only one spot is observed.

Loss on Drying Not more than 0.20% (105°C, 3 hours).

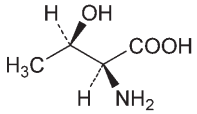
Residue on Ignition Not more than 0.10% .

Assay Proceed as directed in the Assay for DL-Alanine.

Each ml of 0.1 mol/L perchloric acid = 11.91 mg of $C_4H_9NO_3$

L-Threonine

L-トレオニン



$C_4H_9NO_3$ Mol. Wt. 119.12

(2*S*,3*R*)-2-Amino-3-hydroxybutanoic acid [72-19-5]

Content L-Threonine, when calculated on the dried basis, contains 98.0–102.0% of L-threonine ($C_4H_9NO_3$).

Description L-Threonine occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor, and has a slightly sweet taste.

Identification

(1) Proceed as directed in Identification (1) for DL-Threonine.

(2) To 0.5 g of L-Threonine, add 5 ml of water, dissolve by warming, and proceed as directed in Identification (2) for DL-Threonine.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: –26.0 to –29.0° (3.0 g, water, 50 ml, on the dried basis).

(2) Clarity and color of solution Colorless and clear (1.0 g, water 20 ml).

(3) pH 5.0–6.5 (1.0 g, water 20 ml).

(4) Chloride Not more than 0.021% as Cl.

Proceed as directed in Purity (3) for DL-Threonine.

(5) Heavy metals Not more than 20 μg/g as Pb.

Proceed as directed in Purity (4) for DL-Threonine.

(6) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.50 g of L-Threonine, and dissolve it in 5 ml of diluted hydrochloric acid (1 in 4).

Apparatus Use Apparatus B.

(7) Allothreonine Proceed as directed in Purity (6) for DL-Threonine.

Loss on Drying Not more than 0.20% (105°C, 3 hours).

Residue on Ignition Not more than 0.10%.

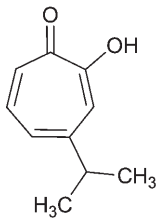
Assay Proceed as directed in the Assay for DL-Alanine.

Each ml of 0.1 mol/L perchloric acid = 11.91 mg of $C_4H_9NO_3$

Thujaplicin (Extract)

Hinokitiol (Extract)

ツヤプリシン



$C_{10}H_{12}O_2$ Mol. Wt. 164.20

2-Hydroxy-4-(1-methylethyl)cyclohepta-2,4,6-trien-1-one [499-44-5]

Definition Thujaplicin (Extract) is obtained from the trunks, branches, or roots of the tree *Thujopsis dolabrata* Siebold et Zuccarini and consists mainly of thujaplicins.

Content Thujaplicin (Extract), when dried, contains 98.0–102% of β- thujaplicin ($C_{10}H_{12}O_2$ = 164.20).

Description Thujaplicin (Extract) occurs as white to yellow crystals, crystalline powder, or lumps. It has a characteristic odor.

Identification Dissolve 0.1 g of Thujaplicin (Extract) in 10 ml of ethanol, and add 1 drop of iron(III) chloride TS. A dark red color develops.

Purity

(1) Clarity of solution Clear (1.0 g, ethanol 5.0 ml).

(2) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Loss on Drying Not more than 0.5 % (1 g, 1.7–2.0 kPa, silica gel, 4 hours).

Residue on Ignition Not more than 0.05 % (2 g).

Assay

Test Solution Weigh accurately about 0.2 g of Thujaplicin (Extract), previously dried, add exactly 1 ml of the internal standard solution, and add ethanol to make exactly 100 ml.

Standard Solution Weigh accurately about 0.2 g of β-thujaplicin for assay, previously dried, add exactly 1 ml of the internal standard solution, and add ethanol to make exactly 100 ml. As the internal standard solution, use a solution prepared by adding absolute ethanol to 1.0 g of diphenyl ether, weighed exactly, to make exactly 5 ml.

Procedure Analyze 0.5 μl portions of the test solution and the standard solution by gas chromatography using the operating conditions given below. Determine the peak area ratios (Q_T and Q_S) of β-thujaplicin to diphenyl ether for the test solution and the standard solution. Calculate the content of β-thujaplicin by the formula:

$$\text{Content (\%) of β-thujaplicin } (C_{10}H_{12}O_2) = \frac{\text{Weight (g) of β-thujaplicin for assay}}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 100$$

Operating Conditions

Detector: Hydrogen flame ionization detector.

Column: A silicate-glass capillary tube (0.25 mm internal diameter and 30 m length) coated with a 0.25-μm thick layer of dimethylpolysiloxane for gas chromatography.

Column temperature: Raise from 100°C to 250°C at a rate of 10°C per minute.

Injection port temperature: 250°C.

Injection method: Split (10:1).

Carrier gas: Helium.

Flow rate: Adjust so that the peak of β-thujaplicin appears about 7 minutes after the injection.

Titanium Dioxide

二酸化チタン

TiO_2 Mol. Wt. 79.87

Titanium dioxide [13463-67-7]

Content Titanium Dioxide, when dried, contains not less than 99.0% of titanium dioxide (TiO_2).

Description Titanium Dioxide occurs as a white powder. It is odorless and tasteless.

Identification To 0.5 g of Titanium Dioxide, add 5 ml of sulfuric acid, and heat gently until fumes of sulfuric acid are evolved. After cooling, add water gradually to make about 100 ml, and filter. To 5 ml of the filtrate, add hydrogen peroxide TS. A yellow-red to orange-red color develops.

Purity

(1) Water-soluble substances Not more than 0.25%.

Weigh 4.0 g of Titanium Dioxide, add 50 ml of water, shake, and allow to stand overnight. Add 2 ml of ammonium chloride solution (1 in 10), and shake. If a precipitate of titanium dioxide is not formed, add another 2 ml of ammonium chloride solution (1 in 10), and allow to stand. After the precipitate is formed, add water to make 200 ml, and filter while shaking. Discard 10 ml of the initial filtrate, transfer 100 ml of the subsequent filtrate into a platinum crucible, previously weighed, evaporate to dryness, ignite to constant weight, and weigh the residue.

(2) Hydrochloric acid-soluble substances Not more than 0.50%.

Weigh 5.0 g of Titanium Dioxide, add 100 ml of diluted hydrochloric acid (1 in 20), shake, heat on a water bath for 30 minutes with occasional stirring, and filter. Wash the residue three times with 10 ml of diluted hydrochloric acid (1 in 20) each time, combine the filtrate and the washings, evaporate to dryness, ignite to constant weight, and weigh the residue.

(3) Heavy metals Not more than 10 μg/g as Pb.

Sample Solution Weigh 10.0 g of Titanium Dioxide, transfer into a 250-ml beaker, and add 50 ml of diluted hydrochloric acid (1 in 20). Cover with a watch glass, heat to boiling, then continue boiling gently for 15 minutes, and centrifuge to precipitate the insoluble residue. Filter the supernatant, wash the beaker used and the residue three times with 10 ml of hot water each time, and filter through the same filter paper. Wash the filter paper with 10 to 15 ml of hot water, combine the filtrate and the washings, cool, and add water to make 100 ml.

Test Solution Measure 20 ml of the sample solution, add one drop of phenolphthalein TS, add ammonia TS dropwise until the color of the solution changes to a slightly pink color, and then add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(4) Arsenic Not more than 1.3 μg/g as As_2O_3.

Test Solution Measure 15 ml of the sample solution prepared in Purity (3).

Apparatus Use Apparatus B.

Loss on Drying Not more than 0.50% (105°C, 3 hours).

Loss on Ignition Not more than 0.50% (dried substance, 775–825°C).

Assay Weigh accurately about 0.15 g of Titanium Dioxide, previously dried, transfer into a 500-ml Erlenmeyer flask, add 5 ml of water, and shake well until a homogeneous milky liquid is obtained. Add 30 ml of sulfuric acid and 12 g of ammonium sulfate, heat gently at first and then strongly to dissolve the sample completely. After cooling, add 120 ml of water and 40 ml of hydrochloric acid, shake well, and add 3 g of aluminum metal bar or wire. Immediately insert one end of a U-tube with a rubber stopper into this solution and the other end in a wide-mouthed bottle containing a saturated solution of sodium hydrogen carbonate, and generate hydrogen. When the aluminum metal dissolves completely and the liquid is transparent purple in color, allow to stand for several minutes, cool to below 50°C in running water, and remove the U-tube with rubber stopper. Add 3 ml of a saturated potassium thiocyanate solution as indicator, and titrate immediately with 0.1 mol/L ferric ammonium sulfate solution to the first faint brown color that persists for about 30 seconds.

$$\text{Content (\%) of titanium dioxide } (TiO_2) = \frac{\left(7.987 \times \dfrac{\text{Volume (ml) of 0.1 mol/L ferric ammonium sulfate consumed}}{}\right)}{\text{Weight (g) of the sample} \times 1{,}000} \times 100$$

d-α-Tocopherol

α-Vitamin E

d-α-トコフェロール

[59-02-9]

Definition *d*-α-Tocopherol is obtained by isolation from vegetable fats and oils, which are produced from oil seeds, or mixed tocopherols (products consisting mainly of *d*-*α*-, *d*-*β*-, *d*-*γ*-, and *d*-δ-tocopherols, obtained from vegetable fats and oils). It consists mainly of *d*-α-tocopherol. It may contain edible fats or oils.

Content *d*-α-Tocopherol contains not less than 40% of total tocopherols, of which not less than 50% consists of *d*-α-tocopherol.

Description *d*-α-Tocopherol is a light yellow to red-brown, clear viscous liquid having a slight, characteristic odor.

Identification Dissolve 0.05 g of *d*-α-Tocopherol in 10 ml of absolute ethanol, add 2 ml of nitric acid, and heat at about 75°C for 15 minutes. An orange to red color develops.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: not less than +24°.

Weigh accurately an amount of *d*-α-Tocopherol equivalent to about 0.1 g of the total tocopherols, transfer into a separating funnel, and dissolve it in 50 ml of diethyl ether. Add 2 g of potassium ferricyanide dissolved in 20 ml of sodium hydroxide solution (1 in 125), and shake for 3 minutes. Wash the diethyl ether layer with four 50-ml portions of water, and collect the diethyl ether layer. Dehydrate the diethyl ether layer by adding about 2 g of anhydrous sodium sulfate, and filter. Evaporate the diethyl ether from the filtrate, immediately dissolve the residue in 5 ml of isooctane, and measure the optical rotation. Calculate the specific rotation of this solution, using the concentration (g/ml) of the total tocopherols in the solution determined as directed in the Assay.

(2) Acid value Not more than 5.0.

Proceed as directed in Purity (2) for Tocotrienol.

(3) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Assay

Test Solution Weigh accurately an amount of *d*-α-Tocopherol equivalent to about 0.05 g of the total tocopherols, transfer into a brown volumetric flask, and dissolve it in hexane to make exactly 100 ml.

Standard Solution Weigh accurately about 0.05 g each of *d*-α-tocopherol for assay, *d*-β-tocopherol for assay, *d*-γ-tocopherol for assay, and *d*-δ-tocopherol for assay into separate brown 100-ml volumetric flasks, and dissolve each in hexane to make standard stock solutions. Transfer exactly an appropriate amount of each of the standard stock solutions into a volumetric flask so that the relative proportion of tocopherols in the resulting solution is almost the same as that in the sample. Mix them to prepare a standard solution.

Procedure Analyze 20 μl portions of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Measure the peak areas of *d*-α-tocopherol, *d*-β-tocopherol, *d*-γ-tocopherol, and *d*-δ-tocopherol for the test solution and the standard solution, and express as $A_{T\alpha}$, $A_{T\beta}$, $A_{T\gamma}$, and $A_{T\delta}$ for the test solution and $A_{S\alpha}$, $A_{S\beta}$, $A_{S\gamma}$, and $A_{S\delta}$ for the standard solution, respectively. Calculate the content of the total tocopherols using the following formula, and then determine the percentage of *d*-α-tocopherol in the total tocopherols.

$$\text{Content (\%) of total tocopherols} = \left(\frac{A_{T\alpha}}{A_{S\alpha}} \times S_\alpha + \frac{A_{T\beta}}{A_{S\beta}} \times S_\beta + \frac{A_{T\gamma}}{A_{S\gamma}} \times S_\gamma + \frac{A_{T\delta}}{A_{S\delta}} \times S_\delta\right) \times \frac{1}{\text{Weight (g) of the sample}} \times 100$$

S_α = amount (g) of *d*-α-tocopherol in 100 ml of the standard solution,

S_β = amount (g) of *d*-β-tocopherol in 100 ml of the standard solution,

S_γ = amount (g) of *d*-γ-tocopherol in 100 ml of the standard solution,

S_δ = amount (g) of *d*-δ-tocopherol in 100 ml of the standard solution.

Operating Conditions

Detector: Ultraviolet spectrophotometer (determination wavelength: 292 nm).

Column: A stainless steel tube of 3–6 mm internal diameter and 15–25 cm length.

Column packing material: 5- to 10-μm silica gel for liquid chromatography.

Column temperature: A constant room temperature.

Mobile phase: A 200:1 mixture of hexane/2-propanol.

Flow rate: Adjust so that the retention time of *d*-α-tocopherol is about 5 minutes.

d-γ-Tocopherol

γ-Vitamin E

d-γ-トコフェロール

Definition *d*-γ-Tocopherol is obtained by isolation from vegetable fats and oils, which are produced from oil seeds, or mixed tocopherols (products consisting mainly of *d*-*α*-, *d*-*β*-, *d*-*γ*-, and *d*-δ-tocopherols, obtained from vegetable fats and oils). It consists mainly of *d*-*γ*-tocopherol. It may contain edible fats or oils.

Content *d*-*γ*-Tocopherol contains not less than 40% of total tocopherols, of which not less than 70% consists of *d*-*γ*- tocopherol.

Description *d*-*γ*-Tocopherol is a light yellow to red-brown clear viscous liquid having a slight, characteristic odor.

Identification Dissolve 0.05 g of *d*-*γ*-Tocopherol in 10 ml of absolute ethanol, add 2 ml of nitric acid, and heat at about

75°C for 15 minutes. An orange to red color develops.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: Not less than +20°.
Proceed as directed in Purity (1) for *d*-α-Tocopherol.

(2) Acid value Not more than 5.0.
Proceed as directed in Purity (2) for Tocotrienol.

(3) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Assay Proceed as directed in the Assay for *d*-α-Tocopherol.

d-δ-Tocopherol

δ-Vitamin E

d-δ-トコフェロール

Definition *d*-δ-Tocopherol is obtained by isolation from vegetable fats and oils, which are produced from oil seeds, or mixed tocopherols (products consisting mainly of *d*-α-, *d*-β-, *d*-γ-, and *d*-δ-tocopherols, obtained from vegetable fats and oils). It consists mainly of *d*-δ-tocopherol. It may contain edible fats or oils.

Content *d*-δ-Tocopherol contains not less than 40% of total tocopherols, of which not less than 60% consists of *d*-δ-tocopherol.

Description *d*-δ-Tocopherol is a light yellow to red-brown, clear viscous liquid having a slight, characteristic odor.

Identification Dissolve 0.05 g of *d*-δ-Tocopherol in 10 ml of absolute ethanol, add 2 ml of nitric acid, and heat at about 75°C for 15 minutes. An orange to red color develops.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: Not less than +20°.
Proceed as directed in Purity (1) for *d*-α-Tocopherol.

(2) Acid value Not more than 5.0.
Proceed as directed in Purity (2) for Tocotrienol.

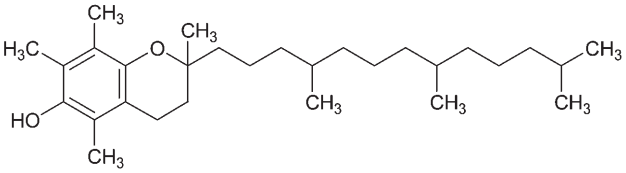
(3) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Assay Proceed as directed in the Assay for *d*-α-Tocopherol.

dl-α-Tocopherol

dl-α-トコフェロール



$C_{29}H_{50}O_2$ Mol. Wt. 430.71
2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-ol

Content *dl*-α-Tocopherol contains not less than 96.0–102.0% of *dl*-α-tocopherol ($C_{29}H_{50}O_2$).

Description *dl*-α-Tocopherol is a light yellow to yellow-brown, viscous liquid. It is odorless.

Identification Proceed as directed in Identification for *d*-α-Tocopherol.

Purity

(1) Specific absorbance $E_{1cm}^{1\%}$ (292 nm): 71.0–76.0.
Weigh accurately about 0.1 g of *dl*-α-Tocopherol, and dissolve it in anhydrous ethanol to make exactly 100 ml. Measure exactly 5 ml of this solution, add anhydrous ethanol to make exactly 100 ml, and measure the absorbance.

(2) Refractive index n_D^{20}: 1.503–1.507.

(3) Clarity of solution Clear (0.10 g, anhydrous ethanol 10 ml).

(4) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Assay

Test Solution and Standard Solution Weigh accurately about 0.05 g each of *dl*-α-Tocopherol and *dl*-α-Tocopherol Reference Standard into separate brown 50-ml volumetric flasks, and add absolute ethanol to make two solutions of exactly 50 ml each. Use them as the test solution and as the standard solution, respectively.

Procedure Analyze 20 μl portions of these solutions by liquid chromatography using the operating conditions given below. Measure peak heights (H_T and H_S) of *dl*-α-Tocopherol for the test solution and the standard solution, and obtain the content by the formula:

$$\text{Content (\%) of } dl\text{-α-tocopherol } (C_{29}H_{50}O_2) = \frac{\left(\dfrac{\text{Weight (g) of}}{dl\text{-α-Tocopherol Reference Standard}}\right)}{\text{Weight (g) of the sample}} \times \frac{H_T}{H_S} \times 100$$

Operating Conditions

Detector: Ultraviolet spectrophotometer (determination wavelength: 292 nm).

Column: A stainless steel tube of 4.6 mm internal diameter and 15 cm length.

Column packing material: 5-μm octadecylsilanized silica gel for liquid chromatography.

Column temperature: A constant temperature around

35ºC.

Mobile phase: A 49:1 mixture of methanol/water.

Flow rate: Adjust so that the retention time of *dl*-α-tocopherol is about 10 minutes.

Column selection: The column should be capable of resolving the peaks of *dl*-α-tocopherol and *dl*-α-tocopherol acetate, in this order, with a resolution rate of 2.6 or more when 20 µl of a solution containing 0.05 g each of *dl*-α-tocopherol and *dl*-α-tocopherol acetate in 50 ml of absolute ethanol is chromatographed using the conditions given above. The relative standard deviation of peak heights of *dl*-α-tocopherol is 0.8% or less in five repetitive tests conducted on the standard solution using the given operating conditions.

Tocotrienol

トコトリエノール

Definition Tocotrienol is obtained by isolating and purifying bran oil from the rice plant *Oryza sativa* Linné or oil from the oil palm *Elaeis guineesis* Jacquin. It consists mainly of tocotrienols. It may contain edible fats or oils.

Content Tocotrienol contains not less than 25% of total tocotrienols.

Description Tocotrienol is a yellow to red-brown, viscous liquid having a slight, characteristic odor.

Identification Dissolve 0.05 g of Tocotrienol in 10 ml of absolute ethanol, add 2 ml of nitric acid, and heat at about 75°C for 15 minutes. An orange to red color develops.

Purity

(1) Specific gravity 0.94–0.99.

(2) Acid value Not more than 5.0.

Test Solution Weigh accurately about 2.5 g of Tocotrienol, and add 50 ml of a 1:1 mixture of ethanol/diethyl ether to which 0.02 mol/L ethanolic potassium hydroxide has been added until the faint pink color of the solution persists for 30 seconds (indicator: phenolphthalein TS).

Procedure Add a few drops of phenolphthalein TS to the test solution, and titrate with 0.02 mol/L ethanolic potassium hydroxide to the first faint pink color that persists for 30 seconds. Determine the acid value, using the formula given below.

$$\text{Acid value} = \frac{\left(\begin{array}{c}\text{Volume (ml) of 0.02 mol/L}\\ \text{ethanolic potassium hydroxide consumed}\end{array}\right) \times 5.611}{\text{Weight(g) of the sample} \times 5}$$

(3) Heavy metals Not more than 20 µg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Arsenic Not more than 2.0 µg/g as As_2O_3 (1.0 g, Method 3, Apparatus B).

Assay

Test Solution Weigh accurately an appropriate amount of Tocotrienol equivalent to about 0.025 g of total tocotrienols into a brown volumetric flask, dissolve it in hexane to make exactly 100 ml.

Standard Solutions Weigh accurately about 0.05 g each of *d*-α-, *d*-β-, *d*-γ-, and *d*-δ-tocopherols for assay in separate brown 100-ml volumetric flasks, and dilute each with hexane to volume to prepare standard stock solutions. Prepare a standard solution, using the standard stock solutions so that it contains *d*-α-, *d*-β-, *d*-γ-, and *d*-δ-tocopherols at almost the same ratio as that of the corresponding tocotrienols in the sample (the approximate ratio of tocotrienols in the sample should be determined by conducting preliminary tests).

Procedure Analyze 20 µl portions of the test solution and the standard solution by liquid chromatography using the operating conditions given below. Measure the peak areas ($A_{T\alpha}$, $A_{T\beta}$, $A_{T\gamma}$, and $A_{T\delta}$) of individual tocotrienols for the test solution and the peak areas ($A_{S\alpha}$, $A_{S\beta}$, $A_{S\gamma}$, and $A_{S\delta}$) of individual tocophenols for the standard solution. Determine the content of each tocotrienol, using the formula given below. The relative retention times of corresponding *d*-α-tocotrienol, *d*-β-tocotrienol, *d*-γ-tocotrienol, and *d*-δ-tocotrienol to *d*-α-tocopherol, *d*-β-tocopherol, *d*-γ-tocopherol, and *d*-δ-tocopherol are about 1.1–1.3.

Operating Conditions

Detector: Ultraviolet absorption spectrophotometer (determination wavelength: 292 nm).

Column: A stainless steel tube of 3–6 mm internal diameter and 15–25 cm length.

Column packing material: 5- to 10-µm silica gel for liquid chromatography.

Column temperature: 40°C.

Mobile phase: A 985:10:5 mixture of hexane/dioxane/2-propanol.

Flow rate: Adjust so that the retention time of *d*-α-tocopherol is about 7–8 minutes.

$$\text{Content (\%) of total tocotrienols} = \left(\frac{A_{T\alpha}}{A_{S\alpha}} \times S_\alpha + \frac{A_{T\beta}}{A_{S\beta}} \times S_\beta + \frac{A_{T\gamma}}{A_{S\gamma}} \times S_\gamma + \frac{A_{T\delta}}{A_{S\delta}} \times S_\delta\right) \times \frac{1}{\text{Weight(g) of the sample}} \times 100$$

S_α = amount (g) of *d*-α-tocopherol in 100 ml of the standard solution,

S_β = amount (g) of *d*-β-tocopherol in 100 ml of the standard solution,

S_γ = amount (g) of *d*-γ-tocopherol in 100 ml of the standard solution,

S_δ = amount (g) of *d*-δ-tocopherol in 100 ml of the standard solution.

Tomato Color

トマト色素

Definition Tomato Color is obtained from the fruits of the tomato plant *Lycopersicon esculentum* Miller and consists mainly of lycopene. It may contain edible fats or oils.

Color Value The Color Value ($E_{1cm}^{10\%}$) of Tomato Color is not less than 300 and is in the range of 95–115% of the labeled value.

Description Tomato Color occurs as a brown to dark red powder, lumps, paste, or liquid having a slight, characteristic

odor.

Identification

(1) Weigh the equivalent of 0.1 g of Tomato Color with a Color Value 300, and dissolve it in 100 ml of ethyl acetate. An orange color develops.

(2) A solution of Tomato Color in hexane exhibits absorption maxima at wavelengths of 438–450 nm, 465–475 nm, and 495–505 nm.

(3) Weigh the equivalent of 0.1 g of Tomato Color with a Color Value 300, and dissolve it in 10 ml of ethyl acetate. Use this solution as the test solution. Analyze a 5-µl portion of the test solution by thin-layer chromatography using a 7:3 mixture of hexane/acetone as the developing solvent. No control solution is used. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the original line, and air-dry the plate. A yellow-red spot of lycopene is observed at an R_f value of about 0.7–0.8. This color immediately disappears when the spot is sprayed with 5% sodium nitrite solution followed by 0.5 mol/L sulfuric acid.

Purity

(1) Heavy metals Not more than 40 µg/g as Pb (0.50 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 8.0 µg/g as Pb (1.25 g, Method 1).

(3) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50g, Method 3, Apparatus B).

Color Value Test

Test Solution Weigh accurately an appropriate amount of Tomato Color, dissolve it in 25 ml of a 1:1 mixture of acetone/cyclohexane, and add hexane to make exactly 100 ml. Measure exactly 2 ml of this solution, and add hexane to make exactly 100 ml. Centrifuge the solution obtained if necessary, and use the supernatant as the test solution.

Procedure Conduct the test according to the operating conditions given below, as directed in the Color Value Test.

Operating Conditions

Solvent: Hexane.

Wavelength: Maximum absorption wavelength of 465–475 nm.

Tragacanth Gum

トラガントガム

[9000-65-1]

Definition Tragacanth Gum is obtained from the exudate of *Astragalus gummifer* Labillardière and consists mainly of polysaccharides.

Description Tragacanth Gum occurs as a white to whitish powder or as a white to light yellowish white, translucent, flattened or laminar flake, and it is odorless.

Identification

(1) To 1 g of powdered Tragacanth Gum, add 50 ml of water. An almost homogeneous, somewhat turbid viscous solution is formed.

(2) Place about 1.0 g of powdered Tragacanth Gum in a watch glass containing 2–3 drops of a 1:1 mixture of water/glycerol and 1 drop of iodine TS. Mix well with the end of a small glass rod, taking care to prevent air bubble formation. Allow to stand for 10 minutes or more and swell it. Apply small amount of swelled sample to a slide glass with the end of a small glass rod, add 1 drop of a 1:1 mixture of water/glycerol. Cover it with a cover glass, being careful not to allow air bubbles to be trapped, and examine by an optical microscope. A few blue granules of starch are found. For microscopic examination, use 10 or 40 times scale as an objective and 10 times scale as an eyepiece.

Purity

(1) Hydrochloric acid-insoluble substances Not more than 2.0%.

Previously, dry a glass filter (1G3) for 30 minutes at 110°C, cool in a desiccators, and weigh accurately. Accurately weigh about 2 g of powdered Tragacanth Gum, add 95 ml of methanol to moisten and swell the powder. Add 60 ml of hydrochloric acid and boiling chips, and heat under a reflux condenser in a water bath for 3 hours with occasional shaking. While warm, filter by suction with a glass filter (1G3), previously dried for 30 minutes at 110°C, cooled in a desiccators, and accurately weighed. Wash the residue well first with warm water and then with 40 ml of methanol again. Dry the residue together with the glass filter for 2 hours at 105°C. Allow to cool in a desiccator, and weigh accurately.

(2) Karaya gum Weigh accurately about 1.0 g of Tragacanth Gum, add 20 ml of water, and heat until it is a homogeneous viscous liquid. Add 5 ml of hydrochloric acid, and boil 5 minutes. No light pink to red color is produced.

(3) Heavy metals Not more than 40 µg/g as Pb (0.50 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(4) Lead Not more than 10 µg/g as Pb (1.0 g, Method 1).

(5) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Loss on Drying Not more than 17.0% (105°C, 5 hours).

Ash Not more than 4.0%.

Acid-insoluble Ash Not more than 0.5%.

Microbial Limits Proceed as directed in the Microbial Limit Tests. The total bacterial count is not more than 10,000/g, and *Escherichia coli* is negative.

Tricalcium Phosphate

Calcium Phosphate, Tribasic
Tertiary Calcium Phosphate

リン酸三カルシウム

Definition Tricalcium Phosphate consists of a mixture of calcium phosphates having an approximate composition of $10CaO{\cdot}3P_2O_5{\cdot}H_2O$.

Content Tricalcium Phosphate, when dried, contains the equivalent of 98.0–103.0% of tricalcium phosphate ($Ca_3(PO_4)_2$=310.18).

Description Tricalcium Phosphate occurs as a white powder.

Identification

(1) Moisten Tricalcium Phosphate with silver nitrate solution (1 in 50). A yellow color develops.

(2) To 0.1 g of Tricalcium Phosphate, add 5 ml of diluted acetic acid (1 in 4), boil, cool, and filter. To the filtrate, add 5 ml of ammonium oxalate solution (1 in 30). A white precipitate is formed.

Purity

(1) Clarity of solution Slightly turbid.

Test Solution Weigh 2.0 g of Tricalcium Phosphate, add 15 ml of water and 5.0 ml of hydrochloric acid, and dissolve by heating for 5 minutes in a water bath.

(2) Carbonate Weigh 2.0 g of Tricalcium Phosphate, add 5 ml of water, and boil. After cooling, add 2 ml of hydrochloric acid. Little or no effervescence occurs.

(3) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of Tricalcium Phosphate, add 5 ml of water and 7 ml of diluted hydrochloric acid (1 in 4), dissolve by heating. After cooling, add ammonia TS until a slight precipitate is formed. Add a small amount of diluted hydrochloric acid (1 in 4) dropwise to dissolve the precipitate, and if necessary, filter through a filter paper for quantitative analysis (5C). Add 10 ml of hydrochloric acid–ammonium acetate buffer (pH 3.5) and water to make 50 ml.

Control Solution To 2.0 ml of Lead Standard Solution, add 10 ml of hydrochloric acid–ammonium acetate buffer (pH 3.5) and water to make 50 ml.

(4) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.50 g of Tricalcium Phosphate, and dissolve it in 5 ml of diluted hydrochloric acid (1 in 4).

Apparatus Use Apparatus B.

Loss on Drying Not more than 10.0% (200°C, 3 hours).

Assay Weigh accurately about 0.3 g of Tricalcium Phosphate, previously dried, dissolve it in 10 ml of diluted hydrochloric acid (1 in 4), and add water to make exactly 200 ml. Proceed as directed in Method 2 in Calcium Salt Determination, using this solution as the test solution.

Each ml of 0.02 mol/L EDTA = 2.068 mg of $Ca_3(PO_4)_2$

Trimagnesium Phosphate

Magnesium Phosphate, Tribasic
Tertiary Magnesium Phosphate

リン酸三マグネシウム

$Mg_3(PO_4)_2 \cdot nH_2O$ (n=8, 5, or 4)

Mol. Wt. octahydrate 406.98
tetrahydrate 334.92

Trimagnesium phosphate octahydrate [13446-23-6]
Trimagnesium phosphate pentahydrate
Trimagnesium phosphate tetrahydrate [13465-22-0]

Definition Trimagnesium Phosphate occurs as several crystalline compounds (octa-, penta-, and tetrahydrates).

Content Trimagnesium Phosphate, when ignited, contains not less than 98.0–101.5% of trimagnesium phosphate anhydrous ($Mg_3(PO_4)_2$=262.86).

Description Trimagnesium Phosphate occurs as a white crystalline powder.

Identification

(1) Dissolve 0.2 g of Trimagnesium Phosphate in 10 ml of dilute nitric acid. To this solution, add a few drops of ammonium molybdate TS. A yellow precipitate is produced. When ammonium TS is added, the precipitate dissolves, and a white precipitate is produced.

(2) Dissolve 0.1 g of Trimagnesium Phosphate by adding 0.7 ml of dilute acetic acid and 20 ml of water. Add 1 ml of iron(III) chloride TS, allow to stand for 5 minutes, and filter. The filtrate responds to all tests for Magnesium Salts in the Qualitative Tests.

Purity

(1) Clarity of solution Turbid.

Test Solution Weigh 2.0 g of Trimagnesium Phosphate, add 16 ml of water and 4.0 ml of dilute hydrochloric acid, and dissolve by heating for 5 minutes on a water bath.

(2) Heavy metals Not more than 30 μg/g as Pb.

Suspend 1.33 g of Trimagnesium Phosphate in 20 ml of water, and adjust the pH to 3–4 with dilute hydrochloric acid to allow to dissolve. Filter the solution, and add water to the filtrate to make exactly 40 ml. Use this solution to prepare the control and test solutions.

Control Solution Add exactly 10 ml of the prepared solution to 2.0 ml of Lead Standard Solution, measured exactly, and add water to make exactly 40 ml.

Test Solution To the remaining 30 ml of the prepared solution, add water to make exactly 40 ml.

(3) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.50 g of Trimagnesium Phosphate, and dissolve it in 5 ml of dilute hydrochloric acid.

Apparatus Use Apparatus B.

(4) Fluoride Not more than 5.0 μg/g as F.

Test Solution Weigh 1.0 g of Trimagnesium Phosphate in a beaker, and dissolve it in 10 ml of diluted hydrochloric acid (1 in 10). Heat the solution, boil for 1 minute, and transfer into a polyethylene beaker, and immediately cool with ice. Add 15 ml of sodium citrate solution (1 in 4) and 10 ml of disodium ethylenediaminetetraacetate solution (1 in 40), and mix them. Adjust the pH of the mixture to 5.4–5.6 with diluted hydrochloric acid (1 in 10) or sodium hydroxide solution (2 in 5). Transfer it into a 100-ml volumetric flask, and add water to make up to volume. Take 50 ml of the obtained solution in a polyethylene beaker.

Control Solution Weigh 2.210 g of sodium fluoride, previously dried at 110°C for 2 hours, in a polyethylene beaker, add 200 ml of water, and dissolve by stirring. Transfer this solution into a 1,000-ml volumetric flask, and add water to make up to volume. Transfer the solution into a polyethylene beaker, and use as the control stock solution. Place exactly 5 ml of the control stock solution into a 1,000-ml volumetric flask, and add water to make up to volume. Transfer exactly 1 ml of this solution into a polyethylene beaker, add 15 ml of sodium citrate solution (1 in 4) and 10 ml of disodium ethylenediaminetetraacetate solution (1 in 40), and mix. Adjust the pH of the mixture to 5.4–5.6 with diluted hydrochloric acid (1 in 10) or sodium hydroxide solution (2 in 5). Transfer the resulting solution into a 100-ml volumetric flask, and add water to make up to volume. Transfer 50 ml of the last solution into a polyethylene beaker.

Procedure Measure the electric potentials of the test solution and the control solution, using a potentiometer connected to a reference electrode and fluorine ion electrode. The electric potential of the test solution is not lower than that of the control solution.

Loss on Ignition

Tetrahydrate: 15–23% (1.0g, 425°C, 3 hours).

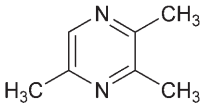
Pentahydrate: 20–27% (1.0g, 425°C, 3 hours).
Octahydrate: 30–37% (1.0g, 425°C, 3 hours).

Assay Weigh accurately about 0.3 g of Trimagnesium Phosphate, previously ignited, and dissolve by adding 50 ml of water and 5 ml of diluted hydrochloric acid (2 in 3). Then add 40 ml of 0.1 mol/L EDTA, and heat in a water bath at 50°C for 30 minutes. After cooling, add about 10 ml of ammonia–ammonium chloride buffer (pH10.7), and titrate with 0.1 mol/L zinc acetate (indicator: 5 drops of eriochrome black T TS). The endpoint is when the blue color of the solution changes to blue-purple. Separately, perform a blank test in the same manner, and make any necessary correction.

Each ml of 0.1 mol/L EDTA solution = 8.762 mg of $Mg_3(PO_4)_2$

2,3,5-Trimethylpyrazine

2,3,5-トリメチルピラジン



$C_7H_{10}N_2$ Mol. Wt. 122.17

2,3,5-Trimethylpyrazine [14667-55-1]

Content 2,3,5-Trimethylpyrazine contains not less than 98.0% of 2,3,5-trimethylpyrazine ($C_7H_{10}N_2$).

Description 2,3,5-Trimethylpyrazine is a colorless to yellow, transparent liquid having a characteristic odor.

Identification Determine the absorption spectrum of 2,3,5-Trimethylpyrazine as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Refractive index n_D^{20}: 1.500–1.509.

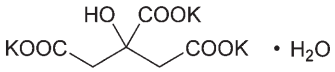
(2) Specific gravity d_{25}^{25}: 0.960–0.990.

Assay Proceed as directed in the Peak Area Percentage Method in the Gas Chromatographic Assay in the Flavoring Substances Tests. Use operating conditions (1).

Tripotassium Citrate

Potassium Citrate

クエン酸三カリウム



$C_6H_5K_3O_7 \cdot H_2O$ Mol. Wt. 324.41

Tripotassium 2-hydroxypropane-1,2,3-tricarboxylate monohydrate [anhydrous 866-84-2]

Content Tripotassium Citrate, when calculated on the dried basis, contains 99.0–101.0% of tripotassium citrate ($C_6H_5K_3O_7$ = 306.39).

Description Tripotassium Citrate occurs as colorless crystals or as a white crystalline powder. It is odorless.

Identification Tripotassium Citrate responds to all tests for Potassium Salt and to test (2) for Citrate in the Qualitative Tests.

Purity

(1) Clarity and color of solution Colorless and almost clear (1.0 g, water 20 ml).

(2) pH 7.6–9.0 (1.0 g, water 20 ml).

(3) Sulfate Not more than 0.024% as SO_4 (1.0 g, Control solution 0.005 mol/L sulfuric acid 0.50 ml).

(4) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Drying Not more than 6.5% (200°C, 2 hours).

Assay Weigh accurately about 0.2 g of Tripotassium Citrate, add 30 ml of acetic acid for nonaqueous titration, and dissolve by warming. Cool, and titrate with 0.1 mol/L perchloric acid. Usually, a potentiometer is used to confirm the endpoint. When crystal violet–acetic acid TS (1 ml) is used as the indicator, the endpoint is when the color of the solution changes from purple through blue to green. Separately, perform a blank test in the same manner, and make any necessary correction. Calculate the content on the dried basis.

Each ml of 0.1 mol/L perchloric acid = 10.21 mg of $C_6H_7K_3O_7$

Tripotassium Phosphate

Potassium Phosphate, Tribasic
Tertiary Potassium Phosphate

リン酸三カリウム

$K_3PO_4 \cdot nH_2O$ (n = 3, 1½, 1 or 0) Mol. Wt. trihydrate 266.31
anhydrous 212.27

Tripotassium phosphate trihydrate
Tripotassium phosphate sesquihydrate
Tripotassium phosphate monohydrate
Tripotassium phosphate [7778-53-2]

Content Tripotassium Phosphate, when ignited, contains not less than 97.0% of tripotassium phosphate (K_3PO_4).

Description Tripotassium Phosphate occurs as colorless to white crystals or lumps or as a white powder.

Identification A solution of Tripotassium Phosphate (1 in 20) responds to all tests for Potassium Salt and for Phosphate in the Qualitative Tests.

Purity

(1) Clarity and color of solution Colorless and very slightly turbid (1.0 g, water 20 ml).

(2) pH 11.5–12.5 (1.0 g, water 100 ml).

(3) Chloride Not more than 0.011% as Cl (1.0 g, Control solution 0.01 mol/L hydrochloric acid 0.30 ml).

(4) Sulfate Not more than 0.019% as SO_4 (1.0 g, Control solution 0.005 mol/L sulfuric acid 0.40 ml).

(5) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of Tripotassium Phosphate, dissolve it in 30 ml of water, neutralize with diluted acetic acid (1 in 20), and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Ignition Not more than 23.0% (120°C for 2 hours, then 300–400°C for 1 hour).

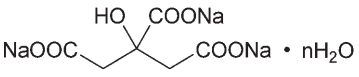
Assay Weigh accurately about 2 g of Tripotassium Phosphate, previously ignited, dissolve it in 50 ml of water, keep at about 15°C, and titrate with 1 mol/L hydrochloric acid (indicator: 3–4 drops of methyl orange–xylene cyanol FF TS).

Each ml of 1 mol/L hydrochloric acid = 106.1 mg of K_3PO_4

Trisodium Citrate

Sodium Citrate

クエン酸三ナトリウム



n = 2 or 0

$C_6H_5Na_3O_7 \cdot nH_2O$ (n = 2 or 0) Mol. Wt. dihydrate 294.10
anhydrous 258.07

Trisodium 2-hydroxypropane-1,2,3-tricarboxylate dihydrate [6132-04-3]
Trisodium 2-hydroxypropane-1,2,3-tricarboxylate [68-04-2]

Definition Trisodium Citrate occurs in two forms: the crystal form (dihydrate) called Trisodium Citrate (crystal) and the anhydrous form called Trisodium Citrate (anhydrous).

Content Trisodium Citrate, when dried, contains 99.0–101.0% of trisodium citrate ($C_6H_5Na_3O_7$).

Description Trisodium Citrate occurs as colorless crystals or as a white powder. It is odorless and has a cool, salty taste.

Identification Trisodium Citrate responds to all tests for Sodium Salt and to test (2) for Citrate in the Qualitative Test.

Purity

(1) Clarity and color of solution Colorless and almost clear (1.0 g, water 20 ml).

(2) pH 7.6–9.0 (1.0 g, water 20 ml).

(3) Sulfate Not more than 0.024% as SO_4 (1.0 g, Control solution 0.005 mol/L sulfuric acid 0.50 ml).

(4) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Drying

Crystal 10.0–13.0% (180°C, 2 hours).

Anhydrous Not more than 1.0% (180°C, 2 hours).

Assay Weigh accurately about 0.2 g of Trisodium Citrate, previously dried, add 30 ml of acetic acid for nonaqueous titration, and dissolve by warming. After cooling, titrate with 0.1 mol/L perchloric acid. Usually, a potentiometer is used to confirm the endpoint. When crystal violet–acetic acid TS (1 ml) is used as the indicator, the endpoint is when the color of the solution changes from purple through blue to green. Separately, perform a blank test in the same manner, and make any necessary correction.

Each ml of 0.1 mol/L perchloric acid = 8.602 mg of $C_6H_5Na_3O_7$

Trisodium Phosphate

Sodium Phosphate, Tribasic
Tertiary Sodium Phosphate

リン酸三ナトリウム

$Na_3PO_4 \cdot nH_2O$ (n = 12, 6 or 0) Mol. Wt. dodecahydrate 380.12
anhydrous 163.94

Trisodium phosphate dodecahydrate [10101-89-0]
Trisodium phosphate hexahydrate
Trisodium phosphate [7601-54-9]

Definition Trisodium Phosphate occurs in two forms: the crystalline form (dodeca- and hexahydrates) called Trisodium Phosphate (crystal) and the anhydrous form called Trisodium Phosphate (anhydrous).

Content Trisodium Phosphate, when dried, contains 97.0–103.0% of trisodium phosphate (Na_3PO_4).

Description Trisodium Phosphate (crystal) occurs as colorless to white crystals or crystalline powder. Trisodium Phosphate (anhydrous) occurs as a white powder or granules.

Identification A solution of Trisodium Phosphate (1 in 20) responds to all tests for Sodium Salt and for Phosphate in the Qualitative Tests.

Purity For Trisodium Phosphate (crystal), dry the sample before performing the tests.

(1) Clarity and color of solution Colorless and very slightly turbid (0.50 g, water 20 ml).

(2) pH 11.5–12.5 (1.0 g, water 100 ml).

(3) Chloride Not more than 0.071% as Cl (0.30 g, Control solution 0.01 mol/L hydrochloric acid 0.60 ml).

(4) Sulfate Not more than 0.058% as SO_4 (0.50 g, Control solution 0.005 mol/L sulfuric acid 0.60 ml).

(5) Heavy metals Not more than 20 μg/g as Pb.

Test Solution Weigh 1.0 g of Trisodium Phosphate, dissolve it in 20 ml of water, neutralize with diluted acetic acid (1 in 20), and add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

Control Solution Measure 2.0 ml of Lead Standard Solution, add 2 ml of diluted acetic acid (1 in 20) and water to make 50 ml.

(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Loss on Drying

Crystal Not more than 58.0% (120°C, 2 hours, then 200°C, 5 hours).

Anhydrous Not more than 5.0% (200°C, 5 hours).

Assay Weigh accurately about 2 g of Trisodium Phosphate, previously dried, dissolve it in 50 ml of water, keep at about 15°C, and titrate with 1 mol/L hydrochloric acid (indicator: 3–4 drops of methyl orange–xylene cyanol FF TS).

Each ml of 1 mol/L hydrochloric acid = 81.97 mg of Na_3PO_4

Trypsin

トリプシン

Definition Trypsin is a proteolytic enzyme derived from pancreas of animals or internal organs of fishes or crustaceans. It may contain lactose or dextrin.

Enzyme Activity Trypsin has the enzyme activity equivalent to not less than 600,000 units per gram.

Description Trypsin occurs as a white to yellowish brown powder or granules or as a light brown to brown liquid or paste.

Purity

(1) Sulfate Not more than 48% as SO_4.

Test Solution Weigh 1.0 g of Trypsin, dissolve it in water to make 1,000 ml. Use 50 ml of the solution as the test solution.

Control Solution Use 50 ml of 0.005 mol/L sulfuric acid.

(2) Lead Not more than 5.0 μg/g as Pb (2.0 g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

Microbial Limits Proceed as directed in the Microbial Limit Tests. The total bacterial count is not more than 50,000/g, and *Escherichia coli* is negative.

Enzyme Activity Determination

(i) *Substrate Solution*

Dissolve 0.0857 g of *N*-benzoyl-L-arginine ethyl ester hydrochloride in water to make exactly 100 ml. Measure exactly 10 ml of this solution, and add phosphate buffer (pH 7.6) to make exactly 100 ml.

(ii) *Sample Solution*

Weigh accurately an amount of Trypsin equivalent to 5,000–6,000 units and dissolve it in 0.001 mol/L hydrochloric acid to make exactly 100 ml.

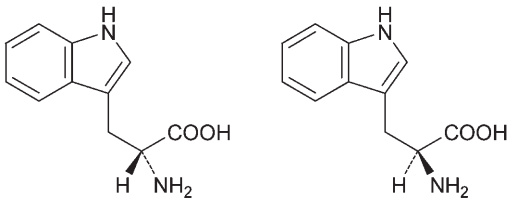
(iii) *Procedure*

Measure exactly 0.20 ml of 0.001 mol/L hydrochloric acid, add 3.0 ml of the substrate solution, and mix. Adjust the absorbance to 0.050 at a wavelength of 253 nm at 25±0.1°C, using water as the reference. Measure exactly 0.20 ml of the sample solution, add 3.0 ml of the substrate solution, and mix. Measure the absorbances at 30-second intervals for 5 minutes under the same conditions as given above in this section. Plot the time (seconds) against the absorbance on a graph. Determine the change (ΔA) in absorbance per minute in a range in which the time-absorbance curve is straight. Calculate the enzyme activity by the formula given below. One unit of the enzyme activity is the quantity of enzyme that changes the absorbance by 0.003 per minute when a test is performed under the conditions given in this section.

$$\text{Enzyme Activity of Trypsin (units/g)} = \frac{\Delta A \times 100}{0.003 \times \text{Weight (mg) of the sample} \times 0.2} \times 1{,}000$$

DL-Tryptophan

DL-トリプトファン



$C_{11}H_{12}N_2O_2$ Mol. Wt. 204.23

(2*RS*)-2-Amino-3-(1*H*-indol-3-yl)propanoic acid [54-12-6]

Content DL-Tryptophan, when calculated on the dried basis, contains 98.0–102.0% of DL-tryptophan ($C_{11}H_{12}N_2O_2$).

Description DL-Tryptophan occurs as white to yellowish-white crystals or crystalline powder. It is odorless or has a slight odor and has a slightly sweet taste.

Identification

(1) To 5 ml of a solution of DL-Tryptophan (1 in 1,000), add 1 ml of ninhydrin solution (1 in 1,000), and heat for 3 minutes. A purple color develops.

(2) To 0.2 g of DL-Tryptophan, add 100 ml of water, and dissolve by warming. To 10 ml of this solution, add 5 ml of *p*-dimethylaminobenzaldehyde TS and 2 ml of diluted hydrochloric acid (1 in 4), and heat in a water bath for 5 minutes. A red-purple to blue-purple color develops.

(3) To 0.2 g of DL-Tryptophan, add 100 ml of water, and dissolve by warming. The solution has no optical rotation.

Purity

(1) Clarity and color of solution Weigh 0.50 g of DL-Tryptophan, and dissolve it in 10 ml of sodium hydroxide solution (1 in 50). The solution is almost clear and not darker in color than Matching Fluid C.

(2) pH 5.5–7.0.

To 0.20 g of DL-Tryptophan, add 100 ml of water, and dissolve by warming. Measure the pH of the resulting solution.

(3) Chloride Not more than 0.021% as Cl.

Test Solution Weigh 0.50 g of DL-Tryptophan, dissolve it in 6 ml of diluted nitric acid (1 in 10), and add water to make 50 ml.

Control Solution Use 0.30 ml of 0.01 mol/L hydrochloric acid.

(4) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 4, Control solution Lead Standard Solution 2.0 ml).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.50 g of DL-Tryptophan, add 5 ml of diluted hydrochloric acid (1 in 20), and dissolve by heating.

Apparatus Use Apparatus B.

Loss on Drying Not more than 0.30% (105°C, 3 hours).

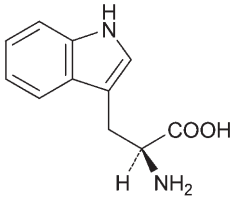
Residue on Ignition Not more than 0.10%.

Assay Weigh accurately about 0.3 g of DL-Tryptophan, and proceed as directed in the Assay for DL-Alanine.

Each ml of 0.1 mol/L perchloric acid = 20.42 mg of $C_{11}H_{12}N_2O_2$

L-Tryptophan

L-トリプトファン



$C_{11}H_{12}N_2O_2$ Mol. Wt. 204.23

(2*S*)-2-Amino-3-(1*H*-indol-3-yl)propanoic acid [73-22-3]

Content L-Tryptophan, when calculated on the dried basis, contains 98.0–102.0% of L-tryptophan ($C_{11}H_{12}N_2O_2$).

Description L-Tryptophan occurs as white to yellowish-white crystals or crystalline powder. It is odorless or has a slight odor and has a slightly bitter taste.

Identification

(1) Proceed as directed in Identification (1) and (2) for DL-Tryptophan.

(2) To 1.0 g of L-Tryptophan, add 100 ml of water, and dissolve by warming. The solution is levorotatory. It is dextrorotatory when made alkaline by adding sodium hydroxide solution (1 in 5).

Purity

(1) Specific rotation $[\alpha]_D^{20}$: −30.0 to −33.0°.

Weigh accurately about 0.5 g of L-Tryptophan, add about 40 ml of water, dissolve by warming, and cool, and then add water to make exactly 50 ml. Measure the angular rotation of this solution, and calculate on the dried basis.

(2) Clarity and color of solution Weigh 0.50 g of L-Tryptophan, and dissolve it in 10 ml of sodium hydroxide solution (1 in 50). The solution is almost clear and not darker in color than Matching Fluid C.

(3) pH 5.5–7.0.

Weigh 1.0 g of L-Tryptophan, add 100 ml of water, and dissolve by warming, and measure the pH.

(4) Chloride Not more than 0.021% as Cl.

Proceed as directed in Purity (3) for DL-Tryptophan.

(5) Heavy metals Not more than 20 μg/g as Pb.

Proceed as directed in Purity (4) for DL-Tryptophan.

(6) Arsenic Not more than 4.0 μg/g as As_2O_3.

Test Solution Weigh 0.50 g of L-Tryptophan, add 3 ml of 1 mol/L hydrochloric acid and 2 ml of water, and dissolve by heating.

Apparatus Use Apparatus B.

Loss on Drying Not more than 0.30% (105°C, 3 hours).

Residue on Ignition Not more than 0.10%.

Assay Weigh accurately about 0.3 g of L-Tryptophan, and proceed as directed in the Assay for DL-Alanine.

Each ml of 0.1 mol/L perchloric acid = 20.42 mg of $C_{11}H_{12}N_2O_2$

Turmeric Oleoresin

Curcumin

ウコン色素

Definition Turmeric Oleoresin is obtained from the rhizomes of the turmeric plant *Curcuma longa* Linné and consists mainly of curcumin. It may contain edible fats or oils.

Color Value The Color Value ($E^{10\%}_{1cm}$) of Turmeric Oleoresin is not less than 1,500 and is in the range of 90–110 % of the labeled value.

Description Turmeric Oleoresin occurs as a yellow to dark red-brown powder, lumps, paste, or liquid having a characteristic odor.

Identification

(1) Weigh the equivalent of 0.1 g of Turmeric Oleoresin with a Color Value 1,500, dissolve it in 200 ml of ethanol. A yellow color with a light-green fluorescence develops.

(2) A solution of Turmeric Oleoresin in ethanol exhibits an absorption maximum at a wavelength of 420–430 nm.

(3) Weigh the equivalent of 1 g of Turmeric Oleoresin with a Color Value 1,500, dissolve it in 100 ml of ethanol, and add hydrochloric acid until the color of the solution turns slightly orange. Use this solution as the test solution. Add boric acid to the test solution. A red-orange color develops.

(4) Weigh the equivalent of 1 g of Turmeric Oleoresin with a Color Value 1,500, dissolve it in 100ml of ethanol, centrifuge the solution at 3,000 rpm for 10 min. Use the supernatant as the test solution. Analyze a 5 µl portion of the test solution by thin-layer chromatography using a 4:4:2:1 mixture of ethanol/3-methyl-1-butanol/water/ammonia solution as the developing solvent. No control solution is used. Use a thin-layer plate coated with silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the original line, and air-dry the plate. Examine both in daylight and under ultraviolet light (around 366 nm). Two or more yellow spots are observed at R_f values of 0.40–0.85. All spots show yellow fluorescence in UV-light.

Purity

(1) Heavy metals Not more than 40 µg /g as Pb (0.50g, Method 2, Control solution Lead standard solution 2.0 ml).

(2) Lead Not more than 10 µg /g as Pb (1.0g, Method 1).

(3) Arsenic Not more than 4.0 µg /g as As_2O_3 (0.50g, Method 3, Apparatus B)

Color Value Test Proceed as directed in the Color Value Test.

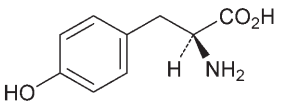
Operating Conditions

Solvent: Ethanol.

Wavelength: Maximum absorption wavelength of 420–430nm.

L-Tyrosine

L-チロシン



$C_9H_{11}NO_3$ Mol.Wt.181.19

(2*S*)-2-Amino-3-(4-hydroxyphenyl)propanoic acid [60-18-4]

Content L-Tyrosine, when calculated on the dried basis, contains 98.0–102.0% of L-tyrosine ($C_9H_{11}NO_3$).

Description L-Tyrosine occurs as white crystals or crystalline powder. It is odorless, and is tasteless or has a very slight characteristic taste.

Identification

(1) To 5 ml of a saturated solution of L-Tyrosine, add 1 ml of ninhydrin solution (1 in 50), and heat for 3 minutes in a water bath. A blue-purple color develops.

(2) To 5 ml of a saturated solution of L-Tyrosine, add 1 ml of iron(III) chloride solution (1 in 20), and heat. A dark red color develops.

Purity

(1) Specific rotation $[\alpha]_D^{20}$: –10.5 to –12.5°.

Weigh accurately about 5 g of L-Tyrosine, and dissolve it in 1 mol/L hydrochloric acid to make exactly 100 ml. Measure the angular rotation of this solution, and calculate on the dried basis.

(2) Clarity and color of solution Colorless and almost clear (1.0 g, 1 mol/L hydrochloric acid 20 ml).

(3) pH 5.0–6.5 (saturated solution).

(4) Chloride Not more than 0.10% as Cl (0.070 g, Control solution 0.01 mol/L hydrochloric acid 0.20 ml).

(5) Heavy metals Not more than 20 µg/g as Pb (1.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(6) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50g, Method 3, Apparatus B).

Loss on Drying Not more than 0.30% (105°C, 3 hours).

Residue on Ignition Not more than 0.10%.

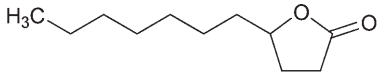
Assay Weigh accurately about 0.3 g of L-Tyrosine, and proceed as directed in the Assay for L-Asparagine.

Each ml of 0.1 mol/L perchloric acid = 18.12 mg of $C_9H_{11}NO_3$.

γ-Undecalactone

Undecalactone
Undecano-1,4-lactone

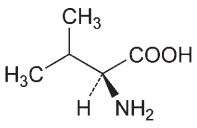
γ-ウンデカラクトン



$C_{11}H_{20}O_2$ Mol. Wt. 184.28
5-Heptyldihydrofuran-2(3*H*)-one [104-67-6]
Content γ-Undecalactone contains not less than 98.0% of γ-undecalactone ($C_{11}H_{20}O_2$).
Description γ-Undecalactone is a colorless to light yellow, transparent liquid having a peach-like odor.
Identification Determine the absorption spectrum of γ-Undecalactone as directed in the Liquid Film Method under Infrared Spectrophotometry, and compare with the Reference Spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.
Purity
(1) Refractive index n_D^{20}: 1.449–1.455.
(2) Specific gravity 0.944–0.948.
(3) Clarity of solution Clear (1.0 ml, 60% (vol) ethanol 5.0 ml).
(4) Acid value Not more than 5.0 (Flavoring Substances Tests).
Assay Weigh accurately about 1 g of γ-Undecalactone, and proceed as directed in the Ester Content Test in the Flavoring Substances Tests.
Each ml of 0.5 mol/L ethanolic potassium hydroxide = 92.14 mg of $C_{11}H_{20}O_2$

L-Valine

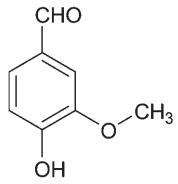
L-バリン



$C_5H_{11}NO_2$ Mol. Wt. 117.15
(2*S*)-2-Amino-3-methylbutanoic acid [72-18-4]
Content L-Valine, when calculated on the dried basis, contains 98.0–102.0% of L-valine ($C_5H_{11}NO_2$).
Description L-Valine occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic order, and has a slight characteristic taste.
Identification To 5 ml of a solution of L-Valine (1 in 1,000), add 1 ml of ninhydrin solution (1 in 1,000), and heat for 3 minutes. A purple color develops.
Purity
(1) Specific rotation $[\alpha]_D^{20}$: +26.5 to +29.0° (4.0 g, diluted hydrochloric acid (1 in 2), 50 ml, on the dried basis).
(2) Clarity and color of solution Colorless and clear (0.50 g, water 20 ml).
(3) pH 5.5–7.0 (1.0 g, water 30 ml).
(4) Chloride Not more than 0.021% as Cl (0.50 g, Control solution 0.01 mol/L hydrochloric acid 0.30 ml).
(5) Heavy metals Not more than 20 μg/g as Pb (1.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).
(6) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 2, Apparatus B).
Loss on Drying Not more than 0.30% (105°C, 3 hours).
Residue on Ignition Not more than 0.10%.
Assay Proceed as directed in the Assay for DL-Alanine.
Each ml of 0.1 mol/L perchloric acid = 11.71 mg of $C_5H_{11}NO_2$

Vanillin

バニリン



$C_8H_8O_3$ Mol. Wt. 152.15
4-Hydroxy-3-methoxybenzaldehyde [121-33-5]
Content Vanillin contains not less than 98.0% of vanillin ($C_8H_8O_3$).
Description Vanillin occurs as white to light yellow needles or crystalline powder having a vanilla-like odor and taste.
Identification
(1) To 0.5 g of Vanillin, add 10 ml of water, dissolve by warming, and add 3 drops of iron(III) chloride solution (1 in 10). A blue-purple color develops. Heat to keep the solution at about 80°C for 5 minutes. The solution turns brown, and a white to gray-white precipitate is formed.
(2) To 1 g of Vanillin, add 5 ml of sodium hydrogen sulfite TS, and dissolve by warming in warm water while shaking. To the obtained solution, add 10 ml of diluted sulfuric acid (1 in 20), warm at 60–70°C for about 5 minutes, and allow to stand. Crystals are deposited.
Purity
(1) Melting point 81–83°C.
(2) Clarity of solution Clear.
Test Solution Weigh 1.0 g of Vanillin, add 20 ml of water, and dissolve by heating to 80°C.
(3) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0 ml).
(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 4, Apparatus B).
Loss on Drying Not more than 0.5% (4 hours).
Residue on Ignition Not more than 0.05%.
Assay Weigh accurately about 1 g of Vanillin, and proceed as directed in Method 2 in the Aldehyde and Ketone Content Test in the Flavoring Substances Tests. In the test, allow the

sample to stand for 15 minutes.

Each ml of 0.5 mol/L hydrochloric acid = 76.07 mg of $C_8H_8O_3$

Vegetable Tannin

植物タンニン

Definition Vegetable Tannin* is obtained from nutgalls or the seed pods of Tara and consists mainly of tannin and tannic acid.

Content Vegetable Tannin, when dried, contains the equivalent of not less than 96% of tannic acid.

Description Vegetable Tannin occurs as a yellowish white to light brown powder having a slight, characteristic odor. It has a strong astringent taste.

Identification

(1) To 5 ml of a solution of Vegetable Tannin (1 in 20), add two drops of iron(III) chloride solution (1 in 10). A bluish black color is formed, and a precipitate is produced on standing.

(2) To three 5-ml portions of a solution of Vegetable Tannin (1 in 20), separately add a drop of albumin TS, a drop of gelatin TS, or 1 ml of starch TS. Each solution produces a precipitate.

(3) Dissolve 1 g of Vegetable Tannin in 100 ml of water, add 5 ml of diluted hydrochloric acid (1 in 2), and heat at 80 to 90°C for 2 hours. Use this solution as the test solution. Separately, dissolve 0.1 g of gallic acid in 100 ml of water, and use this solution as the control solution. Analyze a 5-µl portion each of the test solution and the control solution by thin-layer chromatography using a 5:4:1 mixture of ethyl formate/toluene/formic acid as a developing solvent. Use a thin-layer plate coated with fluorescent silica gel for thin-layer chromatography as the solid support and then dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 10 cm above the original line, and air-dry the plate. Examine under ultraviolet light (around 254 nm). A spot is observed at an R_f value of about 0.35 for each solution and emits blue-purple fluorescence under ultraviolet light.

(4) Dissolve 0.050 g of Vegetable Tannin in 3 ml of water, add 1 ml of calcium hydroxide TS, and shake thoroughly. No yellow or red color develops.

Purity

(1) Heavy metals Not more than 40 µg/g as Pb (0.50 g, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 10 µg/g as Pb (1.0 g, Method 1).

(3) Arsenic Not more than 4.0 µg/g as As_2O_3 (0.50 g, Method 2, Apparatus B).

(4) Gum or dextrin Dissolve 3.0 g of Vegetable Tannin in 15 ml of hot water. The solution is clear or slightly turbid. Cool and filter this solution, and add 5 ml of ethanol to 5 ml of the filtrate. No turbidity occurs.

(5) Resinous substances To 5 ml of the filtrate obtained in Purity (4), add 10 ml of water. No turbidity appears.

Loss on Drying Not more than 7.0% (105°C, 2 hours).

Residue on Ignition Not more than 1.0%.

Assay

Test Solution and Control Solution Weigh 0.100 g of Vegetable Tannin and 0.001 g of gallic acid, and add a 4:1 mixture of water/methanol to each to make two solutions of exactly 100 ml. Use them as the test solution and as the control solution, respectively.

Procedure Analyze 10 µl portions of them by liquid chromatography using the operating conditions given below. Confirm that the peak of gallic acid appears at a retention time of 2.2–2.5 minutes after the injection of the control solution. Measure the total area of all peaks that appear within 30 minutes of the injection of the test solution to normalize to 100, and measure the total area of all peaks that appear in 10–25 minutes as the peak area of tannic acid. Determine the peak area percentage of tannic acid from both total areas, and calculate the content of Vegetable Tannin.

Operating Conditions

Detector: Ultraviolet spectrophotometer (determination wavelength: 280nm).

Column: A stainless steel tube of 4 mm internal diameter and 25 cm length.

Column packing material: 7-µm octadecylsilanized silica gel for liquid chromatography.

Column temperature: Room temperature.

Mobile phase

A: 0.1% (w/v) phosphoric acid.

B: methanol containing 0.1% (w/v) phosphoric acid.

Concentration gradient (A/B): Run a linear gradient from 80% A to 0% A over 30 minutes.

Flow rate: 1.0 ml/min.

Vitamin A Esters of Fatty Acids

Retinol Fatty Acid Esters

ビタミン A 脂肪酸エステル

Definition Vitamin A Esters of Fatty Acids are categorized into two types: a vitamin A ester of acetic acid or a vitamin A ester mainly of palmitic acid.

Content 1 g of Vitamin A Esters of Fatty Acids contains the equivalent of not less than 450 mg of vitamin A and the equivalent of 90–120% of the labeled content of vitamin A. Three hundred mg of vitamin A is equivalent to one million international units.

Description Vitamin A Esters of Fatty Acids occur as light yellow to reddish-light yellow crystals or oily substance having a slight, characteristic odor.

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* Vegetable Tannin is one of the substances belonging to the "Tannin (Extract)" category.

"Tannin (Extract)" is defined in the List of Existing Food Additives as a substance that is obtained from the fruits of the Japanese persimmon (*Diospyros kaki* Thunberg); the astringent skins of the chestnut (*Castanea crenata* Siebold et Zuccarini); the seed coats of the tamarind tree (*Tamarindus indica* Linné); the seed pods of Tara (*Caesalpinia spinosa*); nutgalls of *Rhus javanica* Linné and other species of the genus *Rhus*; nutgalls of *Quercus infectoria* Oliver and other species of the genus *Quercus*; or silver wattle bark. It consists mainly of tannin and tannic acid.

Identification

(1) Prepare a test solution by dissolving an amount of the sample equivalent to 1,500 units of vitamin A in 5 ml of petroleum ether. Analyze a 5 µl portion of the test solution by thin-layer chromatography using a 4:1 mixture of cyclohexane/diethyl ether as the developing solvent. Use a thin-layer plate coated with fluorescent silica gel for thin-layer chromatography as the solid support and then dried at 105°C for 2 hours. Stop the development when the solvent front has ascended to a point about 10 cm above the original line, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm). Spots corresponding to vitamin A, vitamin A ester of acetic acid, and vitamin A ester of palmitic acid are observed at R_f values of about 0.09, 0.45, and 0.62, respectively.

(2) Dissolve 0.05 g of the sample in 2-propanol for vitamin A determination to prepare a solution containing about 3 µg of vitamin A per ml. The solution exhibits an absorption maximum at a wavelength of 324–328 nm.

Purity

(1) Acid value Not more than 2.8.

Weigh accurately about 2 g of the sample, and proceed as directed in the Acid Value Test in the Fats and Related Substances Tests.

(2) Absorbance ratio

Test Solution Weigh accurately an amount of the sample equivalent to 0.060 g of vitamin A, and dissolve it in 2-propanol for vitamin A determination to make exactly 100 ml. Measure exactly 1 ml of this solution, add 2-propanol for vitamin A determination to make exactly 200 ml.

Procedure Measure the absorbances of the test solution at wavelengths of 300 nm, 310 nm, 320 nm, 326 nm, 330 nm, 340 nm, and 350 nm, respectively. Calculate the ratio of the absorbance at each wavelength to the absorbance (A) at 326 nm when the absorbance is expressed as 1,000. Each absorbance ratio is within±0.030 of each value given in the table.

Wavelength (nm)	Ratios of Absorbance	
	Vitamin A ester of acetic acid	Vitamin A ester of palmitic acid
300	0.578	0.590
310	0.815	0.825
320	0.948	0.950
326	1.000	1.000
330	0.972	0.981
340	0.786	0.795
350	0.523	0.527

Assay From the absorbance (A) at 326 nm of the test solution prepared in Purity (2), calculate by the formula:

$$\text{Content of vitamin A (mg)} = \frac{A \times V}{W \times 100} \times 0.570$$

V = total number of milliliters of the test solution,
W = number of grams of the sample in V ml of the test solution.

Vitamin A in Oil

ビタミン A 油

Definition Vitamin A in Oil is a fatty oil obtained from the fresh liver, pyloric appendage, or other parts of aquatic animals; a vitamin A (retinol) concentrate of the fatty oil; vitamin A esters of fatty acids (retinol fatty acid ester); or a product prepared by dissolving any of the former three substances in edible fats or oils.

Content 1 g of Vitamin A in Oil contains the equivalent of not less than 30 mg of vitamin A and the equivalent of 90–120% of the labeled content of vitamin A. Three hundred mg of vitamin A is equivalent to one million international units.

Description Vitamin A in Oil occurs as a light yellow to reddish-light yellow oily substance having a slight, characteristic odor.

Identification Proceed as directed in Identification (1) and (2) for Vitamin A Esters of Fatty Acids.

Purity

(1) Acid value Proceed as directed in Purity (1) for Vitamin A Esters of Fatty Acids .

(2) Absorbance ratio If the sample contains vitamin A esters of fatty acids, proceed as directed in Purity (2) for Vitamin A Esters of Fatty Acids.

Assay

Test Solution Weigh accurately an amount of Vitamin A in Oil that is equivalent to not less than 0.15 mg of vitamin A and that contains not more than 1 g of fat or oil, transfer into a flask, and add 30 ml of aldehyde-free ethanol and 1 ml of a solution of pyrogallol in ethanol (1 in 10). Add 3 ml of potassium hydroxide solution (9 in 10), and heat under a reflux condenser on a water bath for 30 minutes to saponify. Cool quickly to ordinary temperature, add 30 ml of water, and transfer into separating funnel A. Wash the flask with 10 ml of water and then with 40 ml of diethyl ether for vitamin A determination, add the washings to separating funnel A, shake well, and allow to stand. Transfer the aqueous layer into separating funnel B, wash the flask with 30 ml of diethyl ether for vitamin A determination, add the washings to separating funnel B, and shake to extract. Transfer the aqueous layer into the flask, transfer the diethyl ether layer into separating funnel A, transfer the aqueous layer from the flask above into separating funnel B, add 30 ml of diethyl ether for vitamin A determination, and shake to extract. Transfer the diethyl ether layer into separating funnel A, add 10 ml of water, invert the separating funnel gently 2 or 3 times, allow to stand, and remove the separated aqueous layer. Wash three times with 50 ml of water each time, shaking stronger. Wash repeatedly with 50 ml of water each time until the washings no longer shows a color with phenolphthalein TS, and allow to stand for 10 minutes. Remove water as much as possible, transfer the diethyl ether layer into an Erlenmeyer flask, wash the separating funnel twice with 10 ml of diethyl ether for vitamin A determination each time, and add the washings to the Erlenmeyer flask. Add 5 g of anhydrous sodium sulfate, shake, and transfer the diethyl ether extract into an eggplant-shaped flask by decantation. Wash the remaining sodium sulfate more than twice with 10 ml of diethyl ether for vitamin A determination each time,

and add the washings to the eggplant-shaped flask. Concentrate the diethyl ether extract to about 1 ml while shaking in a water bath at 45°C, using an aspirator. Immediately add 2-propanol for vitamin A determination to dissolve, and dilute exactly to obtain a solution containing about 3 μg of vitamin A per ml.

Procedure Measure the absorbances (A_1, A_2, and A_3) of the test solution at wavelengths of 310 nm, 325 nm, and 334 nm, respectively, and calculate the content by the formula:

$$\text{Content (mg/g) of vitamin A} = E_{1cm}^{1\%}\ (325\ \text{nm}) \times 0.549$$

$$E_{1cm}^{1\%}\ (325\ \text{nm}) = \frac{A_2}{W} \times \frac{V}{100} \times f$$

$$f = 6.815 - 2.555 \times \frac{A_1}{A_2} - 4.260 \times \frac{A_3}{A_2}$$

f = correction factor,
V = total number of milliliters of the test solution,
W = number of grams of the sample in V ml of the test solution.

When the sample contains vitamin A esters of fatty acids, proceed as directed in the Assay for Vitamin A Esters of Fatty Acids.

Storage Standards Store in a hermetic, light-resistant container under inert gas.

Xanthan Gum

キサンタンガム

[11138-66-2]

Definition Xanthan Gum is obtained from the culture fluid of *Xanthomonas campestris* and consists mainly of polysaccharides. It may contain glucose, lactose, dextrin, or maltose.

Content Xanthan Gum, when dried, contains 72.0–108.0% of xanthan gum.

Description Xanthan Gum occurs as a white to brownish powder having a slight odor.

Identification Place 300 ml of water into a 500-ml beaker, heat to 80°C, and add a mixture of 1.5g of Xanthan Gum and 1.5 g of carob bean gum powder while magnetically stirring at high speed. Stir at 60°C or higher until the mixture dissolve, and continue to stir for at least 30 minutes at 60°C or higher. Allow to cool to room temperature for 2 hours. Then cool the mixture at a temperature under 4°C, and an elastic gel is formed. A 1% solution of Xanthan Gum prepared in the same manner without carob bean gum does not form an elastic gel.

Purity

(1) Total nitrogen Not more than 1.5% (about 0.2 g, Semi-micro Kjeldahl Method).

(2) Lead Not more than 2.0 μg/g as Pb (5.0 g, Method 1).

(3) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 3, Apparatus B).

(4) 2-Propanol Not more than 0.05%.

(i) Apparatus Use the apparatus specified in Purity (9) for Processed Eucheuma Algae.

(ii) Method

Test Solution Weigh accurately about 2 g of Xanthan Gum in an eggplant-shaped flask (A), add 200 ml of water, a few boiling chips, and 1 ml of silicon resin, and stir well. Place exactly 4 ml of the internal standard solution in a volumetric flack (E), and set up the apparatus. Moisten the joint parts with water. Distill it at a rate of 2 to 3 ml/minute, taking care not to allow bubbles to be trapped in the delivery tube with a spray trap (C), and collect about 90 ml of distillate. To the distillate, add water to make exactly 100 ml. Use *tert*-butanol solution (1 in 1,000) as the internal standard solution.

Standard Solution Weigh accurately about 0.5 g of 2-propanol, and add water to make exactly 50 ml. Measure exactly 5 ml of this solution, and add water to make exactly 50 ml. Then place exactly 2 ml of the second solution and 8 ml of the internal standard solution into a 200-ml volumetric flask, and add water to volume.

Procedure Analyze 2.0 μl portions of the test solution and the standard solution by gas chromatography using the operating conditions below. Determine the peak area ratios (Q_T and Q_S) of 2-propanol to *tert*-butanol for the test solution and the standard solution. Obtain the content of 2-propanol by the formula:

$$\text{Content (\%) of 2-propanol} = \frac{\text{Weight (g) of 2-propanol}}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 0.2$$

Operating Conditions

Detector: Flame-ionization detector.

Column: A glass tube of 3 mm internal diameter and 2 m length.

Column packing material: 180- to 250-μm styrene-divinylbenzene porous polymer for gas chromatography.

Column temperature: A constant temperature at about 120°C.

Injection port temperature: A constant temperature at about 200°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust so that the retention time of 2-propanol is about 10 minutes.

Loss on Drying Not more than 15.0% (105°C, 2.5 hours).

Ash Not more than 16.0% (Use the sample dried at 105°C for 4 hours).

Microbial Limits Proceed as directed in the Microbial Limit Tests. The total bacterial count is not more than 10,000/g, and *Escherichia coli* is negative.

Assay Dry a glass filter (1G4) under reduced pressure at 80°C for 30 minutes, allow to cool in a desiccator, and weigh accurately. Weigh accurately about 0.5 g of dried Xanthan Gum, add 10 ml of potassium hydroxide solution (1 in 25) to dissolve, and add 90 ml of water. To this solution, add 15 ml of diluted hydrochloric acid (1 in 3) and 300 ml of absolute ethanol, and stir vigorously. Allow to stand for 2 hours, and centrifuge with 4,000 rpm for 10 minutes, and remove the supernatant. Add absolute ethanol again, and repeat the same procedures until the supernatant is free of chlorides. Filter the precipitate obtained through the glass filter, with absolute ethanol. Wash the residue with acetone, dry under the reduced pressure at 80°C for 1.5 hours, allow

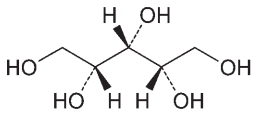
to cool in a desiccator, and weigh accurately. Calculate the content by the formula:

$$\text{Content (\%) of xanthan gum} = \frac{\text{Weight (g) of the residue}}{\text{Weight (g) of the sample}} \times 100$$

Xylitol

Xylit

キシリトール



$C_5H_{12}O_5$ Mol. Wt. 152.15

meso-Xylitol [87-99-0]

Content Xylitol, when calculated on the anhydrous basis, contains 98.5–101.0% of xylitol ($C_5H_{12}O_5$).

Description Xylitol occurs as white crystals or crystalline powder. It is odorless and has a cool, sweet taste.

Identification

(1) Dissolve 5 g of Xylitol in 10 ml of a 1:1 mixture of hydrochloric acid/formalin. Heat at 50°C for 2 hours, and add 25 ml of ethanol. Crystals are deposited. Collect the crystals by filtration, add 10 ml of water, and dissolve by warming. Add 50 ml of ethanol to produce crystals. Collect the deposited crystals by filtration, recrystallize twice from ethanol, and dry at 105°C for 2 hours. The melting point is 195–201°C.

(2) Determine the absorption spectrum of Xylitol, previously dried in a phosphorus(V) oxide desiccator under reduced pressure for 24 hours, as directed in the Potassium Bromide Disk Method under Infrared Spectrophotometry, and compare with the Reference Spectrum or the Xylitol Reference Standard spectrum. Both spectra exhibit similar intensities of absorption at the same wavenumbers.

Purity

(1) Melting point 92–96°C.

(2) Clarity of solution Clear (1.0 g, water 2.0 ml).

(3) pH 5.0–7.0 (1.0 g, water 10 ml).

(4) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 2, Control solution Lead Standard Solution 2.0ml).

(5) Lead Not more than 1.0 μg/g as Pb (10.0 g, Method 1).

(6) Arsenic Not more than 4. 0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

(7) Nickel Not more than 2.0 μg/g as Ni.

Weigh 50.0 g of Xylitol, dissolve it in a 1:1 mixture of water/dilute acetic acid to make 500 ml. Use this solution as solution A.

Test Solution Transfer 100 ml of solution A to a separating funnel, add 2.0 ml of 1% (w/v) ammonium pyrrolidine dithiocarbamate and 10 ml of methyl isobutyl ketone, shake, and collect the methyl isobutyl ketone layer.

Control Solution Transfer 100 ml of solution A into each of 3 separating funnels. To the funnels, add 0.5, 1.0, and 1.5 ml of Nickel Standard Solution, respectively, and then proceed as directed for the test solution.

Procedure Perform the test on the test solution and the control solutions as directed under Flame Atomic Absorption Spectrophotometry using the operating conditions below, and determine the content of Nickel using the Standard Addition Method.

Operating Conditions

Light source: Nickel hollow cathode lump.

Wavelength of analytical line: 232.0 nm.

Supporting gas: Air.

Combustible gas: Acetylene.

(8) Other sugar-alcohols Not more than 1.0%.

Calculate each content (%) of L-arabinitol, galactitol, D-mannitol, and D-sorbitol as directed in the Assay. The total of these contents is the content (%) of other sugar-alcohols.

Control Solution Weigh accurately about 0.01 g of each sugar-alcohol standards, and dissolve it in water to make exactly 100 ml.

(9) Reducing sugars Not more than 0.2% as D-glucose.

Weigh 1.0 g of Xylitol, transfer into a flask, and dissolve it in 25 ml of water. Add 40 ml of Fehling's TS, boil gently for 3 minutes, and allow to stand to form a precipitate of cuprous oxide. Filter the supernatant through a glass filter (1G4). Add immediately warm water in the flask, wash the precipitate, filter the washings through the same glass filter, and discard the filtrate. Repeat the washing and filtering process until the washings are no longer alkaline. Immediately add 20 ml of ferric sulfate TS to the precipitate in the flask, and dissolve. Filter through the above glass filter, wash with water, and combine the filtrate and the washings. Heat to 80°C, and add 0.6 ml of 0.02 mol/L potassium permanganate. The pink color of the solution does not disappear immediately.

Water Content Not more than 0.50% (1.0 g, Direct Titration).

Residue on Ignition Not more than 0.10%.

Assay

Test Solution Weigh accurately about 2 g of Xylitol, and dissolve it in water to make exactly 100 ml. Measure exactly 1 ml of this solution, and add exactly 1 ml of the internal standard solution. Evaporate in a water bath at 60°C under reduced pressure to dryness. Add 1.0 ml of dehydrated pyridine and 1.0 ml of acetic anhydride. Heat under a reflux condenser in a water bath for 1 hour, and cool. Use the resulting solution as the test solution. As the internal standard solution, use a solution prepared by diluting about 0.2 g of erythritol, accurately weighed, with water to exactly 25 ml.

Control Solution Weigh accurately about 0.2 g of Xylitol Reference Standard, dissolve it in water to make exactly 10 ml. Measure exactly 1 ml of this solution, and proceed as directed for the test solution.

Procedure Analyze both the test solution and the control solution by gas chromatography using the operating conditions below. Determine the peak area ratios (Q_T and Q_S) of xylitol derivative to erythritol derivative for the test solution and the control solution, respectively. Determine the content of xylitol by the formula below, and calculate on the anhydrous basis.

Content (%) of xylitol ($C_5H_{12}O_5$)

$$= \frac{\text{Weight (g) of Xylitol Reference Standard} \times 10}{\text{Weight (g) of the sample}} \times \frac{Q_T}{Q_S} \times 100$$

Operating Conditions

Detector: Flame-ionization detector.

Column: A silicate glass capillary tube (0.25 mm internal diameter and 30 m length) coated with a 0.25-μm thick layer of 14% cyanopropyl phenyl–86% dimethylpolysiloxane for gas chromatography.

Column temperature: Maintain the temperature at 180°C for 2 minutes, thereafter raise at a rate of 10°C/minute to 220°C, and then maintain at 220°C for 15 minutes.

Injection port temperature: 250°C.

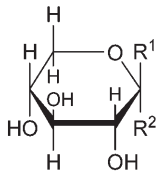
Injection method: Sprit (20:1).

Carrier gas: Helium.

Flow rate: Adjust so that the peak of the erythritol derivative appears about 6 minutes after the injection.

D-Xylose

D-キシロース



α-D-Xylopyranose: R^1 = H, R^2 = OH
β-D-Xylopyranose: R^1 = OH, R^2 = H

$C_5H_{10}O_5$ Mol. Wt. 150.13

D-Xylopyranose [58-86-6]

Content D-Xylose, when dried, contains 98.0–101.0% of D-xylose ($C_5H_{10}O_5$).

Description D-Xylose occurs as colorless to white crystals or as a white crystalline powder. It is odorless and has a sweet taste.

Identification

(1) Add 2–3 drops of a solution of D-Xylose (1 in 20) to 5 ml of boiling Fehling's TS. A red precipitate is formed.

(2) Dissolve 1 g of D-Xylose in 25 ml of freshly boiled and cooled water. The solution is dextrorotatory.

(3) To 1 g of D-Xylose, add 3 ml of water, dissolve by warming, add 3 ml of a 5:2 mixture of diluted hydrochloric acid (1 in 4)/a solution of diphenylamine in ethanol (1 in 40), and heat in a water bath for 5 minutes. A yellow to light orange color develops.

(4) Dissolve 0.5 g of D-Xylose in 20 ml of water, add 30 ml of phenylhydrazine hydrochloride–sodium acetate TS and 10 ml of diluted acetic acid (1 in 20), heat in a water bath for about 2 hours to form a precipitate, and recrystallize the precipitate from water. The melting point is 160–163°C.

Purity

(1) Clarity and color of solution Colorless and almost clear (4.0 g, water 20 ml).

(2) Free acid Weigh 1.0 g of D-Xylose, dissolve it in 10 ml of freshly boiled and cooled water, add 1 drop of phenolphthalein TS, and add 1 drop of 0.2 mol/L sodium hydroxide. The color of the solution is pink.

(3) Sulfate Not more than 0.005% as SO_4.

Test Solution Weigh 1.0 g of D-Xylose, and dissolve it in 30 ml of water.

Control Solution Use 0.10 ml of 0.005 mol/L sulfuric acid.

(4) Heavy metals Not more than 10 μg/g as Pb (2.0 g, Method 1, Control solution Lead Standard Solution 2.0 ml).

(5) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

(6) Other saccharide

Test Solution Weigh 0.5 g of D-Xylose, and dissolve it in water to make 1,000 ml.

Procedure Analyze 0.1 ml of the test solution by paper chromatography using a 6:4:3 mixture of 1-butanol/pyridine/water as the developing solvent. No control solution is used. For the filter paper, use a No. 2 filter paper for chromatography. Stop the development when the solvent front has ascended to a point about 15 cm above the point on which the test solution was applied, and mark the front point. After the filter paper is air-dried, again develop with the same developing solvent, and stop the development when the solvent front reaches the point marked. Repeat the developing process once more, spray the filter paper with the color developing reagent, dry at 100–125°C for 5 minutes, and observe from above in daylight. Only one pink spot is observed. Prepare a color developing reagent as follows: Weigh 0.93 g of aniline and 1.66 g of phthalic anhydride, and dissolve them in 100 ml of water saturated 1-butanol.

Loss on Drying Not more than 1.0% (105°C, 3 hours).

Residue on Ignition Not more than 0.05% (5 g).

Assay Weigh accurately about 1 g of D-Xylose, previously dried, and dissolve it in water to make exactly 500 ml. Measure exactly 10 ml of this solution, transfer into a flask with a ground-glass stopper, and add exactly 50 ml of sodium metaperiodate solution (1 in 400). Add 1 ml of sulfuric acid, and heat in a water bath for 15 minutes. After cooling, add 2.5 g of potassium iodide, shake well, allow to stand in a dark and cold place for 15 minutes, and titrate with 0.1 mol/L sodium thiosulfate (indicator: starch TS). Separately, perform a blank test in the same manner, and make any necessary correction.

Each ml of 0.1 mol/L sodium thiosulfate = 1.877 mg of $C_5H_{10}O_5$

Yeast Cell Wall

酵母細胞壁

Definition Yeast Cell Wall is obtained from the yeast *Saccharomyces cerevisiae* and consists mainly of polysaccharides.

Description Yeast Cell Wall occurs as an off-white to brownish-red powder or suspension having a slight, characteristic odor.

Identification

(1) If the sample is a powder, prepare a suspension by magnetically stirring 1g of it with 100 ml of water at high speed. If the sample is a suspension, use as is for testing. Examine the suspension sample with a 200–400 power microscope. Egg-shaped or flat single cells with a long axis diameter of 1 to 12 μm or their fragments, are observed.

(2) To 1 g of a powder sample or a previously dried suspension sample, add 50 ml of phosphate buffer (pH 6.8), magnetically stire at high speed, and allow to stand for 30 minutes. It swells.

Purity

(1) Heavy metals Not more than 20 μg/g as Pb (1.0 g of a powder sample or previously dried suspension sample, Method 2, Control solution Lead Standard Solution 2.0 ml).

(2) Lead Not more than 5.0 μg/g as Pb (2.0 g of a powder sample or previously dried suspension sample, Method 1).

(3) Arsenic Not more than 2.0 μg/g as As_2O_3 (1.0 g of a powder sample or previously dried suspension sample, Method 3, Apparatus B).

(4) Total nitrogen Not more than 5.6% (on the dried basis, about 1.0 g, Semi-micro Kjeldahl Method).

(5) Starch To 1.0 g of a powder sample or previously dried suspension sample, add 1 drop of iodine TS, and examine microscopically. Little or no black-purple stained particles are observed.

Loss on Drying

Powder sample: Not more than 8.0% (120°C, 2 hours).

Suspension sample: Not more than 92.0% (120°C, 2 hours).

Ash Not more than 10.0% (1.0 g of a powder sample or previously dried suspension sample).

Microbial Limits Proceed as directed in the Microbial Limit Tests. The total bacterial count is not more than 10,000/g, and *Escherichia coli* is negative.

Yucca Foam Extract

ユッカフォーム抽出物

Definition Yucca Foam Extract is obtained from *Yucca brevifolia* Engelmann or *Yucca schidigera* Roezl ex Ortgies and consists mainly of saponins.

Content Yucca Foam Extract, when calculated on the anhydrous basis, contains not less than 3.0% of yucca saponins.

Description Yucca Foam Extract occurs as a yellow to brown powder or as a brown liquid having a characteristic odor.

Identification

(1) Weigh an amount of Yucca Foam Extract equivalent to 0.6 g on the anhydrous basis, add 10 ml of a 9:1 mixture of methanol/water, shake vigorously, and filter. Analyze 1 μl of the filtrate as the test solution by thin-layer chromatography using a 40:16:8:1 mixture of ethyl acetate/ethanol/water/acetic acid as the developing solvent. No control solution is used. Use a thin-layer plate for yucca foam extract, previously dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 8 cm above the original line. Air-dry the plate, spray with *p*-anisaldehyde–sulfuric acid TS, and heat at 110°C for 10 minutes. At least four yellow-green to blue-green spots are observed at R_f values of about 0.4–0.6.

(2) Measure 3 ml of solution A prepared in the Assay, evaporate the solvent, and dissolve the residue in 0.1 ml of ethyl acetate. Use this solution as the test solution. Use Solution B, prepared in the Assay, as the control solution. Analyze 2-μl portions of the test solution and the control solution by thin-layer chromatography using a 2:1 mixture of hexane/ethyl acetate as the developing solvent. Use a thin-layer plate for yucca foam extract, previously dried at 110°C for 1 hour. Stop the development when the solvent front has ascended to a point about 8 cm above the original line, and air-dry the plate. Spray with *p*-anisaldehyde–sulfuric acid TS, and heat at 110°C for 10 minutes. The spot from the test solution corresponds in color tone and R_f value to the yellow-green to blue-green spot from the control solution.

Purity

(1) pH 3.5–5.0 (1.0 g on the anhydrous basis, water 100 ml).

(2) Heavy metals Not more than 20 μg/g as Pb (1.0 g on the anhydrous basis, Method 2, Control solution Lead Standard Solution 2.0 ml).

(3) Arsenic Not more than 2.0 μg/g as As_2O_3 (1.0 g on the anhydrous basis, Method 3, Apparatus B).

Water Content

Liquid sample: Not more than 60% (0.1 g, Direct Titration).

Powder sample: Not more than 8.0% (0.1 g, Direct Titration).

Residue on Ignition Not more than 5.0% (2 g on the anhydrous basis).

Assay

Test Solution Weigh accurately an amount of Yucca Foam Extract equivalent to about 0.2 g on the anhydrous basis, and dissolve it in 5 ml of water. Pour this solution into a glass tube of 15 mm internal diameter packed with 20 ml of styrene-divinylbenzene absorption resin. Wash the resin with 100 ml of water and 100 ml of a 3:2 mixture of water/methanol at a flow rate of not more than 2 ml/minute. Next, elute with 100 ml of a 9:1 mixture of methanol/water, and evaporate the solvent in the eluate. Dissolve the residue in ethanol to make exactly 20 ml. Measure exactly 10 ml of this solution, add 10 ml of 2 mol/L hydrochloric acid, and heat under a reflux condenser for 3 hours in a water bath. After cooling, extract twice with 80 ml of diethyl ether each time, and combine the diethyl ether phases. Then wash the diethyl ether phase with 20 ml of water. Dehydrate with 20 g of anhydrous sodium sulfate, and evaporate the diethyl ether. Dissolve the residue in ethyl acetate to make exactly 50 ml. Refer to the resulting solution as solution A. Measure exactly 1 ml of solution A, and add ethyl acetate to make exactly 10 ml.

Standard Solution and Blank Test Solution Weigh accurately an amount of sarsasapogenin for assay equivalent to about 5 mg on the anhydrous basis, dissolve it in ethyl acetate to make exactly 5 ml. Refer to this solution as Solution B. Measure exactly 1 ml of Solution B, and add ethyl acetate to make exactly 200 ml. Use this solution as the standard solution. As the blank test solution, use ethyl acetate.

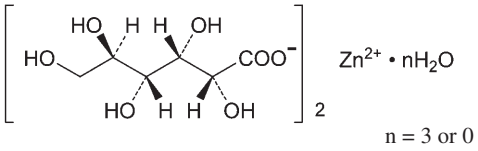
Procedure Measure 2 ml each of the test solution, the standard solution, and the blank test solution. To each, add 1 ml of 0.5% *p*-anisaldehyde–ethylacetate TS and 1 ml of a

1:1 mixture of sulfuric acid/ethyl acetate, and shake gently for exactly 10 minutes in a 60°C water bath. After cooling for exactly 10 minutes in a water bath at room temperature, immediately measure the absorbances (A_T, A_S, and A_0) of the test solution, standard solution, and blank test solution at 430 nm, using ethyl acetate as the reference solution. Determine the yucca saponin content by the formula:

$$\text{Content (\%) of yucca saponin} = \frac{\text{Weight (g) of sarsasapogenin}}{\text{Anhydrous basis weight (g) of the sample}} \times \frac{A_T - A_0}{A_S - A_0} \times 2.10 \times 100$$

Zinc Gluconate

グルコン酸亜鉛



$C_{12}H_{22}O_{14}Zn \cdot nH_2O$ (n = 3 or 0) Mol. Wt. trihydrate 509.75
Anhydrous 455.70

Monozinc bis(D-gluconate) trihydrate
Monozinc bis(D-gluconate) [82139-35-3]

Content Zinc Gluconate, when calculated on the anhydrous basis, contains 97.0–102.0% of zinc gluconate ($C_{12}H_{22}O_{14}Zn$).

Description Zinc Gluconate occurs as a white crystalline powder or granules.

Identification

(1) A solution of Zinc Gluconate (1 in 20) responds to all tests for Zinc Salt in the Qualitative Tests.

(2) Measure 5 ml of a solution of Zinc Gluconate in warm water (1 in 10), and proceed as directed in Identification (2) for Glucono-δ-Lactone.

Purity

(1) Lead Not more than 10 μg/g as Pb.

Test Solution Weigh 1.00 g of Zinc Gluconate, dissolve it in 1 ml of nitric acid and 20 ml of water, and add water to make exactly 100 ml.

Procedure Proceed as directed in Method 2 in the Lead Limit Test.

(2) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

(3) Reducing sugars Not more than 1.0% as D-glucose.

Weigh 1.0 g of Zinc Gluconate, transfer into a 250-ml Erlenmeyer flask, dissolve it in 10 ml of water, and add 25 ml of alkaline cupric citrate TS. Cover with a small beaker, boil gently for exactly 5 minutes, and cool quickly to room temperature. To this solution, add 25 ml of diluted acetic acid (1 in 10), exactly 10 ml of 0.05 mol/L iodine, 10 ml of diluted hydrochloric acid (1 in 4), and 3 ml of starch TS, in that order. Titrate the excess iodine with 0.1 mol/L sodium thiosulfate. The volume of the sodium thiosulfate solution consumed is not less than 6.3 ml.

Water Content Not more than 11.6% (0.2 g, Direct Titration).

Assay Weigh accurately about 0.7 g of Zinc Gluconate, add 100 ml of water, and dissolve by warming if necessary. Add 5 ml of ammonia–ammonium chloride buffer (pH 10.7), and titrate with 0.05 mol/L EDTA (indicator: 0.1 ml of eriochrome black T TS) until the color of the solution changes to blue. Calculate the content on the anhydrous basis.

Each ml of 0.05 mol/L EDTA = 22.79 mg of $C_{12}H_{22}O_{14}Zn$

Zinc Sulfate

硫酸亜鉛

$ZnSO_4 \cdot 7H_2O$ Mol. Wt. 287.58

Zinc sulfate heptahydrate [7446-20-0]

Content Zinc Sulfate, when calculated on the anhydrous basis, contains not less than 98.0% of zinc sulfate ($ZnSO_4$ = 161.47).

Description Zinc Sulfate occurs as colorless crystals or as a white crystalline powder. It is odorless.

Identification Zinc Sulfate responds to all tests for Zinc Salt and for Sulfate in the Qualitative Tests.

Purity

(1) Free acid Weigh 0.25 g of Zinc Sulfate, dissolve it in 5 ml of water, and add 1 drop of methyl orange TS. No red color develops.

(2) Lead Not more than 10 μg/g as Pb.

Test Solution Weigh 1.00 g of Zinc Sulfate, dissolve it by adding 1 ml of nitric acid and 20 ml of water, and add water to make exactly 100 ml.

Procedure Proceed as directed in Method 2 in the Lead Limit Test.

(3) Alkali metal and alkali-earth metals Not more than 0.50%.

Weigh 2.0 g of Zinc Sulfate, dissolve it in 150 ml of water, and add ammonium sulfide TS until the precipitate is no longer formed. Add water to make 200 ml, and filter through a dry filter paper. Discard the initial 20 ml of filtrate, measure the subsequent 100 ml of filtrate, evaporate to dryness, ignite at 450–550°C to constant weight, and weigh the residue.

(4) Arsenic Not more than 4.0 μg/g as As_2O_3 (0.50 g, Method 1, Apparatus B).

Water Content Not more than 43.5% (0.1 g, Direct Titration).

Assay Weigh accurately about 0.4 g of Zinc Sulfate, add 100 ml of water, and dissolve by warming if necessary. Add 5 ml of ammonia–ammonium chloride buffer (pH 10.7), and titrate with 0.05 mol/L EDTA (indicator: 0.1 ml of eriochrome black T TS) until the color of the solution changes to blue. Calculate on the anhydrous basis.

Each ml of 0.05 mol/L EDTA = 8.074 mg of $ZnSO_4$

STANDARDS FOR MANUFACTURING

STANDARDS FOR USE

STANDARDS FOR LABELING

E. STANDARDS FOR MANUFACTURING

Standards applying generally to all food additives

1. Acid Clay, Bentonite, Diatomaceous Earth, Kaolin, Magnesium Carbonate, Sand, Silicon Dioxide, Talc, or any other similar water-insoluble mineral substance shall not be used for manufacturing or processing any other food additive, except when the substance is indispensable for manufacturing or processing the additive.

2. Unless otherwise specified, preparations of additives shall be manufactured using only additives (confined to substances designated under Article 10 of the Food Sanitation Law, natural flavoring agents, substances that are generally provided for eating or drinking as foods and that are used as additives, and substances appearing in the *List of Existing Food Additives*: Ministry of Health and Welfare Notification No. 120, April 1996) and foods (for the additives or foods for which specifications are established under Article 11 Paragraph 1 of the Law, the specifications shall be meet; water shall be potable).

3. If food additives are manufactured by making use of microorganisms obtained by recombinant DNA technologies, the methods to be used shall be verified to comply with the standards specified by the Minister of Health, Labour and Welfare.

4. If food additives are manufactured or processed, no spinal column of specified cattle shall be used as ingredients of the food additives. However, this provision shall not apply to cases where fat and oil derived from spinal columns of specified cattle are hydrolyzed, saponified, or transesterified under high temperature and high pressure before they are used as ingredients.

Standards applying specifically to individual food additives and preparations

Kansui (confined to chemically synthesized substances)
Kansui shall be manufactured or processed by making use of Potassium carbonate (anhydrous), Sodium carbonate, Sodium hydrogen carbonate, or potassium or sodium salts of phosphoric acids which comply with the corresponding specifications, alone, or as a mixture of two or more of the compounds, as an aqueous solution of a single compound or mixture, or as a single compound or mixture distilled with wheat flour.

Absinth Extract, Capsicum Water-soluble Extract, Carrot Carotene, Clove Extract, Essential Oil-removed Fennel Extract, Gardenia Yellow, Garlic Extract, Ginger Extract, Horseradish Extract, Licorice Extract, Licorice Oil Extract, Mustard Extract, Onion Color, Orange Color, Oregano Extract, Paprika Color, Pepper Extract, Perilla Extract, Rosemary Extract, Sage Extract, Sesame Seed Oil Unsaponified Matter, Spice Extracts, Tamarind Color, Tannin (extract), Turmeric Oleoresin, Wasabi Extract, and natural flavoring agents (hereinafter referred to as the substances obtained only from ajowan, allspice, angelica, anise, asafetida, basil, caraway, cardamom, carrot, caper, capsicum, cassia, celery, chamomile, chervil, Chinese pepper, chive, cinnamon, clove, coriander, cress, cumin, curry leaves, dill, fennel, fenugreek, gardenia, garlic, ginger, hemp seeds, horseradish, horsemint, hyssop, Japanese pepper, juniperberry, laurel, lavender, lemon balm, lemongrass, licorice, linden, marjoram, mint, mustard, *myoga* (*Zingiber mioga*), nigella, nutmeg, onion, orange peel, oregano, paprika, parsley, pepper, peppermint, perilla, poppy seeds, rose, rosemary, saffron, salvia, sassafras, savory, sesame seeds, shallot, sorrel, spearmint, star anise, tamarind, tarragon, thyme, turmeric, vanilla, *wasabi* (Japanese horseradish), or wormwood.)

When the food additives listed above are manufactured or processed, no solvents other than those appearing in the following list shall be used for extracting. In addition, methanol and 2-propanol shall not remain in any of the food additives at more than 50 μg/g each, acetone at more than 30 μg/g, dichloromethane and 1,1,2-trichloroethene at more than 30 μg/g as the total amount of both solvents, and hexane at more than 25 μg/g.

A list of usable solvents
Acetone; Butane; 1-Butanol; 2-Butanol; Carbon dioxide; Cyclohexane; Dichloromethane; Diethyl ether; Ethanol; Ethyl acetate; Ethyl methyl ketone; Edible fats and oils; Glycerol; Hexane; Methanol; Methyl acetate; Nitrous oxide; Propane; 1-Propanol; 2-Propanol; Propylene glycol; 1,1,1,2-Tetrafluoroethane; 1,1,2-Tichloroethane; and Water.

F. STANDARDS FOR USE

Standards applying generally to all food additives

1. Unless otherwise specified, if standards for use are established for food additives used as ingredients of an additive preparation, the existing standards are deemed to be standards for the preparation.

2. When a food listed in column 2 of the following table that contains additives listed in column 1 of the same table is used in the process of the manufacturing or processing of any of the foods listed in column 3 in the same table, the additives contained in that food are considered to be used in the food listed in column 3.

Column 1	Column 2	Column 3
Sulfur Dioxide Potassium Pyrosulfite Sodium Hydrosulfite Sodium Pyrosulfite Sodium Sulfite	*Amanatto* (dried candied beans), Candied cherries (i.e., candied, pitted cherries, or such cherries which are frosted with crystal sugar or which are immersed in syrup), Dijon mustard, dried fruits (excluding raisin), dried potatoes, food molasses, frozen unheated crabs, fruit wines, gelatin, *Kanpyo* (dried gourd strips), *Konnyaku-ko* (Konjac powder), miscellaneous alcoholic beverages, natural fruit juice (confined to products to be consumed in 5-fold or more dilution), prawn, simmered beans, starch syrup, tapioca starch for saccharification.	All foods excluding those listed in column 2
Sodium Saccharin	Flour paste (hereinafter in section F, referred to as any paste food which is prepared by adding sugar, fats/oils, powder milk, eggs, or wheat flour to main ingredients such as wheat flour, starch, nuts or their processed products, cocoa, chocolate, coffee, fruit pulps, or fruit juice, pasteurized, and used for bread or confectionery as fillings or toppings).	Confections
Potassium Sorbate Sorbic Acid	*Miso* (fermented soybean paste).	*Miso-zuke* (*miso*-pickled foods)
All food additives	All foods.	Milk and milk products (excluding ice cream) prescribed in Article 2 of the Ministerial Ordinance concerning Compositional Specifications for Milk and Milk Products, ETC. (Ministry of Health and Welfare Ordinance No. 52, 1951)

Standards applying specifically to individual food additives

Substance Name	Major Use Category	Target Foods	Maximum Limit	Other Requirements
Acesulfame Potassium	Sweetener	*An* (sweetened bean paste).	2.5 g/kg	These maximum limits do not apply to foods approved to have special-dietary-use labeling.
		Confections (excluding chewing gum).	2.5 g/kg	
		Chewing gum.	5.0 g/kg	
		Edible ices (including sherbets, flavored ices, and other similar foods).	1.0 g/kg	

Substance Name	Major Use Category	Target Foods	Maximum Limit	Other Requirements
Acesulfame Potassium (continued)		Fermented milk.*	0.50 g/kg	* Applied to dilutions, in the case of concentrated products.
		Flour paste.	1.0 g/kg	
		Foods with nutrition claims (tablets only).	6.0 g/kg	
		Fruit wines (all kinds of wines).*	0.50 g/kg	
		Ice cream.	1.0 g/kg	
		Jams.	1.0 g/kg	
		Lactic acid bacterial beverages.*	0.50 g/kg	
		Milk drinks.*	0.50 g/kg	
		Other miscellaneous alcoholic beverages.	0.50 g/kg	
		Moist cakes (hereafter refer to fine bakery products with cream, jam, fruits and the like; Japanese unbaked cakes).	2.5 g/kg	
		Nonalcoholic beverages.	0.50 g/kg	
		Pickles.	1.0 g/kg	
		Sugar substitutes.**	15 g/kg	** Products used by directly adding to drinks, such as coffee and tea, as substitutes for sugar.
		Tare (a dip or sauce mainly for Japanese or Chinese foods).	1.0 g/kg	
		Other foods.	0.35 g/kg	
Acetaldehyde	Flavoring agent	All foods.		Only for flavoring.
Acetone	Extracting agent	Fats and oils. Guarana nuts.		Only for extracting components from such nuts in the manufacturing of guarana beverages or for fractionating components of fats or oils. Shall be removed before the preparation of the final food.
Acetophenone	Flavoring agent	All foods.		Only for flavoring.
Acid Clay	Processing agent	See “Water-insoluble minerals.”		
Activated Acid Clay	Processing agent	See “Water-insoluble minerals.”		
Aliphatic Higher Alcohols (excluding substances generally recognized as highly toxic)	Flavoring agent	All foods.		Only for flavoring.
Aliphatic Higher Aldehydes (excluding substances generally recognized as highly toxic)	Flavoring agent	All foods.		Only for flavoring.

Substance Name	Major Use Category	Target Foods	Maximum Limit	Other Requirements
Aliphatic Higher Hydrocarbons (excluding substances generally recognized as highly toxic)	Flavoring agent	All foods.		Only for flavoring.
Allyl Cyclohexylpropionate	Flavoring agent	All foods.		Only for flavoring.
Allyl Hexanoate	Flavoring agent	All foods.		Only for flavoring.
Allyl Isothiocyanate	Flavoring agent	All foods.		Only for flavoring.
Aluminum Ammonium Sulfate	Raising agent Processing agent			Not permitted in *miso* (fermented soy bean paste).
Aluminum Potassium Sulfate	Raising agent Processing agent			Not permitted in *miso* (fermented soy bean paste).
Ammonium Persulfate	Flour treatment agent	Wheat flour.	0.30 g/kg	
Amyl Alcohol	Flavoring agent	All foods.		Only for flavoring.
α-Amylcinnamaldehyde	Flavoring agent	All foods.		Only for flavoring.
Anisaldehyde	Flavoring agent	All foods.		Only for flavoring.
Annatto, Water-soluble Potassium Norbixin Sodium Norbixin	Food color	See the corresponding section for individual standards.		
Aromatic Alcohols	Flavoring agent	All foods.		Only for flavoring.
Aromatic Aldehydes (excluding substances generally recognized as highly toxic)	Flavoring agent	All foods.		Only for flavoring.
Beet Red	Food color	See “Food colors other than chemically synthesized products.”		
Bentonite	Processing agent	See “Water-insoluble minerals.”		
Benzaldehyde	Flavoring agent	All foods.		Only for flavoring.
Benzoic Acid	Preservative	Caviar. Margarine. Nonalcoholic beverages. Soy sauce. Syrup.	2.5 g/kg 1.0 g/kg 0.60 g/kg 0.60 g/kg 0.60 g/kg	When the additive is used in margarine with Sorbic Acid or Potassium Sorbate, or a preparation containing either of these two additives, the total amount of them as benzoic acid and as sorbic acid shall not be more than 1.0 g/kg.
Benzoyl Peroxide	Flour treatment agent	Wheat flour.		Shall be used only as diluted Benzoyl Peroxide by mixing with one or more of Alum, calcium salts of Phosphoric Acid, Calcium Sulfate, Calcium Carbonate, Magnesium Carbonate, and Starch.
Benzyl Acetate	Flavoring agent	All foods.		Only for flavoring.
Benzyl Alcohol	Flavoring agent	All foods.		Only for flavoring.

Substance Name	Major Use Category	Target Foods	Maximum Limit	Other Requirements
Benzyl Propionate	Flavoring agent	All foods.		Only for flavoring.
Biotin	Dietary supplement	Foods with health claims.		
Black Currant Color	Food color	See "Food colors other than chemically synthesized products."		
d-Borneol	Flavoring agent	All foods.		Only for flavoring.
Butanol	Flavoring agent	All foods.		Only for flavoring.
Butyl Acetate	Flavoring agent	All foods.		Only for flavoring.
Butyl Butyrate	Flavoring agent	All foods.		Only for flavoring.
Butyl *p*-Hydroxybenzoate	Preservative		as *p*-hydroxybenzoic acid	
		Fruit sauce.	0.20 g/kg	
		Nonalcoholic beverages.	0.10 g/kg	
		Fruits and fruit vegetables.	0.012 g/kg of rinds	
		Soy sauce.	0.25 g/L	
		Syrup.	0.10 g/kg	
		Vinegar.	0.10 g/L	
Butylated Hydroxyanisole (BHA)	Antioxidant		as BHA	When BHA is used in combination with BHT, the total amount of both shall not exceed the corresponding limit.
		Butter.	0.2 g/kg	
		Fats & oils.	0.2 g/kg	
		Fish & shellfish (dried).	0.2 g/kg	
		Fish & shellfish (salted).	0.2 g/kg	
		Fish & shellfish (frozen) (except frozen fish/shellfish and oysters for raw consumption).	1 g/kg of dip	
		Mashed potato (dried).	0.2 g/kg	
		Whale meat (frozen) (except frozen products for raw consumption).	1 g/kg of dip	
Butylated Hydroxytoluene (BHT)	Antioxidant		as BHT	When BHT is used in combination with BHA, the total amount of both shall not exceed the corresponding limit.
		Butter.	0.2 g/kg	
		Chewing gum.	0.75 g/kg	
		Fats & oils.	0.2 g/kg	
		Fish & shellfish (dried).	0.2 g/kg	
		Fish & shellfish (salted).	0.2 g/kg	
		Fish & shellfish (frozen) (excluding frozen fish/shellfish and oysters for raw consumption).	1 g/kg of dip	
		Mashed potato (dried).	0.2 g/kg	
		Whale meat (frozen) (except frozen products for raw consumption).	1 g/kg of dip	
Butyric Acid	Flavoring agent	All foods.		Only for flavoring.

Substance Name	Major Use Category	Target Foods	Maximum Limit	Other Requirements
Calcium Carbonate	Dietary supplement Processing agent	 Chewing gum. Other foods.	as calcium 10% 1.0% These limits are not applied to foods approved to be labeled as "special. dietary use."	Only when indispensable for manufacturing or processing the food, or when used for nutritive purposes.
Calcium Carboxy-methylcellulose	Thickening agent	All foods.	2.0%	When used with one or more of the following additives, the total amount shall not be more than 2.0%: Methyl Cellulose, Sodium Carboxymethyl-cellulose, Sodium Carboxymethylstarch, and Sodium Starch Phosphate.
Calcium Chloride	Dietary supplement Tofu coagulator	All foods.	1.0% as calcium Not applied to foods approved to be labeled as "special dietary use."	Only when indispensable for manufacturing or processing the food, or when used for nutritive purposes.
Calcium Citrate	Dietary supplement	All foods.	1.0% as calcium Not applied to foods approved to be labeled as "special dietary use."	
Calcium Dihydrogen Phosphate	Dietary supplement Processing agent	All foods.	1.0% as calcium. Not applied to foods approved to be labeled as "special dietary use."	Only when indispensable for manufacturing or processing the food, or when used for nutritive purposes.
Calcium Dihydrogen Pyrophosphate	Dietary supplement Processing agent	All foods.	1.0% as calcium Not applied to foods approved to be labeled as "special dietary use."	Only when indispensable for manufacturing or processing the food, or when used for nutritive purposes.
Calcium Disodium Ethylenediamine-tetraacetate	Antioxidant	 Canned and bottled nonalcoholic beverages. Other canned and bottle foods.	as EDTA-$CaNa_2$ 0.035 g/kg 0.25 g/kg	
Calcium Ferrocyanide	Anticaking agent	Salt only.	0.020 g/kg as anhydrous sodium ferrocyanide.	When it is used together with either or both of Potassium Ferrocyanide and Sodium Ferrocyanide, the sum of those shall not be more than 0.020 g/kg of salt as anhydrous sodium ferrocyanide.

Substance Name	Major Use Category	Target Foods	Maximum Limit	Other Requirements
Calcium Gluconate	Dietary supplement	All foods.	1.0% as calcium Not applied to foods approved to be labeled as "special dietary use."	Only for nutritive purposes.
Calcium Glycerophosphate	Dietary supplement	All foods.	1.0% as calcium Not applied to foods approved to be labeled as "special dietary use."	Only for nutritive purposes.
Calcium Hydroxide	Dietary supplement Processing agent	All foods.	1.0% as calcium Not applied to foods approved to be labeled as "special dietary use."	Only when indispensable for manufacturing or processing the food, or when used for nutritive purposes.
Calcium Lactate	Dietary supplement Processing agent	All foods.	1.0% as calcium Not applied to foods approved to be labeled as "special dietary use."	
Calcium Monohydrogen Phosphate	Dietary supplement Processing agent	All foods.	1.0% as calcium Not applied to foods approved to be labeled as "special dietary use."	Only when indispensable for manufacturing or processing the food, or when used for nutritive purposes.
Calcium Pantothenate	Dietary supplement	All foods.	1.0% as calcium Not applied to foods approved to be labeled as "special dietary use."	
Calcium Propionate	Preservative	 Bread and cakes. Cheese.	as propionic acid 2.5 g/kg 3.0 g/kg	When the additive is used in cheese with Sorbic Acid or one of its salts, the total amount of them as propionic acid and as sorbic acid shall not be more than 3.0 g/kg.
Calcium Stearoyl Lactylate	Emulsifier	Bread. Butter cake. Confections (baked and fried wheat flour products only, excluding butter cakes and sponge cakes). Macaroni and other such products. Mixed powder: for bread. for confections (fried wheat flour products only).	4.0 g/kg 5.5 g/kg 4.0 g/kg 4.0 g/kg of dry noodles 5.5 g/kg 5.5 g/kg	

Substance Name	Major Use Category	Target Foods	Maximum Limit	Other Requirements
Calcium Strearoyl Lactylate (continued)		for confections (baked wheat flour products only, excluding butter cakes and sponge cakes).	5.0g/kg	
		for moist cakes.*	10g/kg	* In this section, moist cakes refer to rice flour products only.
		for sponge cakes, butter cakes, and steamed bread.	8.0 g/kg	
		for steamed *manjyu* (bun made by steaming wheat flour dough).	2.5 g/kg	
		Moist cakes.	6.0g/kg	
		Noodles (raw noodles and instant noodles excluding other dry noodles).	4.5 g/kg of boiled noodles	
		Sponge cakes.	5.5 g/kg	
		Steamed bread (bread made by steaming wheat flour dough).	5.5 g/kg	
		Steamed *manjyu*.	2.0 g/kg	
Calcium Sulfate	Dietary supplement Tofu coagulator	All foods.	1.0% as calcium Not applied to foods approved to be labeled as "special dietary use."	Only when indispensable for manufacturing or processing the food, or when used for nutritive purposes.
Caramel I	Food color	See “Food colors other than chemically synthesized products.”		
Caramel II	Food color	See “Food colors other than chemically synthesized products.”		
Caramel III	Food color	See “Food colors other than chemically synthesized products.”		
Caramel IV	Food color	See “Food colors other than chemically synthesized products.”		
β-Carotene	Food color Dietary supplement			Not permitted in fresh fish/shellfish (including fresh whale meat), *konbu* (kelp)/*wakame* (both Laminariales algae), legumes/pulses, meat, *nori* (laver), tea, or vegetables. Store in a hermetic, light-resistant container under inert gas, in a cool place.
Carrot Carotene	Food color	See “Food colors other than chemically synthesized products.”		
Carthamus Red	Food color	See “Food colors other than chemically synthesized products.”		
Carthamus Yellow	Food color	See “Food colors other than chemically synthesized products.”		
Chloride Dioxide	Flour treatment agent	Wheat flour.		
Chlorophyll	Food color	See “Food colors other than chemically synthesized products.”		
1,8-Cineole	Flavoring agent	All foods.		Only for flavoring.
Cinnamaldehyde	Flavoring agent	All foods.		Only for flavoring.
Cinnamic Acid	Flavoring agent	All foods.		Only for flavoring.

Substance Name	Major Use Category	Target Foods	Maximum Limit	Other Requirements
Cinnamyl Acetate	Flavoring agent	All foods.		Only for flavoring.
Cinnamyl Alcohol	Flavoring agent	All foods.		Only for flavoring.
Citral	Flavoring agent	All foods.		Only for flavoring.
Citronellal	Flavoring agent	All foods.		Only for flavoring.
Citronellol	Flavoring agent	All foods.		Only for flavoring.
Citronellyl Acetate	Flavoring agent	All foods.		Only for flavoring.
Citronellyl Formate	Flavoring agent	All foods.		Only for flavoring.
Cochineal Extract	Food color	See "Food colors other than chemically synthesized products."		
Copper Chlorophyll	Food color		as copper	
		Agar jelly in *mitsumame* packed into cans or plastic containers. (*mitsumame*: foods prepared by mixing agar jelly, cut fruits, green beans, etc. with sugar syrup)	0.0004 g/kg	
		Chewing gum.	0.050 g/kg	
		Chocolate.	0.0010 g/kg	
		Fish-paste products (excluding *surimi*).	0.030 g/kg	
		Fruits and vegetables for preservation.*	0.10 g/kg	* Foods which are processed for preserving, including dried foods, salted foods, pickled foods in vinegar, and preserved foods in syrup.
		Konbu (kelp).	0.15 g/kg of dry kelp	
		Moist cakes (excluding bread with sweet fillings or toppings).	0.0064 g/kg	
Copper Gluconate	Dietary supplement	Breast-milk substitute.	0.60 mg/L as copper* when formulated into a standard concentration	* Not apply to cases where these additives are used in formulated dried milk under approval of the Minister of Health, Labour and Welfare.
		Foods with health claim.	15 mg as copper/ recommended daily intake for each food	
Cupric Sulfate	Dietary supplement	Breast-milk substitute.	0.60 mg/L as copper when formulated into a standard concentration	Not apply to cases where these additives are used in formulated dried milk under approval of the Minister of Health, Labour and Welfare.
Cyclohexyl Acetate	Flavoring agent	All foods.		Only for flavoring.
Cyclohexyl Butyrate	Flavoring agent	All foods.		Only for flavoring.
L-Cysteine Monohydrochloride	Antioxidant	Bread. Fruit juice.		
Decanal	Flavoring agent	All foods.		Only for flavoring.

Substance Name	Major Use Category	Target Foods	Maximum Limit	Other Requirements
Decanol	Flavoring agent	All foods.		Only for flavoring.
Diatomaceous Earth	Processing agent	See “Water-insoluble minerals.”		
Diluted Benzoyl Peroxide	Flour treatment agent	Wheat flour.	0.30 g/kg	
Diphenyl	Antimolding agent		as maximum residue limit, less than:	
		Grapefruit.	0.070 g/kg*	* Only when used by infiltrating into a piece of paper inserted in a package for storage or transpotation.
		Lemon.	0.070 g/kg*	
		Orange.	0.070 g/kg*	
Disodium Ethylene-diaminetetraacetate	Antioxidant		as EDTA-$CaNa_2$	
		Canned and bottle nonalcoholic beverages.	0.035 g/kg	Shall be chelated with calcium ion before the preparation of the finished food.
		Other canned and bottled foods.	0.25 g/kg	
Disodium Glycyrrhizinate	Sweetener	*Miso* (fermented soybean paste).		
		Soy sauce.		
Dunaliella Carotene	Food color	See "Food colors other than chemically synthesized products."		
Erythorbic Acid	Antioxidant	All foods.		Not permitted for nutritive purposes in fish paste products (excluding *surimi*) and bread.
				Only for antioxidizing purposes in other foods.
Ester Gum	Chewing gum base	Chewing gum.		Only as chewing gum base.
Esters	Flavoring agent	All foods.		Only for flavoring.
Ethers	Flavoring agent	All foods.		Only for flavoring.
Ethyl Acetate	Flavoring agent	All foods (flavoring only).		Only for flavoring, except when:
	Solvent	Ethanol.		1. Used for denaturing ethanol which is used for the removal astringency of persimmons, the manufacture of crystalline fructose, the preparation of granules or tablets of spices, or the manufacture of *konnyakuko* (Konjac powder), or which is used as a solvent for Butylated Hydroxytoluene or Butylated Hydroxyanisole, or as an ingredient for the manufacture of vinegar.

Substance Name	Major Use Category	Target Foods	Maximum Limit	Other Requirements
Ethyl Acetate (continued)		Yeast extract. Vinyl acetate resin.		2. Used for accelerating yeast-autolysis in the extract (water-soluble fraction obtained by autolysis of yeast), or 3. Used as a solvent for vinyl acetate resin. Ethyl Acetate used in manufacturing yeast extract shall be removed before the completion of the final food.
Ethyl Acetoacetate	Flavoring agent	All foods.		Only for flavoring.
Ethyl Butyrate	Flavoring agent	All foods.		Only for flavoring.
Ethyl Cinnamate	Flavoring agent	All foods.		Only for flavoring.
Ethyl Decanoate	Flavoring agent	All foods.		Only for flavoring.
2-Ethyl-3,(5 or 6)-dimethylpyrazine	Flavoring agent	All foods.		Only for flavoring.
Ethyl Heptanoate	Flavoring agent	All foods.		Only for flavoring.
Ethyl Hexanoate	Flavoring agent	All foods.		Only for flavoring.
Ethyl *p*-Hydroxybenzoate	Preservative	Same as for Butyl *p*-Hydroxybenzoate.		
Ethyl Isovalerate	Flavoring agent	All foods.		Only for flavoring.
2-Ethyl-3-methylpyrazine	Flavoring agent	All foods.		Only for flavoring.
Ethyl Octanoate	Flavoring agent	All foods.		Only for flavoring.
Ethyl Phenylacetate	Flavoring agent	All foods.		Only for flavoring.
Ethyl Propionate	Flavoring agent	All foods.		Only for flavoring.
Ethylvanillin	Flavoring agent	All foods.		Only for flavoring.
Eugenol	Flavoring agent	All foods.		Only for flavoring.
Fatty Acids	Flavoring agent	All foods.		Only for flavoring.
Ferrocyanides: Calcium Ferrocyanide Pottasium Ferrocianide Sodium Ferrocyanide	Anticaking agent	Salt only.	See the corresponding sections for individual standards.	
Ferrous Gluconate	Color adjuvant	Table olive.	0.15 g/kg as iron	
	Dietary supplement	Dried milk for pregnant and lactating women. Breast-milk substitutes. Weaning foods.		

Substance Name	Major Use Category	Target Foods	Maximum Limit	Other Requirements
Food Blue No. 1 (Brilliant Blue FCF) and its Aluminum Lake	Food color			Not permitted in fish pickles, fresh fish/shellfish (including whale meat), *kasutera* (a type of pound cake), *kinako* (roasted soybean flour), *konbu* (kelp)/*wakame* (both Laminariales algae), legumes/pulses, marmalade, meat, meat pickles, *miso* (fermented soybean paste), noodles (including wantan), *nori* (laver) and its similar products, soy sauce, sponge cakes, tea leaves, vegetables, or whale meat pickles.
Food Blue No. 2 (Indigo Carmine) and its Aluminum Lake	Food color			
Food Green No. 3 (Fast Green FCF) and its Aluminum Lake	Food color			
Food Red No. 2 (Amaranth) and its Aluminum Lake	Food color			
Food Red No. 3 (Erythrosin) and its Aluminum Lake	Food color			
Food Red No. 40 (Allura Red) and its Aluminum Lake	Food color			
Food Red No. 102 (New Coccine)	Food color			
Food Red No. 104 (Phloxine)	Food color			
Food Red No. 105 (Rose Bengal)	Food color			
Food Red No. 106 (Acid Red)	Food color			
Food Yellow No. 4 (Tartrazine) and its Aluminum Lake	Food color			
Food Yellow No. 5 (Sunset Yellow) and its Aluminum Lake	Food color			
Food colors other than chemically synthesized products: Beet Red Black Currant Color Caramel I Caramel II Caramel III Caramel IV Carrot Carotene Carthamus Red Carthamus Yellow Chlorophyl Cochineal Extract Dunaliella Carotene Gardenia Blue Gardenia Red Gardenia Yellow Grape Skin Color Haematococcus Algae Color Lac Color	Food color			Not permitted in fresh fish/shellfish (including fresh whale meat), *konbu* (dried kelp)/*wakame*(both Laminariales algae), legumes/pulses, meat, *nori* (laver), tea leaves, or vegetables, except when gold is used on *nori*.

Substance Name	Major Use Category	Target Foods	Maximum Limit	Other Requirements
Food colors other than chemically synthesized products (continued) Marigold Color Monascus Color Palm Oil Carotene Paprika Color Purple Corn Color Purple Sweet Potato Color Red Cabbage Color Spirulina Color Tomato Color Turmeric Oleoresin Other colors from natural souces				Not permitted in fresh fish/shellfish (including fresh whale meat), *konbu* (dried kelp)/*wakame*(both Laminariales algae), legumes/pulses, meat, *nori* (laver), tea leaves, or vegetables, except when gold is used on *nori*.
Furfural and its derivatives (excluding substances generally recognized as highly toxic)	Flavoring agent	All foods.		Only for flavoring.
Gardenia Blue	Food color	See “Food colors other than chemically synthesized products.”		
Gardenia Red	Food color	See “Food colors other than chemically synthesized products.”		
Gardenia Yellow	Food color	See “Food colors other than chemically synthesized products.”		
Geraniol	Flavoring agent	All foods.		Only for flavoring
Geranyl Acetate	Flavoring agent	All foods.		Only for flavoring.
Geranyl Formate	Flavoring agent	All foods.		Only for flavoring.
Grape Skin Color	Food color	See “Food colors other than chemically synthesized products.”		
Guaiac Resin	Antioxidant	Fats and oils. Butter.	1.0 g/kg 10 g/kg	
Haematococcus Algae Color	Food color	See “Food colors other than chemically synthesized products.”		
Hexane	Extracting agent			Only for extracting fat/oil in manufacturing edible fat/oil. Shall be removed before the completion of the final product.
Hexanoic Acid	Flavoring agent	All foods.		Only for flavoring.
Hydrochloric Acid	Processing agent			Shall be neutralized or removed before the preparation of the final food.
Hydrogen Peroxide	Sterilizer	All foods.		Shall be removed or decomposed before the completion of the final food.
Hydroxycitronellal	Flavoring agent	All foods.		Only for flavoring.

Substance Name	Major Use Category	Target Foods	Maximum Limit	Other Requirements
Hydroxycitronellal Dimethylacetal	Flavoring agent			Only for flavoring.
Hypochlorous Acid Water	Sterilizer			Shall be removed before the completion of the final product.
Imazalil	Antimolding agent		as maximum residue limit	
		Banana.	0.0020 g/kg	
		Citrus fruits except mandarin orange.	0.0050 g/kg	
Indole and its derivatives	Flavoring agent	All foods.		Only for flavoring.
Ion Exchange Resins	Processing agent			Shall be removed before the preparation of the final food.
Ionone	Flavoring agent	All foods.		Only for flavoring.
Iron Sesquioxide	Food color	Banana (stem only). *Konnyaku* (konjac).		For banana, can be used only on stems.
Isoamyl Acetate	Flavoring agent	All foods.		Only for flavoring.
Isoamyl Alcohol	Flavoring agent	All foods.		Only for flavoring.
Isoamyl Butyrate	Flavoring agent	All foods.		Only for flavoring.
Isoamyl Formate	Flavoring agent	All foods.		Only for flavoring.
Isoamyl Isovalerate	Flavoring agent	All foods.		Only for flavoring.
Isoamyl Phenylacetate	Flavoring agent	All foods.		Only for flavoring.
Isoamyl Propionate	Flavoring agent	All foods.		Only for flavoring.
Isobutanol	Flavoring agent	All foods.		Only for flavoring.
Isobutyl *p*-Hydroxybenzoate	Preservative	Same as for Butyl *p*-Hydroxybenzoate.		
Isobutyl Phenylacetate	Flavoring agent	All foods.		Only for flavoring.
Isoeugenol	Flavoring agent	All foods.		Only for flavoring.
Isopropanol	Flavoring agent	All foods.		Only for flavoring.
Isopropyl Citrate	Antioxidant		as monoisopropyl citrate	
		Butter.	0.10 g/kg	
		Fats and oils.	0.10 g/kg	
Isopropyl *p*-Hydroxybenzoate	Preservative	Same as for Butyl *p*-Hydroxybenzoate.		
Isothiocyanates (excluding substances generally recognized as highly toxic)	Flavoring agent	All foods.		Only for flavoring.
Kaolin	Processing agent	See “Water-insoluble minerals.”		
Ketones	Flavoring agent	All foods.		Only for flavoring.
Lac Color	Food color	See “Food colors other than chemically synthesized products.”		

Substance Name	Major Use Category	Target Foods	Maximum Limit	Other Requirements
Lactones (excluding substances generally recognized as highly toxic)	Flavoring agent	All foods.		Only for flavoring.
Linalool	Flavoring agent	All foods.		Only for flavoring.
Linalyl Acetate	Flavoring agent	All foods.		Only for flavoring.
Liquid Paraffin	Processing agent	Bread.	0.10% as maximum residue limit	Only for releasing dough in dividing using an automatic dispenser or in baking.
Magnesium Stearate	Processing agent	Capsule- and tablet-form foods with health claims.		
Maltol	Flavoring agent	All foods.		Only for flavoring.
D-Mannitol	Antisticking agent	Candies.	40%	
		Chewing gum.	20%	
		Furikake (sprinkles only products containing granules).	50% of granules	
		Rakugan (dried rice-flour cakes).	30%	
		Tsukudani konbu (Kelp boiled down in soy sauce.	25% as maximum residue limit	
	Chomiryo (seasoning)*	All foods.		* When used as a blend with Potassium Chloride and Glutamates for seasoning foods or enhancing their original flavor, no limits are specified (only cases where D-Mannitol does not exceed 80% of the sum of Potassium Chloride, Glutamates, and D-Mannitol).
Marigold Color	Food color	See "Food colors other than chemically synthesized products."		
dl-Menthol	Flavoring agent	All foods.		Only for flavoring.
l-Menthol	Flavoring agent	All foods.		Only for flavoring.
l-Menthyl Acetate	Flavoring agent	All foods.		Only for flavoring.
p-Methylacetophenone	Flavoring agent	All foods.		Only for flavoring.
Methyl Anthranilate	Flavoring agent	All foods.		Only for flavoring.
Methyl Cellulose	Thickening agent	All foods.	2.0%	When used with one or more of the following additives, the total amount shall not be more than 2.0%: Calcium Carboxymethylcellulose, Sodium Carboxymethylcellulose, Sodium Carboxymethylstarch, and Sodium Starch Phosphate.

Substance Name	Major Use Category	Target Foods	Maximum Limit	Other Requirements
Methyl Cinnamate	Flavoring agent	All foods.		Only for flavoring.
Methyl *N*-Methyl-anthranilate	Flavoring agent	All foods.		Only for flavoring.
Methyl β-Naphthyl Ketone	Flavoring agent	All foods.		Only for flavoring.
5-Methylquinoxaline	Flavoring agent	All foods.		Only for flavoring.
Methyl Salicylate	Flavoring agent	All foods.		Only for flavoring.
Monascus Color	Food color	See "Food colors other than chemically synthesized products."		
Monocalcium Di-L-Glutamate	Seasoning	All foods.	1.0% as calcium Not applied to foods approved to be labeled as "special dietary use."	
Morpholine Salts of Fatty Acids	Coating agent	Rinds of fruits or vegetables.		Only as coating agent.
Natamycin	Processing agent	Natural cheese.	less than 0.020 g/kg	Confined to the surface of hard and semi-hard cheeses.
Nicotinamide	Dietary supplement			Not permitted in fresh fish/shellfish (including fresh whale meat) or meat.
Nicotinic Acid	Dietary supplement			Not permitted in fresh fish/shellfish (including fresh whale meat) or meat.
Nitrous Oxide	Propellant	Whip cream products only.		
γ-Nonalactone	Flavoring agent	All foods.		Only for flavoring.
Nordihydroguaiaretic Acid	Antioxidant	Butter. Oil.	0.10 g/kg 0.10 g/kg	
Octanal	Flavoring agent	All foods.		Only for flavoring.
Oxalic Acid	Processing agent			Shall be removed before the completion of the final food.
Palm Oil Carotene	Food color	See "Food colors other than chemically synthesized products."		
Paprika Color	Food color	See "Food colors other than chemically synthesized products."		
l-Perillaldehyde	Flavoring agent	All foods.		Only for flavoring.
Perlite	Processing agent	See "Water-insoluble minerals."		
Phenethyl Acetate	Flavoring agent	All foods.		Only for flavoring.
Phenol Ethers (excluding substances generally recognized as highly toxic)	Flavoring agent	All foods.		Only for flavoring.
Phenols (excluding substances generally recognized as highly toxic)	Flavoring agent	All foods.		Only for flavoring.

Substance Name	Major Use Category	Target Foods	Maximum Limit	Other Requirements
o-Phenylphenol	Antimolding agent	Citrus fruits.	0.010g /kg as maximum residue limit of *o*-phenylphenol	
Piperonal	Flavoring agent	All foods.		Only for flavoring.
Piperonyl Butoxide	Insecticide	Cereal grains.	0.024 g/kg	
Polybutene	Chewing gum base	Chewing gum.		Only as chewing gum base.
Polyisobutylene	Chewing gum base	Chewing gum.		Only as chewing gum base.
Polyvinyl Acetate	Chewing gum base Film-forming agent	Chewing gum. Rinds of fruits and vegetables.		Only as chewing gum base or film-forming agent.
Polyvinylpolypyrrolidone	Filtration aid			Only as filtration aid. Shall be removed before the completion of the final food.
Potassium Bromate	Flour treatment agent	Bread (only products made of wheat flour).	0.030 g/kg of wheat flour	Shall be decomposed or removed before the completion of the final food.
Potassium Carbonate	Raising agent Processing agent			
Potassium Ferrocyanide	Anticaking agent	Salt only.	0.020 g/kg as anhydrous sodium ferrocyanide	When it is used together with either or both of Calcium Ferrocyanide and Sodium Ferrocyanide, the sum of those shall not be more than 0.020 g/kg of salt as anhydrous sodium ferrocyanide.
Potassium Hydrogen Sulfite Solution	Antioxidant Bleaching agent Preservative	Same as for Potassium Pyrosulfite		
Potassium Hydroxide	Processing agent			Shall be neutralized or removed before the completion of the final product.
Potassium Hydroxide Solution	Processing agent			
Potassium Nitrate	Fermentation regulator	Cheese. Sake (rice wine).	0.20 g/L of raw milk 0.10 g/L of raw mash	
	Color fixative	Meat products. Whale meat bacon.	0.070 g/kg as maximum residue limit of NO_2 0.070 g/kg as maximum residue limit of NO_2	

Substance Name	Major Use Category	Target Foods	Maximum Limit	Other Requirements
Potassium Norbixin (Annato, water-soluble)	Food color			Not permitted in fresh fish/shellfish (including whale meat), *konbu* (kelp)/*wakame* (both Laminariales algae) and their similar products, legumes/pulses, meat, *nori* (laver) and its similar products (except when gold is used on *nori*), tea leaves, or vegetables.
Potassium Pyrosulfite	Antioxidant Bleaching agent Preservative		as maximum residue limit of SO_2 less than:	Not permitted in legumes/pulses, or sesame seeds, or vegetables.
		Amanattoh (dried candied beans).	0.10 g/kg	
		Candied cherries.[1]	0.30 g/kg	1. Candied cherries: candied, pitted cherries, which are frosted with crystal sugar or immersed in syrup.
		Dijon mustard.[2]	0.50 g/kg	
		Dried fruits (excluding raisin).	2.0 g/kg	
		Dried potato.	0.50 g/kg	2. Dijion mustard: mustard obtained by grinding and filtering mustard seeds.
		Food molasses.	0.30 g/kg	
		Frozen raw crab.	0.10 g/kg of shelled crab	3. For "other foods," when a food product is produced using foods listed in the left column that meet the corresponding use standards and when the product contains 0.30 g/kg or more of SO_2 residue, the amount of residue shall be applied to the product.
		Fruit wines (all kinds of wines) (excluding squeezed fruit juice containing 1% or more alcohol of by volume that is used for fruit wine, and its concentrates).	0.35 g/kg	
		Gelatin.	0.50 g/kg	
		Kanpyo (dried gourd strips).	5.0 g/kg	
		Konnyakuko (powdered konjac).	0.90 g/kg	
		Other miscellaneous alcoholic beverages.	0.35 g/kg	
		Mizuame (starch syrup).	0.20 g/kg	
		Molasses.	0.30 g/kg	
		Natural fruit juice (confined to products to be consumed in 5-fold or more).	0.15 g/kg	
		Prawn.	0.10 g/kg of shelled crab	
		Raisin.	1.5 g/kg	
		Simmered beans.	0.10 g/kg	
		Tapioca starch for saccharification.	0.25 g/kg	

Substance Name	Major Use Category	Target Foods	Maximum Limit	Other Requirements
Potassium Pyrosulfite (continued)		Other foods (excluding cherries used for candied cherry, hop used for brewing beer, fruit juice used for manufacturing fruit wine, and squeezed fruit juice containing 1% or more alcohol of by volume that is used for fruit wine, and its concentrates).[3]	0.030 g/kg	
Potassium Sorbate	Preservative		as sorbic acid	
		Amazake (beverages made from rice fermented using *koji (A. oryzae)*, confined to products to be consumed in 3-fold or more dilution).	0.30 g/kg	
		An (sweetened bean paste).	1.0 g/kg	
		Candied cherries.	1.0 g/kg	
		Cheese.[1]	3.0 g/kg	1. For cheese, when the additive is used with Propionic Acid, Calcium Propionate, or Sodium Propionate, the total amount of them as propionic acid and as sorbic acid shall not be more than 3.0 g/kg.
		Dried fish/shellfish (excluding smoking cuttlefish and octopus).	1.0 g/kg	
		Dried prune.	0.50 g/kg	
		Fermented milk (as raw materials for lactic acid bacterial drinks).	0.30 g/kg	
		Fish-paste products (excluding surimi).	2.0 g/kg	
		Flour paste.	1.0 g/kg	
		Fruit juice for confections.	1.0 g/kg	
		Fruit paste for confections.	1.0 g/kg	
		Fruit wines (all kinds of wines).	0.20 g/kg	
		Other miscellaneous alcoholic beverages.	0.20 g/kg	
		Gnocchi.[2]	1.0 g/kg	2. Gnocchi: cooked dumplings made of boiled potatoes or wheat flour.
		Jams.	1.0 g/kg	
		Kasu-zuke (lees-pickled foods).	1.0 g/kg	
		Ketchup.	0.50 g/kg	
		Koji-zuke (*koji* (*A. oryzae*)-pickled foods).	1.0 g/kg	
		Lactic acid bacterial beverages (excluding sterilized beverages).	0.050 g/kg	

Substance Name	Major Use Category	Target Foods	Maximum Limit	Other Requirements
Potassium Sorbate (continued)		Lactic acid bacterial beverages (as ingredients of lactic acid bacterial beverages, excluding sterilized beverages).	0.30 g/kg	
		Margarine.[3]	1.0 g/kg	3. For margarine, when the additive is used with Benzoic Acid or Sodium Benzoate, the total amount of them as benzoic acid and as sorbic acid shall not be more than 1.0 g/kg.
		Meat products.	2.0 g/kg	
		Miso (fermented soy bean paste).	1.0 g/kg	
		Miso-zuke (*miso*-pickled foods).[4]	1.0 g/kg	
		Salted foods.	1.0 g/kg	4. For *miso-zuke*, when the additive is used, the total amount of Sorbic Acid used in the product, and Sorbic Acid and its salts contained in miso as ingredient shall not be more than 1.0 g/kg.
		Sea urchin products.	2.0 g/kg	
		Shoyu-zuke (soy sauce-pickled foods).	1.0 g/kg	
		Simmered beans.	1.0 g/kg	
		Smoked cuttlefish/squid & octopus.	1.5 g/kg	
		Soup (excluding potage-type soup).	0.50 g/kg	
		Su-zuke (vinegar-pickled foods).	0.50 g/kg	
		Syrup.	1.0 g/kg	
		Takuan-zuke (rice bran-pickled radish).	1.0 g/kg	
		Tare (a dip or sauce mainly for Japanese or Chinese foods).	0.50 g/kg	
		Tsukudani (foods boiled down in soy sauce).	1.0 g/kg	
		Tsuyu (a sauce mainly for Japanese noodles).	0.50 g/kg	
		Whale meat products.	2.0 g/kg	
Preparations of Tar Colors				Not permitted in fish pickles, fresh fish/shellfish (including whale meat), *kasutera* (a type of pound cake), *kinako* (roasted soybean flour), *konbu* (kelp)/*wakame* (both Laminariales algae), legumes/pulses, marmalade, meat, meat pickles, *miso* (fermented soybean paste), noodles (including wantan), *nori* (laver) and its similar products, soy sauce, sponge cakes, tea leaves, vegetables, or whale meat pickles.

Substance Name	Major Use Category	Target Foods	Maximum Limit	Other Requirements
Propanol	Flavoring agent	All foods.		Only for flavoring.
Propionic Acid	Preservative	Same as for Calcium Propionate.		
	Flavoring agent	All foods.		
Propyl Gallate	Antioxidant	Butter.	0.10 g/kg	
		Fats and oils.	0.20 g/kg	
Propyl *p*-Hydroxybenzoate	Preservative	Same as for Butyl *p*-Hydroxybenzoate.		
Propylene Glycol	Quality sustainer Processing agent	Crust of Chinese pastry (s*hao mai*, spring roll, *wonton*, *zaio-z*).	1.2%	
		Smoked cuttlefish.	2.0%	
		Raw noodles.	2.0%	
		Other foods.	0.60%	
Propylene Glycol Alginate	Thickener	All foods.	1.0%	
Purple Corn Color	Food color	See "Food colors other than chemically synthesized products."		
Purple Sweet Potato Color	Food color	See "Food colors other than chemically synthesized products."		
Red Cabbage Color	Food color	See "Food colors other than chemically synthesized products."		
Saccharin	Sweetener	Chewing gum base.	0.050 g/kg	
Sand	Processing agent	See "Water-insoluble minerals."		
Silicon Dioxide (fine)	Processing agent	All foods.	2.0%	Not permitted in breast-milk substitutes or weaning foods.
Silicon Dioxide	Filtration aid	All foods.		Only as filtration aid. Shall be removed before the completion of the final food.
Silicone Resin	Antifoaming agent	All foods.	0.050 g/kg	Only for defoaming.
Sodium Benzoate	Preservative		as benzoic acid	
		Caviar.	2.5 g/kg	
		Fruit paste and fruit juice, including concentrated juice, used for manufacturing confections.	1.0 g/kg	For margarine, when the additive is used with Sorbic Acid or Potassium Sorbate or a preparation containing one of these two additives, the total amount of them as benzoic acid and as sorbic acid shall not be more than 1.0 g/kg.
		Margarine.	1.0 g/kg	
		Nonalcoholic beverages.	0.60 g/kg	
		Soy sauce.	0.60 g/kg	
		Syrup.	0.60 g/kg	

Substance Name	Major Use Category	Target Foods	Maximum Limit	Other Requirements
Sodium Carboxymethylcellulose	Thickener	All foods.	2.0%	When used with one or more of the following additives, the total amount shall not be more than 2.0%: Calcium Carboxymethylcellulose, Methyl Cellulose, Sodium Carboxymethylstarch, and Sodium Starch Phosphate.
Sodium Carboxymethylstarch	Thickener	All foods.	2.0%	When used with one or more of the following additives, the total amount shall not be more than 2.0%: Calcium Carboxymethylcellulose, Methyl Cellulose, Sodium Carboxymethylcellulose, and Sodium Starch Phosphate.
Sodium Chlorite	Bleaching agent	Cherries.		Shall be decomposed or removed before the preparation of the final food.
		Citrus peels for confections.		
		Eggs (for shells).	0.50 g/kg of dipping liquid	
		Fuki (Japanese butterbur).		
Sodium Chlorite Solution	Bleaching agent	Grapes.		
		Peaches.		
		Seasoned *kazunoko* (herring roes, excluding salted products).	0.50 g/kg of dipping liquid	
		Vegetables for raw consumption.	0.50 g/kg of dipping liquid	
Sodium Chondroitin Sulfate	Humectant	Fish sausage.	3.0 g/kg	
		Mayonnaise.	20 g/kg	
		Dressing.	20 g/kg	
Sodium Copper Chlorophyllin	Food color		as copper	
		Agar jelly in *mitsumame* (prepared by mixing agar jelly, cut fruits, green beans, etc. with sugar syrup) packed into cans or plastic-containers.	0.0004 g/kg	
		Candies.	0.020 g/kg	
		Chewing gum.	0.050 g/kg	
		Chocolate.	0.0064 g/kg	* Fruit and vegetable preserves include fruits and vegetables that are dried, salted, pickled foods in vinegar, and preserved in syrup.
		Fish-paste products (excluding surimi).	0.040 g/kg	
		Fruit and vegetable preserves.*	0.10 g/kg	

Substance Name	Major Use Category	Target Foods	Maximum Limit	Other Requirements
Sodium Copper Chlorophyllin (continued)		*Konbu* (kelp), dried. Moist cakes (excluding bread with sweet fillings or toppings). Syrup.	0.15 g/kg of dry kelp 0.0064 g/kg 0.064 g/kg	
Sodium Dehydroacetate	Preservative	 Butter. Cheese. Margarine.	as dehydroacetic acid 0.50 g/kg 0.50 g/kg 0.50 g/kg	
Sodium Erythorbate	Antioxidant only	Foods other than fish paste and bread.		Not permitted for nutritive purposes in fish paste products (excluding *surimi*) and bread. Only for antioxidizing purposes in other foods.
Sodium Ferrocyanide	Anticaking agent	Salt only.	0.020 g/kg as anhydrous sodium ferrocyanide	When it is used together with either or both of Calcium Ferrocyanide and Potassium Ferrocyanide, the sum of those shall not be more than 0.020 g/kg of salt as anhydrous sodium ferrocyanide.
Sodium Hydrogen Sulfite Solution	Antioxidant Bleaching agent Preservative	Same as for Potassium Pyrosulfite.		
Sodium Hydrosulfite	Antioxidant Bleaching agent Preservative	Same as for Potassium Pyrosulfite.		
Sodium Hydroxide	Processing agent			Shall be neutralized or removed before the preparation of the final food.
Sodium Hydroxide Solution	Processing agent			
Sodium Hypochlorite	Bleaching agent Sterilizer			Not permitted in sesame.
Sodium Iron Chlorophyllin	Food color			Not permitted in fresh fish/shellfish (including fresh whale meat), *konbu* (kelp)/*wakame* (both Laminariales algae), legumes/pulses, meat, *nori* (laver), tea leaves, or vegetables.
Sodium Methoxide	Processing agent			Shall be decomposed before the completion of the final product, and then the methanol produced during the decomposition process be removed.
Sodium Nitrate	Color fixative	Same as for Potassium Nitrate.		

Substance Name	Major Use Category	Target Foods	Maximum Limit	Other Requirements
Sodium Nitrite	Color fixative		as maximum residue limit of nitrite	
		Fish ham.	0.050 g/kg	
		Fish sausage.	0.050 g/kg	
		Ikura (salted salmon roes).	0.0050 g/kg	
		Meat products.	0.070 g/kg	
		Sujiko (salted and aged salmon roes).	0.0050 g/kg	
		Tarako (salted cod roes).	0.0050 g/kg	
		Whale meat bacon.	0.070 g/kg	
Sodium Norbixin (Annato, water-soluble)	Food color			Same as for Potassium Norbixin.
Sodium Oleate	Film-forming agent	Rinds of fruits and fruit vegetables.		Only for coating.
Sodium *o*-Phenylphenate	Antimolding agent	Citrus fruits.	0.010 g/kg as maximum residue limit of *o*-phenylphenol	
Sodium Polyacrylate	Thickener	All foods.	0.20%	
Sodium Propionate	Preservative	Same as for Calcium Propionate.		
Sodium Pyrosulfite	Antioxidant Bleaching agent Preservative	Same as for Potassium Pyrosulfite.		
Sodium Saccharin	Sweetener		as maximum residue limit of sodium saccharine less than:	
		An (sweetened bean paste).	0.20 g/kg	The maximum limits given left do not apply to foods approved or recognized to have special dietary use labeling.
		Confections (including liquid-form and powdered-form as ingredients).	0.10 g/kg	
		Edible ices (including liquid-form and powdered-form as ingredients).*	0.30 g/kg	* Edible ices include sherbets, flavored ices, and other similar products).
		Fermented milk (excluding those used as ingredients of lactic acid bacterial beverages).	0.20 g/kg	
		Fermented milk (only for ingredients of lactic acid bacterial beverages).	1.5 g/kg	
		Fish paste.	0.30 g/kg	
		Fish/shellfish (processed, excluding fish paste, *tsukudani* (foods boiled down with soy sauce), pickles, and canned or bottled products).	1.2 g/kg	

Substance Name	Major Use Category	Target Foods	Maximum Limit	Other Requirements
Sodium Saccharin (continued)		Fish/shellfish (canned or bottled processed foods).	0.20 g/kg	
		Flour paste.	0.20 g/kg	
		Ice cream products (including liquid-form and powdered-form as ingredients).	0.20 g/kg	
		Jams.	0.20 g/kg	
		Kasu-zuke (lee-pickled foods).	1.2 g/kg	
		Koji-zuke (*koji* (*A. oryzae*)-pickled foods).	2.0 g/kg	
		Lactic acid bacterial drinks.	0.30 g/kg	
		Lactic acid bacterial drinks as ingredients.	1.5 g/kg	
		Milk drinks.	0.30 g/kg	
		Miso (fermented soybean paste).	0.20 g/kg	
		Miso-zuke (*miso*-pickled foods).	1.2 g/kg	
		Nonalcoholic beverages.	0.30 g/kg	
		Nonalcoholic beverages (powdered).	1.5 g/kg	
		Nonalcoholic beverages (only products consumed in 5-fold or more dilution).	1.5 g/kg	
		Pickles (preserved or pickled foods, excluding those listed in this column).	0.20 g/kg	
		Processed sea weeds.	0.50 g/kg	
		Sauces.	0.30 g/kg	
		Shoyu-zuke (soy sauce-pickled foods).	1.2 g/kg	
		Simmered beans.	0.50 g/kg	
		Soy sauce.	0.50 g/kg	
		Su-zuke (vinegar-pickled foods).	2.0 g/kg	
		Syrup.	0.30 g/kg	
		Takuan-zuke (rice bran-pickled radishes).	2.0 g/kg	
		Tsukudani (foods boiled down with soy sauce).	0.50 g/kg	
		Vinegar.	0.30 g/kg	
		Vinegar (used in 3-fold or more dilution).	0.90 g/kg	
		Other foods.	0.20 g/kg	

Substance Name	Major Use Category	Target Foods	Maximum Limit	Other Requirements
Sodium Starch Phosphate	Processing agent	All foods.	2.0%	When used with one or more of the following additives, the total amount shall not be more than 2.0%: Calcium Carboxymethylcellulose, Methyl Cellulose, Sodium Carboxymethylcellulose, and Sodium Carboxymethylstarch.
Sodium Sulfite	Antioxidant Bleaching agent Preservative	Same as for Potassium Pyrosulfite.		
Sorbic Acid			as sorbic acid	
		Amazake (beverages made from rice fermented using *koji* (*A. oryzae*), confined to products to be consumed in 3-fold or more dilution).	0.30 g/kg	
		An (sweetened bean paste).	1.0 g/kg	
		Candied cherries.	1.0 g/kg	
		Cheese.[1]	3.0 g/kg	1. For cheese, when the additive is used with Propionic Acid, Calcium Propionate, or Sodium Propionate, the total amount of them as propionic acid and as sorbic acid shall not be more than 3.0g/kg.
		Dried fish/shellfish (excluding smoking cuttlefish and octopus).	1.0 g/kg	
		Dried prune.	0.50 g/kg	
		Fermented milk (as raw materials for lactic acid bacterial drinks).	0.30 g/kg	
		Fish-paste products (excluding *surimi*).	2.0 g/kg	
		Flour paste products	1.0 g/kg	
		Fruit wines (all kinds of fruit wines).	0.20 g/kg	
		Other miscellaneous alcoholic beverages.	0.20 g/kg	
		Gnocchi.[2]	1.0 g/kg	2. For gnocchi, see in the section of "Potassium Sorbate."
		Jams.	1.0 g/kg	
		Kasu-zuke (lees-pickled foods).	1.0 g/kg	
		Ketchup.	0.50 g/kg	
		Koji-zuke (*koji* (*A. oryzae*)-pickled foods).	1.0 g/kg	
		Lactic acid bacterial beverages (excluding sterilized beverages).	0.050 g/kg	

Substance Name	Major Use Category	Target Foods	Maximum Limit	Other Requirements
Sorbic Acid (continued)		Lactic acid bacterial beverages (as ingredients of lactic acid bacterial beverages, excluding sterilized beverages).	0.30 g/kg	
		Margarine.[3]	1.0 g/kg	3. For margarine, when the additive is used with Benzoic Acid or Sodium Benzoate, the total amount of them as benzoic acid and as sorbic acid shall not be more than 1.0 g/kg
		Meat products.	2.0 g/kg	
		Miso (fermented soy bean paste).	1.0 g/kg	
		Miso-zuke (*miso*-pickled foods).[4]	1.0 g/kg	
		Salted foods.	1.0 g/kg	
		Sea urchin products.	2.0 g/kg	
		Shoyu-zuke (soy sauce-pickled foods).	1.0 g/kg	4. For *miso-zuke*, when the additive is used, the total amount of Sorbic Acid used in the product, and Sorbic Acid and its salts containing in *miso* as ingredient shall not be more than 1.0 g/kg.
		Simmered beans.	1.0 g/kg	
		Smoked cuttlefish and octopus.	1.5 g/kg	
		Soup (excluding potage-type soup).	0.50 g/kg	
		Su-zuke (vinegar-pickled foods).	0.50 g/kg	
		Syrup.	1.0 g/kg	
		Takuan-zuke (rice bran-pickled radish).	1.0 g/kg	
		Tare (a dip or sauce mainly for Japanese or Chinese foods).	0.50 g/kg	
		Tsukudani (foods boiled down in soy sauce).	1.0 g/kg	
		Tsuyu (a sauce mainly for Japanese noodles).	0.50 g/kg	
		Whale meat products.	2.0 g/kg	
Spirulina Color	Food color	See "Food colors other than chemically synthesized products."		
Sucralose	Sweetener	Chewing gums.	2.6 g/kg	These maximum limits do not apply to foods approved to be labeled as special dietary use. *Applied to dilutions, in the case of concentrated products.
		Confections (excluding chewing gum).	1.8 g/kg	
		Fruit wines (all kinds of wines).*	0.40 g/kg	
		Jams.	1.0 g/kg	
		Lactic acid bacterial beverages.*	0.40 g/kg	
		Milk drinks.*	0.40 g/kg	
		Other miscellaneous alcoholic beverages.*	0.40 g/kg	
		Moist cakes.	1.8 g/kg	

Substance Name	Major Use Category	Target Foods	Maximum Limit	Other Requirements
Sucralose (continued)		Nonalcoholic beverages.* Sake (rice wine).* Sake (compounded).* Sugar substitutes.** Other foods.	0.40 g/kg 0.40 g/kg 0.40 g/kg 12 g/kg 0.58 g/kg	** Products used by directly adding to drinks, such as coffee and tea.
Sulfuric Acid	Processing agent			Shall be neutralized or removed before the completion of the final food.
Sulfur Dioxide	Antioxidant Bleaching agent Preservative	Same as for Potassium Pyrosulfite.		
Terpene Hydrocarbons	Flavoring agent	All foods.		Only for flavoring.
Terpineol	Flavoring agent	All foods.		Only for flavoring.
Terpinyl Acetate	Flavoring agent	All foods.		Only for flavoring.
2,3,5,6-Tetramethylpyrazine	Flavoring agent	All foods.		Only for flavoring.
Thiabendazole	Antimolding agent	 Banana (whole). Banana (pulp). Citrus fruits.	as maximum residue limit 0.0030 g/kg 0.0004 g/kg 0.010 g/kg	
Thioethers (excluding substances generally recognized as highly toxic)	Flavoring agents	All foods.		Only for flavoring.
Thiols (excluding substances generally recognized as highly toxic)	Flavoring agents	All foods.		Only for flavoring.
Titanium Dioxide	Food color			Only for coloring. Not permitted in fish pickles, fresh fish/shellfish (including whale meat) *kasutera* (a type of pound cake), *kinako* (roasted soybean flour), *konbu* (kelp)/*wakame* (both Laminariales algae), legumes/pulses, marmalade, meat, meat pickles, *miso* (fermented soybean paste), noodles (including wantan), *nori* (laver), soy sauce, sponge cakes, tea leaves, vegetables, or whale meat pickles.

Substance Name	Major Use Category	Target Foods	Maximum Limit	Other Requirements
dl-α-Tocopherol	Antioxidant	All foods.		Only for antioxidizing, except when it is included in a preparation of β-Carotene, Vitamin A, Vitamin A Esters of Fatty Acids, or Liquid Paraffin.
Tomato Color	Food color	See "Food colors other than chemically synthesized products."		
Tricalcium Phosphate	Dietary supplement Fermentation aid	All foods.	1.0% as Ca Not applied to foods approved to be labeled as "special. dietary use."	Only when indispensable for manufacturing or processing the food, or when used for nutritive purposes.
2,3,5-Trimethylpyrazine	Flavoring agent	All foods.		Only for flavoring.
Turmeric Oleoresin	Food color	See "Food colors other than chemically synthesized products."		
γ-Undecalactone	Flavoring agent	All foods.		Only for flavoring.
Vanillin	Flavoring agent	All foods.		Only for flavoring.
Water-insoluble Minerals: Acid Clay Activated Acid Clay Bentonite Diatomaceous Earth Kaolin Perlite Sand Talc* Other similar substances	 Processing agent Chewing gum base*	 All foods.	as maximum residue limit 0.50% 5.0% (Applied only when talc is used solely in chewing gum.)	Only when indispensable for manufacturing or processing the food. When two or more of the additives listed in this section are used together, the total of each residue amount shall be 0.50% or less.
Zinc Gluconate	Dietary supplement	Breast-milk substitutes. Foods with health claim.	6.0 mg/L as zinc, when formulated into a standard concentration 15 mg as zinc/ recommended daily intake of each food	Not applied to cases where the additive is used in formulated dried milk under approval by the Minister of Health, Labour and Welfare.
Zinc Sulfate	Dietary supplement	Breast-milk substitutes.	6.0 mg/L as zinc, when formulated into a standard concentration	Not applied to cases where the additive is used in formulated dried milk under approval by the Minister of Health, Labour and Welfare.

G. STANDARDS FOR LABELING

Any food additive offered for sale shall be labeled with the items given below in a conspicuous place on the container or package (or on the wrapping when the product is wrapped for retail sale) in a manner that is easily readable without opening the container or package. The labeling shall be in Japanese and be done using words easily readable and understandable for ordinary buyers and users.

1. Name (limited to the name mentioned in Table 1 for a food additive (excluding additives stated in Table 4) listed in Table 1 based on the Enforcement Regulations under the Food Sanitation Law (Ministry of Health and Welfare Ordinance No. 23, 1948))

2. The date (including the year) preceded by the words indicating "use-by date" for a food additive whose quality may rapidly deteriorate when stored under the specified storing conditions. For a food additive other than those mentioned above, the date (including the year) preceded by the words indicating "date of minimum durability."

3. The address of the manufacturing plant (the address of the business office of the importers for an imported product) and the name of the manufacturer (or the name of the importer for an imported product) (the corporate name shall be declared when the manufacturer or importer is a corporation). The labeling may be replaced with the description of the address and name of the manufacturer and the specific code for the manufacturing plant (characters used for codes shall be limited to Arabic numerals, Roman letters, *hiragana*, *katakana*, or their combination), which has been submitted by the manufacturer to the Minister of Health, Labour and Welfare; or with the description of the address and name of the seller, a statement indicating that said person is the seller of the product, and the description of the specific code for the manufacturing plant, which has been submitted by both seller and manufacturer in their joint names to the Minister of Health, Labour and Welfare.

4. The ingredients and the content of each (percentage by weight), for an additive preparation (excluding additives used for the purpose of flavoring). If an ingredient is vitamin A derivative, the percentage by weight as vitamin A shall be declared.

5. The method of storing which meets the standards, for a food additive for which storage standards are established. In case of food additives for which storage standards are not established, the following labeling may be omitted: the method of storing and a statement to the effect that it should be stored at ordinary temperature.

6. The method of use that meets the standards, for a food additive for which standards for use are established.

7. The words "food additive."

8. A statement to the effect that it was derived from a specified material or ingredient, for a food additive listed in Table 6 based on the Enforcement Regulations under the Food Sanitation Law.

9. The name of the color actually produced in food, preceded by the word "preparation," for a tar color preparation.

10. The percentage by weight or color value of the additive, for an additive for which the labeled content or color value is stipulated in the specifications.

11. The percentage by weight as vitamin A, for a vitamin A derivative.

12. A statement to the effect that the product is an L-phenylalanine compound or that the product contains L-phenylalanine, for Aspartame or a preparation containing the substance.

INDEX

INDEX

Page numbers in **bold type** relate to monograph titles.

B

C

D

E

F

G

H

I

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z

INDEX IN JAPANESE
(Monograph Titles Only)

オ

カ

キ

ク

ケ

コ

サ

シ

ス

セ

ソ

タ

チ

ツ

テ

ト

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ノ

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