

B. GENERAL TESTS

Coloring Matter Tests

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

1. Water-insoluble substances

Hereinafter in the Monographs, such a specification as "not more than 0.20% (Coloring Matter Tests)" 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 it accurately.

Weigh exactly 2.0 g of a sample, add 200 ml of boiling water and 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 it accurately.

2. Chloride and Sulfate

Hereinafter in the Monographs, such a specification as "not more than 5.0 % as the total content (Coloring Matter Tests)" indicates that when determined as directed in the following procedure, the total content of sodium chloride and sodium sulfate is not more than 5.0 %.

Procedure Weigh accurately about 0.1 g of a sample, and dissolve in water to make exactly 100 ml. Use this solution as the test solution. Measure exactly 0.2 ml, 1 ml, 10 ml, and 50 ml of each of the Chloride Ion Standard Stock Solutions and Sulfate Ion Standard Stock Solution, add water to make 100 ml each, and use these solutions as the standard solutions. Proceed as directed under Ion Chromatography under the following operating conditions, using 20 µl of each of the test solution, the standard solutions, and the standard stock solutions. Then determine the peak heights or peak areas of the chloride ion and sulfate ion of 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 of 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: Conductometer.

Packing material of column: Porous anion exchanger.

Column: Stainless or plastic tube of 4.6 - 6.0 mm in internal diameter and 5 - 10 cm in length.

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Guard Column: A column with the same internal diameter and packing material as the above column.

Eluant: The solution of 2.5 mmol/l phthalic acid and 2.4 mmol/l tris(hydroxymethyl) amino methane (pH 4.0).

Temperature: 40 .

Flow rate: 1.5 ml/min.

3. Iodide

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

Procedure Weigh accurately about 30 mg of a sample, and dissolve in water to make exactly 10 ml, and use this solution as the test solution. Measure exactly 0.5 ml, 1 ml, 10 ml, and 50 ml of the Iodide Ion Standard Stock Solution, add water to make exactly 100 ml each, and use these solutions as the standard solutions. Proceed as directed under Ion Chromatography under the same conditions specified under Chloride and Sulfate, using 100 μ l each of the test solution, the standard solutions, and the standard stock solution. Determine the peak heights or peak areas of iodide ion of the standard solutions and the standard stock solutions to make the calibration curve. Determine the peak height or area of iodide ion of 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 immediately after its preparation.

4. Bromides

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

Procedure Weigh accurately about 50 mg of a sample, dissolve in water to make exactly 10 ml, and use this solution as the test solution. Measure exactly 0.5 ml, 1 ml, 10 ml, and 50 ml of Bromide Ion Standard Stock Solution, add water to make exactly 100 ml each, and use these solutions as the standard solution. Proceed as directed in Ion Chromatography under the same conditions specified in Chloride and Sulfate, using 20 μ l each of the test solution, the standard solutions, and the original standard solution. Determine the peak heights or peak areas of bromide ion of the standard solutions and the standard stock solutions to make the calibration curve. Determine the peak height or peak area of bromide ion of 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. Avoid direct sunlight during the procedure, use light-resistant containers for the

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preparation of the test solution, and perform the test immediately after its preparation.

5. Heavy Metals

Hereinafter in the Monographs, such a specification as "not more than 20 $\mu\text{g/g}$ as Pb (Coloring Matter Tests, heavy metals (5))" indicates that when determined as directed in (5) of the following procedures, the content of heavy metals as Pb is not more than 20 $\mu\text{g/g}$.

Procedure Unless otherwise specified, weigh 2.5 g of sample, transfer into a platinum, quartz, or porcelain crucible, moisten with a little quantity of sulfuric acid, heat gradually and almost incinerate at a temperature as low as possible. Add again 1 ml of sulfuric acid, heat gradually until the white fumes of sulfuric acid are no longer evolved. Place in an electric furnace, ignite at 450 - 550 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 4) and 5 ml of water, combine the filtrate and the washings to prepare solution A, add water to make 50 ml. Use this solution as the sample solution for chromium and manganese.

For preparing the sample solution for other heavy metals, proceed as follows: Dry the residue obtained on the filter paper above together with the filter paper at 105 , place in a platinum crucible, and ignite at about 450 to incinerate. Add 2 g of anhydrous sodium carbonate, heat to fuse, cool, add 10 ml of water, and acidify with the drops of hydrochloric acid. Transfer it into a beaker, wash the crucible with a small amount of water, collect the washings into the beaker, stir vigorously, combine with solution A prepared above, add again water to make 50 ml.

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

(1) Zinc

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

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

For the test solution and the control solution, proceed as directed under Atomic Absorption Spectrophotometry under 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

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Test Solution Unless otherwise specified, measure 5.0 ml of the sample solution, add 5 ml of diluted hydrochloric acid (1 : 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 : 4), and water to make 50 ml.

For the test solution and control solution, proceed as directed under Atomic Absorption Spectrophotometry under 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, add 10 ml of diluted hydrochloric acid (1 : 4) and water to make 50 ml.

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

For the test solution and the control solution, proceed as directed under Atomic Absorption Spectrophotometry under 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.

Supportive gas: Air.

Combustible gas: Acetylene.

(4) Manganese

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

Control Solution Measure 4.0 ml of the blank test solution, add 1.0 ml of Manganese Standard Solution, add 10 ml of diluted hydrochloric acid (1 : 4) and water to make 50 ml.

For the test solution and the control solution, proceed as directed under Atomic Absorption Spectrophotometry under 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.

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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 dropwise ammonia TS until the solution changes to pink color and add 2 ml of diluted acetic acid (1 : 4). Filter through a filter paper if necessary, wash the filter paper with water, add water to make 50 ml.

Control Solution Measure 20 ml of the blank test solution, place in 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.

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**

Hereinafter in the Monographs, such a specification as "not more than 4.0 $\mu\text{g/g}$ as As_2O_3 (Coloring Matter Tests)" indicates that when determined as directed in the following procedure, the content of arsenic as As_2O_3 is not more than 4.0 $\mu\text{g/g}$.

Procedure

Test Solution Weigh exactly 0.50 g of a sample, transfer into a quartz or porcelain crucible, add 20 ml of the solution of magnesium nitrate in ethanol (1 : 50), ignite ethanol, and heat gradually to incinerate at 450 - 550 °C. If a carbonized material still remains, moisten with 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, add water to make 25 ml.

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

Perform tests for the test solution and control solution by the method using Apparatus C as directed under the Arsenic Limit Test. The absorbance of Test Solution is not more than the absorbance of Standard Solution.

7. **Other Coloring Matters**

Hereinafter in the Monographs, such a specification as "(Coloring Matter Tests, Other Coloring Matters (1))" indicates that the test is to be performed as directed in (1) of the following procedure.

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

(2) Proceed as directed in (1) using a mixture of 25% ethanol and 5% ammonia

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solution (1:1) as a developing solvent.

(3) Weigh 0.30 g of a sample, 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 mixture of acetone, isoamyl acetate, isoamyl alcohol, water and propionic acid (20:13:5:5:2) as a developing solvent. When the developing solvent ascends about 30 cm, stop the development.

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

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

Standard Solution Dry the specified subsidiary colors for 24 hours in a vacuum desiccator, weigh 10.0 mg of each color, dissolve in the specified solution to make exactly 100ml, respectively. Use them as the standard stock solutions, respectively. Measure the specified amount of each standard stock solution, place the plural solutions in a flask, and add the specified solution to make exactly a 100ml-solution

Procedure Measure 20 μ l of each of the test solution and standard solution. For each solution, perform Liquid Chromatography under the operating conditions below. Then, measure the peak area for each of the standard solutions, and make a calibration curve for each coloring matter. Measure the peak area of each subsidiary color in the test solution. Obtain the content of each color using the calibration curves, and calculate total amount of the subsidiary colors.

Operating Conditions

Detector: Visible range absorption detector (measurement wavelength directed in the individual monograph).

Column packing material: 5- μ m octadecylsilylated silica gel.

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

Column temperature: 30 .

Flow rate: 1 ml/min.

9. Unreacted raw materials and products of side reactions

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

Standard Solution Dry the specified unreacted raw materials and products of side reactions for 24 hours in a vacuum desiccator, weigh 10.0 mg of each substance, dissolve in the specified solution to make exactly 100ml, respectively. Use them as standard stock solutions, respectively. Measure 5.0 ml of each standard stock solution, place these solutions in a flask together, add the specified solution to make exactly 100ml. Similarly, taking 2.0 ml of each and 1.0 ml of each, prepare two separate standard solutions in the same manner.

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Procedure Measure 20 μl of each of the test solution and standard solutions. For each solution, perform Liquid Chromatography under the operating conditions below. Then, measure the peak area for each standard solution, and make a calibration curve for each substance. Measure the peak area of each unreacted raw materials and products of side reactions in the test solution. Obtain the content of each unreacted raw material and reaction intermediate using the calibration curve.

Operating Conditions

Detector: Ultraviolet region absorption detector (measuring wavelength: directed in the Monographs).

Column packing material: 5- μm octadecylsilanized silica gel.

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

Column temperature: 30 .

Flow rate: 1 ml/min.

10. Un sulfonated primary aromatic amines

(1) Hereinafter in the Monographs, such a specification as "not more than 0.010 % as aniline (Coloring Matter Tests)" indicates that when determined as directed in the following procedure, the content of un sulfonated primary aromatic amines is not more than 0.010 % as aniline.

Procedure *Test Solution* Weigh accurately about 2 g of the sample, transfer into a separating funnel containing 100 ml of water. Add 50 ml of water to dissolve. Add 5 ml of sodium hydroxide solution (4 100) and 50 ml of ethyl acetate, shake and extract. Separate the ethyl acetate layer. Add 50 ml of ethyl acetate into the water layer, shake and extract. Combine two ethyl acetate layers, wash with sodium hydroxide solution (4 1000) until the color of the solution disappears. Extract un sulfonated primary aromatic amines three times from the ethyl acetate solution using three 10-ml portions of diluted hydrochloric acid (3 10). Combine three hydrochloric acid layers, add water to make exactly 100 ml. Use this solution as solution A. Transfer 10 ml of solution A into a test tube, allow to stand for 10 minutes in ice. Add 1 ml of potassium bromide solution (1 2) and 0.05 ml of sodium nitrite solution (1 30), mix, and allow to stand for 10 minutes in ice. Transfer this mixed solution using water into a 25-ml volumetric flask containing 1 ml of 0.05 mol/l disodium salt of 3-hydroxy-2,7-naphthalenedisulfonic acid solution and 10 ml of sodium carbonate solution (1 10), and add water to make exactly 25 ml. Allow to stand for 15 minutes in a dark place.

Control Solution Weigh 10.0 mg of aniline, add 30 ml of diluted hydrochloric acid (3 10), and dissolve. Add water to make exactly 100 ml. Measure 2.0 ml of this solution, and add diluted hydrochloric acid (1 10) to make 100 ml. Proceed for his solution in the same manner as directed for the test solution.

Reference Solution To measure the absorbance of the test solution, use the

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following reference solution: Transfer 10 ml of solution A into a 25-ml volumetric flask, add 1 ml of 0.05 mol/l disodium salt of 3-hydroxy-2,7-naphthalenedisulfonic acid solution and 10 ml of sodium carbonate solution (1 : 10), and add water to make exactly 25 ml. To measure the absorbance of the control solution, use the following reference solution: To 10 ml of diluted hydrochloric acid (1 : 10), add 1 ml of 0.05 mol/l disodium salt of 3-hydroxy-2,7-naphthalenedisulfonic acid solution and 10 ml of sodium carbonate solution (1 : 10), and add water to make exactly 25 ml.

The absorption of the test solution at 510 nm is not more than that of the control solution.

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

Procedure *Test Solution* Weigh accurately about 1 g of a 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 : 100) and 50 ml of ethyl acetate, shake and extract. Separate the ethyl acetate layer. Add 50 ml of ethyl acetate into the water layer, shake and extract. Combine two ethyl acetate layers, wash with sodium hydroxide solution (4 : 1000) until the color of the solution disappears. To the ethyl acetate layer, add 0.5 ml of diluted sulfuric acid (0.15 : 1000). Dry under pressure at 45 °C, immediately add 1.0 ml of a mixture of same volume-portions of monosodium dihydrogen phosphate solution (0.3 : 100) and methanol.

Standard Solution Weigh 10 mg of -naphthylamine, and dissolve in 3 ml of diluted hydrochloric acid (3 : 10). Add water to make exactly 10 ml, and use the solution as the standard stock solution. To 1.0 ml of the standard stock solution, add ammonium acetate solution (1.54 : 1000) to make exactly 100 ml. To 1.0 ml, 2.0 ml, 5.0 ml and 10.0 ml of this solution, add the mobile phase directed in the operating conditions below to make exactly 100 ml, respectively.

With 100 µl of each of the test solution and standard solution, perform Liquid Chromatography under the operating conditions below. Measure the peak areas of the standard solutions, and make a calibration curve. Then, measure the area of the peak for the test solution appearing at the retention time of -naphthylamine, and calculate the content of -naphthylamine using the calibration curve.

Operating Conditions

Detector: Ultraviolet region absorbance detector (measuring wavelength: 304 nm).

Column packaging material: 5-µm octadecylsilanized silica gel.

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

Column temperature: 40 °C.

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Mobile phase: To 500 ml of methanol, add ammonium acetate solution (1.54 1000) to make 1000 ml.

Flow rate: 1 ml/min.

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

Procedure *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 (4 100) and 50 ml of ethyl acetate, shake and extract. Separate the ethyl acetate layer. Add 50 ml of ethyl acetate to the water layer, shake and extract. Combine two ethyl acetate layers, wash with sodium hydroxide solution (4 1000) until the color of the solution disappears. To this ethyl acetate layer, add 0.5 ml of diluted sulfuric acid (0.15 1000). Dry under pressure at 45 °C, and immediately add 1.0 ml of a mixture of equal volumes of monosodium dihydrogen phosphate solution (0.3 100) and methanol.

Standard Solution Weigh 100 mg of *p*-cresidine, dissolve in 30 ml of diluted hydrochloric acid (3 10). Add water to make exactly 100 ml, and use the solution as the standard stock solution. To 10 ml of the standard stock solution, add ammonium acetate solution (1.54 1000) to make exactly 100 ml. To 1.0 ml, 2.0 ml, 5.0 ml and 10.0 ml of this solution, add the mobile phase directed in following operating conditions to make exactly 100 ml, respectively.

With 100 µl each of the test solution and the standard solutions, perform Liquid Chromatography under the operating conditions below. Measure the peak areas of standard solutions, and make a calibration curve. Measure the area of the peak appearing at the retention time of *p*-cresidine, and calculate the content of *p*-cresidine using the calibration curve.

Operating Conditions

Detector: Ultraviolet region absorbance detector (measuring wavelength: 290 nm).

Column packaging material: 5-µm octadecylsilanized silica gel.

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

Column temperature: 40 °C.

Mobile phase: To 400 ml of methanol, add ammonium acetate solution (1.54 1000) to make 1000 ml.

Flow rate: 1 ml/min.

11. Assay

(1) Titanium Trichloride Method

(i) Measure exactly the specified quantity of the test solution, transfer into a

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500-ml wide-mouthed Erlenmeyer flask, add 15 g of sodium citrate and water to make about 200 ml. While passing carbon dioxide gas through this solution and vigorously boiling this solution, titrate with 0.1 mol/l titanium trichloride. The end point of the titration is the time when the proper 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. Use 10 ml of diluted Light Green SF Yellow (1 : 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 end point of the titration is the time when the proper color of the sample disappears and an orange color appears.

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