Analytical Methods for Residual Compositional Substances of Agricultural Chemicals, Feed Additives, and Veterinary Drugs in Food*

Department of Food Safety
Ministry of Health, Labour and Welfare

* This document is an annex of the Director Notice about Analytical Methods for Residual Compositional Substances of Agricultural Chemicals, Feed Additives, and Veterinary Drugs in Food (Syoku-An No.0124001, January 24, 2005. Final amendments were made on May 26, 2006.).

General Rules

1. Terminology

(1) The term "Substances to be analyzed" refers to substances that should be analyzed using the analytical methods stipulated in this document. The relevant compositional substances of agricultural chemicals, feed additives or veterinary drugs (hereinafter referred to as "agricultural chemicals") (including substances formed through chemical reactions with these substances) and any related substances (such as their salts, or optical isomers) are listed in the Tables in item 6 (1), item 7 (1), and item 9 (1), Section A *General Compositional Standards for Food*, Part 1 *Food* under the *Specifications and Standards for Food, Food Additives, Etc.*(Ministry of Health and Welfare Notification No. 370, 1959; hereinafter referred to as "the Notification No. 370")

(2) The term "Analytical Value" means a value taken from a food listed in the Notification No. 370 that will be compared with the maximum residue limit of the substances to be analyzed of the agricultural chemicals.

(3) The term "Nuts and Seeds" refers to oil seeds, nuts, cacao beans, and coffee beans.

(4) The term "Limit of quantitation" refers to the minimum amount or concentration of a substance to be analyzed from a sample with which the amount or concentration of the target substance can be determined. For chromatography, the substances to be analyzed which show the ratio of S (peak height) / N (baseline noise) = 10 are represented as the concentrations of compositional substances of agricultural chemicals.

(5) The word "Type" refers to the source of the testing method used for analysis, which is classified as follows:

- A: Official analytical methods stipulated by Ministerial Ordinance Concerning Composiotional Standards, Etc. for Milk and Milk Products (Ministry of Health and Welfare Ordinance No. 52, 1951), Notification No. 370 and announcement (excluding type C)
- B: Analytical methods stipulated by the governmental organizations, etc., of foreign countries (excluding Type A)
- C: Analytical methods established by the investigational commission of experts in Japan
- D: Analytical methods described in the references (excluding types A to C)

2. Reagents

Reagents used for analyses by the testing methods pertain specifically to those listed in this Notice in Section C *Reagents*, Part II *Food Additives* in the Notification No. 370 or the following attachment.

Reagents designated as "special grade" in this Notice must meet the requirements for "special grade" specified in the Japan Industrial Standards for the reagents.

3. Samples

Unless otherwise specified, a sample to be used for analysis by the method stipulated in this document should be prepared according to the following steps.

(1) For grains, beans, nuts and seeds, pulverize a sample into particles that can be passed through a 420-μm standard sieve.

(2) For fruits, vegetables and herbs, precisely weigh out approximately 1 kg to be used as the sample, cut into small pieces, and homogenize. If necessary, add a sufficient amount of water during homogenization.

(3) For tea and hops, pulverize a sample into particles that can be passed through a 420-μm standard sieve. For tea types other than green powdered tea, homogenize the pulverized samples.

(4) For spices, follow the sample preparation method for “nuts and seeds” or “fruits” according to the shape of the sample.

(5) For animal muscle, remove the fat from the sample as much as possible, cut into small pieces, and homogenize.

(6) For animal fat, remove the muscle from the sample as much as possible, cut it into small pieces, and homogenize.

(7) For animal liver, kidney, or other edible offal, cut the sample into small pieces and homogenize.

(8) For milk and honey, homogenize a sample by thoroughly mixing.

(9) For fish, cut edible parts into small pieces and homogenize.

(10) For shellfish, remove the shell from the sample, cut the meat into small pieces, and homogenize.

(11) For crustaceans, cut the whole into small pieces for a small sample or cut into small pieces after removing the outrmost shell for a large sample, and homogenize..

(12) For eggs, use only the white and york of the sample. Mix thoroughly, and homogenize the mixture (except if a maximum residue limit in the white or york is established).

4. Precautions during Analysis

(1) To perform an analysis using a method not stipulated in this Notice, the method should have an accuracy, precision or limit of quantitation comparable to or higher than that of the corresponding analytical method stipulated in this Notice, and specificity.

(2) To obtain an analytical value, the number of decimal places of the measured value should be one more than that of the corresponding maximum residue limit. Round off the measured value to the last decimal place of the maximum residue limit.

(Attachment)

Acrylamide copolymer bonded glycerylpropylsilanized silica gel mini column (360 mg)

360 mg of acrylamide copolymer bonded glycerylpropylsilanized silica gel is packed in a polyethylene column tube with an inner diameter of 8 to 9 mm or a column with comparable separation efficiency.

Acetonitrile

Use a rotary vacuum evaporator on 300 mL of acetonitrile to concentrate to the point of dryness. Dissolve the residue in 5 mL of *n*-hexane. For analysis, inject 5 μL of the solution into a gas chromatograph equipped with an electron capture detector. Peaks other than that of *n*-hexane in the resulting chromatogram should be as high as or lower than the peaks of γ-BHC at 2×10^{-11} g.

Acetone

Use a rotary vacuum evaporator on 300 mL of acetone to concentrate to the point of dryness. Dissolve the residue in 5 mL of *n*-hexane. For analysis, inject 5 μL of the solution into a gas chromatograph equipped with an electron capture detector. Peaks other than that of *n*-hexane in the resulting chromatogram should be as high as or lower than the peaks of γ-BHC at 2×10^{-11} g.

Aminopropylsilanized silica gel mini column (360 mg)

360 mg of aminopropylsilanized silica gel is packed in a polyethylene column tube with an inner diameter of 8 to 9 mm or a column with comparable separation efficiency.

Aminopropylsilanized silica gel mini column (500 mg)

500 mg of aminopropylsilanized silica gel is packed in a polyethylene column tube with an inner diameter of 8 to 9 mm or a column with comparable separation efficiency.

Aminopropylsilanized silica gel mini column (1,000 mg)

1,000 mg of aminopropylsilanized silica gel is packed in a polyethylene column tube with an inner diameter of 12 to 13 mm or a column with comparable separation efficiency.

Sodium sulfite

Sodium sulfite (special grade)

Potassium sulfite

Potassium sulfite (special grade)

Argon

Purity of 99.998 v/v% or higher

Isopropylether

Isopropylether (special grade)

Ethanol

Use a rotary vacuum evaporator on 300 mL of ethanol to concentrate to the point of dryness. Dissolve the residue in 5 mL of *n*-hexane. For analysis, inject 5 μL of the solution into a gas chromatograph equipped with an electron capture detector. Peaks other than that of *n*-hexane in the resulting chromatogram should be as high as or lower than the peaks of γ-BHC at 2×10^{-11} g.

Ethylsilanized silica gel mini column (1,000 mg)

1,000 mg of ethysilanized silica gel is packed in a polyethylene column tube with an inner diameter of 12 to 13 mm or a column with comparable separation efficiency.

3 mol/L Ether solution of ethylmagnesiumbromide

3 mol/L Ether solution of ethylmagnesiumbromide

Ethylenediamine-*N*-propylsilanized silica gel mini column (500 mg)

500 mg of ethylenediamine-*N*-propylsilanized silica gel is packed in a polyethylene column tube with an inner diameter of 8 to 9 mm or a column with comparable separation efficiency.

Ether

Use a rotary vacuum evaporator on 300 mL of ether to concentrate to the point of dryness. Dissolve the residue in 5 mL of *n*-hexane. For analysis, inject 5 μL of the solution into a gas chromatograph equipped with an electron capture detector. Peaks other than that of *n*-hexane in the resulting chromatogram should be as high as or lower than the peaks of γ-BHC at 2×10^{-11} g.

Ferrous chloride

Ferrous chloride (special grade)

Sodium chloride

Sodium chloride (special grade). If the reagent is found to include any substance that may interfere with the analysis of substances to be analyzed from agricultural chemicals, wash with a solvent such as *n*-hexane before use.

Basic alumina mini column (1,710 mg)

1,710 mg of basic alumina is packed in a polyethylene column tube with an inner diameter of 8 to 9 mm or a column with comparable separation efficiency.

Guanidine hydrochloride

Guanidine hydrochloride (special grade)

Pyridine hydrochloride

Pyridine hydrochloride (special grade). If the reagent is found to include any substance that may interfere with the analysis of target compositional substances from agricultural chemicals, wash with a solvent such as *n*-hexane before use.

Octadecylsilanized silica gel mini column (360 mg)

360 mg of octadecylsilanized silica gel is packed in a polyethylene column tube with an inner diameter of 8 to 9 mm or a column with comparable separation efficiency.

Octadecylsilanized silica gel mini column (500 mg)

500 mg of octadecylsilanized silica gel is packed in a polyethylene column tube with an inner diameter of 8 to 9 mm or a column with comparable separation efficiency.

Octadecylsilanized silica gel mini column (850 mg)

850 mg of octadecylsilanized silica gel is packed in a polyethylene column tube with an inner diameter of 8 to 9 mm or a column with comparable separation efficiency.

Octadecylsilanized silica gel mini column (1,000 mg)

1,000 mg of octadecylsilanized silica gel is packed in a polyethylene column tube with an inner diameter of 12 to 13 mm or a column with comparable separation efficiency.

Octadecylsilanized silica gel mini column (light shielded, 1,000 mg)

1,000 mg of octadecylsilanized silica gel is packed in a polyethylene column tube, with an inner diameter of 12 to 13 mm, that is wrapped with a light-shielding material. A column with comparable separation efficiency can also be used.

Octadecylsilanized silica gel mini column (5,000 mg)

5,000 mg of octadecylsilanized silica gel is packed in a polyethylene column tube with an inner diameter of 19 mm or a column with comparable separation efficiency.

Trimethyl-ortho-formate

Trimethyl-ortho-formate (first grade)

Trimethyl-ortho-acetate

Purity of 98% or higher

Sodium perchlorate

Sodium perchlorate (special grade)

Sodium peroxide

Sodium peroxide (special grade)

Active carbon

Active carbon (for chromatography)

Active carbon mini column (500 mg)

500 mg of graphite carbon is packed in a polyethylene column tube with an inner diameter of 12 to 13 mm or a column with comparable separation efficiency.

Glass fiber filter paper

Glass fiber filter paper for chemical analysis

Alumina for column chromatography (basic)

Alumina made for column chromatography (basic, particle size of 50 to 200 μm).

Alumina for column chromatography (neutral)

Alumina made for column chromatography (neutral, particle size of 63 to 200 μm).

Synthetic magnesium silicate for column chromatography

Heat synthetic magnesium silicate made for column chromatography (particle size of 150 to 250 μm) at 130°C for 12 hours or longer. Cool down to room temperature in a desiccator.

Silica gel for column chromatography (particle size of 63 to 200 μm)

Heat silica gel made for column chromatography (particle size of 63 to 200 μm) at 130°C for 12 hours or longer. Cool down to room temperature in a desiccator.

Silica gel for column chromatography (particle size of 150 to 425 μm)

Heat silica gel made for column chromatography (particle size of 150 to 425 μm) at 130°C for 12 hours or longer. Cool down to room temperature in a desiccator.

Column medium

Perform acid treatment and silane finish on diatomaceous earth made for gas chromatography (particle size of 150 to 177 μm)

Carboxymethylsilanized silica gel mini column (1,000 mg)

1,000 mg of carboxymethylsilanized silica gel is packed in a polyethylene column tube with an inner diameter of 10 to 12 mm or a column with comparable separation efficiency.

Ammonium citrate

Ammonium citrate (dibasic) (special grade)

Tripotassium citrate

Tripotassium citrate (special grade)

Graphite carbon/aminopropylsilanized silica gel layered mini column (500 mg/500 mg)

500 mg of graphite carbon and 500 mg of aminopropylsilanized silica gel are packed in two layers in a polyethylene column tube with an inner diameter of 12 to 13 mm or a column with comparable separation efficiency.

Glycerylpropylsilanized silica gel mini column (360 mg)

360 mg of glycerylpropylsilanized silica gel is packed in a polyethylene column tube with an inner diameter of 8 to 9 mm or a column with comparable separation efficiency.

β-Glucosidase

Requires the activity to liberate 4 to 12 μmol/min of glucose from salicin at pH 5.0 at 37°C per 1 mg β-glucosidase.

***m*-Chloroperbenzoic acid**

Purity of 70% or higher

Chloroform

Use a rotary vacuum evaporator on 300 mL of chloroform to concentrate to the point of dryness. Dissolve the residue in 5 mL of *n*-hexane. For analysis, inject 5 μL of the solution into a gas chromatograph equipped with an electron capture detector. Peaks other than that of *n*-hexane in the resulting chromatogram should be as high as or lower than the peaks of γ-BHC at 2×10^{-11} g.

Diatomaceous earth

Diatomaceous earth for chemical analysis

High purity nitrogen

Purity of 99.999 v/v% or higher

Synthetic magnesium silicate mini column (900 mg)

900 mg of synthetic magnesium silicate is packed in a polyethylene column tube with an inner diameter of 8 to 9 mm or a column with comparable separation efficiency.

Synthetic zeolite

Synthetic zeolite with a pore size of 0.3 nm

Ammonium acetate

Purity of 97% or higher

Ethyl acetate

Use a rotary vacuum evaporator on 300 mL of ethyl acetate to concentrate to the point of dryness. Dissolve the residue in 5 mL of *n*-hexane. For analysis, inject 5 μL of the solution into a gas chromatograph equipped with an electron capture detector. Peaks other than that of *n*-hexane in the resulting chromatogram should be as high as or lower than the peaks of γ-BHC at 2 x 10^{-11} g.

Borontrifluoride etherate complex

Purity of 99% or higher

Diethylene glycol

Purity of 98% or higher

Diethylene glycol monoethyl ether

Purity of 99% or higher

Cyclohexylsilanized silica gel mini column (light shielded, 1,000 mg)

1,000 mg of cyclohexylsilanized silica gel is packed in a polyethylene column tube, with an inner diameter of 12 to 13 mm, that is wrapped with a light-shielding material. A column with comparable separation efficiency may also be used.

Cyclohexylsilanized silica gel mini column (2,000 mg)

2,000 mg of cyclohexylsilanized silica gel is packed in a polyethylene column tube with an inner diameter of 15 to 16 mm or a column with comparable separation efficiency.

Cyclohexylsilanized silica gel mini column (light shielded, 2,000 mg)

2,000 mg of cyclohexylsilanized silica gel is packed in a polyethylene column tube, with an inner diameter of 15 to 16 mm, that is wrapped with a light-shielding material. A column with comparable separation efficiency may also be used.

Dichloromethane

Use a rotary vacuum evaporator on 300 mL of dichloromethane to concentrate to the point of dryness. Dissolve the residue in 5 mL of *n*-hexane. For analysis, inject 5 μL of the solution into a gas chromatograph equipped with an electron capture detector. Peaks other than that of *n*-hexane in the resulting chromatogram should be as high as or lower than the peaks of γ-BHC at 2 x 10^{-11} g.

Dichloromethane (Special Grade)

Dichloromethane (special grade)

Dichlorodimethylsilane

Purity of 98% or higher

Dibutylhydroxytoluene

Dibutylhydroxytoluene (special grade)

***p*-Dimethylaminobenzaldehyde**

p-Dimethylaminobenzaldehyde (special grade)

Silicon antifoam

Silicon processed for antifoaming

Silica gel mini column (500 mg)

500 mg of silica gel made for column chromatography is packed in a polyethylene column tube with an inner diameter of 8 to 9 mm or a column with comparable separation efficiency.

Silica gel mini column (690 mg)

690 mg of silica gel made for column chromatography is packed in a polyethylene column tube with an inner diameter of 8 to 9 mm or a column with comparable separation efficiency.

Silica gel mini column (light shielded, 690 mg)

690 mg of silica gel made for column chromatography is packed in a polyethylene column tube, with an inner diameter of 8 to 9 mm, that is wrapped with a light-shielding material. A column with comparable separation efficiency may also be used.

Silica gel mini column (1,000 mg)

1,000 mg of silica gel made for column chromatography is packed in a polyethylene column tube with an inner diameter of 8 to 9 mm or a column with comparable separation efficiency.

Sodium borohydride

Purity of 98% or higher

Styrene-divinylbenzene copolymer column

Styrene-divinylbenzene copolymer made for gel permeation chromatography is packed in a stainless steel column tube with an inner diameter of 20 mm and a length of 300 mm. A column with comparable separation efficiency may also be used.

Styrene-divinylbenzene copolymer mini column (265 mg)

265 mg of styrene-divinylbenzene copolymer is packed in a polyethylene column tube with an inner diameter of 8 to 9 mm or a column with comparable separation efficiency.

Sulfamic acid

Sulfamic acid (special grade)

Cellulase

Requires the activity to liberate 29 μmol/min of glucose from cellulose at pH 5.0 at 37°C per 1 mg cellulase.

Porous diatomaceous earth column (20 mL capacity)

A fraction of granular porous diatomaceous earth made for column chromatography at a 20-mL capacity, is packed in a polyethylene column tube with an inner diameter of 20 to 30 mm or a column with comparable separation efficiency.

Neutral alumina mini column (1,710 mg)

1,710 mg of neutral alumina is packed in a polyethylene column tube with an inner diameter of 9 to 10 mm or a column with comparable separation efficiency.

Tetrahydrofuran

Tetrahydrofuran (special grade)

Sodium dodecyl sulfate

Purity of 85% or higher

Triethylamine

Triethylamine (special grade)

Trisodium pentacyanoamine ferroate

Trisodium pentacyanoamine ferroate (special grade)

2,2,2-Trifluoroethanol

2,2,2-Trifluoroethanol (special grade)

Trimethylaminopropylsilanized silica gel mini column (500 mg)

500 mg of trimethylaminopropylsilanized silica gel is packed in a polyethylene column tube with an inner diameter of 8 to 9 mm or a column with comparable separation efficiency.

Trimethylaminopropylsilanized silica gel mini column (1,000 mg)

1,000 mg of trimethylaminopropylsilanized silica gel is packed in a polyethylene column tube with an inner diameter of 6 to 9 mm or a column with comparable separation efficiency.

Trimethylaminopropylsilanized silica gel/benzenesulfonic-silanized silica gel mixture mini column (200 mg)

200 mg of a mixture of trimethylaminopropylsilanized silica gel and benzenesulfonic-silanized silica gel is packed in a polyethylene column tube with an inner diameter of 8 to 9 mm. A column with comparable separation efficiency may also be used.

Sodium benzenthiolate

Purity of 98% or higher

***o*-Nitrobenzaldehyde**

o-Nitrobenzaldehyde (special grade)

Lactic acid

Lactic acid (special grade)

Phenolphthalein test solution

1 g of phenolphthalein dissolved in 100 mL of ethanol.

***o*-Phthalaldehyde**

Purity of 99% or higher

Potassium fluoride

Potassium fluoride (special grade)

Propylsulphonylsilanized silica gel mini column (1,000 mg)

1,000 mg of propylsulphonylsilanized silica gel is packed in a polyethylene column tube with an inner diameter of 12 to 13 mm or a column with comparable separation efficiency.

Fluorescamine

Purity of 98% or higher

9-Fluorenylmethylchloroformate

9-Fluorenylmethylchloroformate (special grade)

***n*-Hexane**

Use a rotary vacuum evaporator on 300 mL of *n*-hexane to evaporate until 5 mL is left. For analysis, inject 5 μL of the solution into a gas chromatograph equipped with an electron capture detector. Peaks other than that of *n*-hexane in the resulting chromatogram should be as high as or lower than the peaks of γ-BHC at 2 x 10^{-11} g.

Potassium peroxodisulfate

Potassium peroxodisulfate (first grade)

Benzenesulfonylpropylsilanized silica gel mini column (500 mg)

500 mg of benzenesulfonylpropylsilanized silica gel is packed in a polyethylene column tube with an inner diameter of 8 to 9 mm or a column with comparable separation efficiency.

3-Pentanone

3-Pentanone (special grade)

***n*-Pentane**

Purity of 99% or higher

Water

Distilled water. If the distilled water is found to include any substance that may interfere with analysis of the target compositional substances from agricultural chemicals, wash with a solvent such as *n*-hexane before use.

Chloroacetic anhydride

Purity of 99% or higher

Fluoroacetic anhydride

Purity of 99% or higher

Sodium sulfate (anhydrous)

Sodium sulfate (anhydrous) (special grade). If the reagent is found to include any substance that may interfere with analysis of the target compositional substances from agricultural chemicals, wash with a solvent such as *n*-hexane before use.

Methanol

Use a rotary vacuum evaporator on 300 mL of methanol to concentrate to the point of dryness. Dissolve the residue in 5 mL of *n*-hexane. For analysis, inject 5 μL of the solution into a gas chromatograph equipped with an electron capture detector. Peaks other than that of *n*-hexane in the resulting chromatogram should be as high as or lower than the peaks of γ-BHC at 2 x 10^{-11} g.

Methylisobutylketone

Methylisobutylketone (special grade)

1-Methylimidazole

Purity of 99% or higher

Methyl orange test solution

0.1 g of Methyl orange dissolved in 100 mL of water.

***N*-Methyl-*N*-nitroso-*p*-toluenesulfonamide**

Purity of 98% or higher

***N*-Methylbistrifluoroacetamide**

Purity of 95% or higher

2-Mercaptoethanol

Purity of 99% or higher

3-Mercaptopropionic acid

Purity of 98% or higher

Monoethanolamine

Monoethanolamine (special grade)

Molecular sieves

Naturally occurring alkali metal sodium silicate or alkaline earth sodium silicate

Potassium iodide starch paper

Potassium iodide starch paper

Iodotrimethylsilane

Purity of 95% or higher

Sodium tetraborate

Sodium tetraborate (special grade)

Sodium lauryl sulfate

Sodium lauryl sulfate (special grade)

Monopotassium hydrogen phosphate

Monopotassium hydrogen phosphate (special grade)

Dipotassium hydrogen phosphate

Dipotassium hydrogen phosphate (special grade)

Tetra-*n*-butylammonium phosphate

Tetra-*n*-butylammonium phosphate (special grade)

Multiresidue Method for Agricultural Chemicals by GC/MS (Agricultural Products)

1. Substances to be analyzed

See Table 1.

2. Apparatus

Gas chromatograph/mass spectrometer (GC/MS)

3. Reagents

Use the reagents listed in Section 2 of the General Rules except for the following:

0.5 mol/L Phosphate buffer (pH 7.0): Dissolve 52.7 g of dipotassium hydrogenphosphate (K_2HPO_4) and 30.2 g of potassium dihydrogenphosphate (KH_2PO_4) in approximately 500 mL of water. Adjust the pH of the solution to 7.0 with 1 mol/L sodium hydroxide or 1 mol/L hydrogen chloride. Add water to make a 1 L solution.

Reference standards of agricultural chemicals: reference standards of known purity

4. Procedure

1) Extraction

i) Grains, beans, nuts and seeds

Add 20 mL of water to 10.0 g of the sample and let stand for 15 minutes.

Add 50 mL of acetonitrile and homogenize the sample. Filter the sample by suction. Add 20 mL of acetonitrile to the residue on the filter paper, and perform homogenization and suction filtration. Mix both filtrates. Add acetonitrile to make a 100 mL solution.

Measure 20 mL of the extracted solution. Add 10 g of sodium chloride and 20 mL of 0.5 mol/L phosphate buffer (pH 7.0). Shake for 10 minutes. Let stand until the solution is clearly separated into layers. Discard the aqueous layer.

Condition an octadecylsilanized silica gel mini column (1000 mg) with 10 mL of acetonitrile. Apply the above-mentioned acetonitrile layer to the column. Elute the column with 2 mL of acetonitrile. Collect the entire volume of effluent, dry over sodium sulfate (anhydrous), and filter. Concentrate the filtrate to dryness at 40°C or lower. Dissolve the residue in 2 mL of acetonitrile/toluene (3:1).

ii) Fruits, vegetables, herbs, tea and hops

For fruits, vegetables and herbs, weigh out 20.0 g of the sample. For tea and hops, weigh out 5.00 g of the sample and let stand in 20 mL of water for 15 minutes.

Add 50 mL of acetonitrile and homogenize the sample. Filter the sample by suction. Add 20 mL of acetonitrile to the residue on the filter paper, and perform homogenization and suction filtration. Mix both filtrates. Add acetonitrile to the filtrate to make a 100 mL solution.

Measure 20 mL of the extracted solution. Add 10 g of sodium chloride and 20 mL of 0.5 mol/L phosphate buffer (pH 7.0) and shake. Let stand until the solution is clearly separated into layers. Discard the aqueous layer. Dry the acetonitrile layer over sodium sulfate (anhydrous) and filter. Concentrate the filtrate to dryness at 40°C or lower. Dissolve the residue in 2 mL of acetonitrile/toluene (3:1).

2) Clean-up

Condition a graphite carbon/aminopropylsilanized silica gel layered mini column (500 mg/500 mg) with 10 mL of acetonitrile/toluene (3:1). Apply the solution obtained during the Extraction step to the column. Elute the column with 20 mL of acetonitrile/toluene (3:1) and collect the entire volume of effluent. Concentrate the effluent to 1 mL or less at 40°C or lower. Add 10 mL of acetone to the concentrated solution and concentrate to 1 mL or less at 40°C or lower. Add 5 mL of acetone to the concentrated solution and concentrate to dryness. Dissolve the residue in acetone/*n*-hexane (1:1) to make a 1 mL solution. Use this as the test solution.

5. Calibration curves

Prepare an acetone solution of the reference standard for each of the agricultural chemicals, and mix them all. Dilute portions of the mixture with acetone/*n*-hexane (1:1) to the appropriate concentrations of the reference standards. Inject 2 μL of each diluted portion into a GC/MS. Use peaks in the resulting chromatograms to prepare calibration curves using the peak height or peak area method.

6. Determination

Inject 2 μL of the test solution into the GC/MS for analysis. Determine the content of each of the agricultural chemicals, using GC/MS results and the calibration curve prepared in the above step 5.

7. Confirmation

Perform GC/MS measurements.

8. Measuring conditions

GC/MS
Column: 5% Phenyl-methyl silicon (0.25 mm I.D. x 30 m length x 0.25 μm film thickness)
Column temperature: 50°C (1 min) - 25°C/min heating - 125°C (0 min) - 10°C/min heating - 300°C (10 min)
Injector temperature: 250°C
Carrier gas: Helium
Ionization mode (voltage): EI (70 eV)
Major monitoring ions (*m/z*): See Table 1.
Expected retention times: See Table 1.

9. Limit of quantitation

See Table 1.
Examples of limit of measurement (ng) are listed.

10. Other

1) Test procedure outline

Extract each agricultural chemical, etc., from the sample using acetonitrile. Dry the extracts after salting-out. For fruits and vegetables, clean-up samples with a graphite-carbon/ aminopropylsilanized silica gel layered mini column. For grains, beans, nuts and seeds, clean-up with an octadecylsilanized silica gel mini column, followed by a graphite-carbon/aminopropylsilanized silica gel layered mini column. Perform measurement and confirmation by GC/MS.

2) Notes

i) In Table 1, substances that can be analyzed by the above-described method are arranged in the order of the Japanese syllabary, with the exclusion of those comprised of compounds which may be regulated, such as metabolites. When isomers of the same substance have different retention times, their names and times are shown separately in different rows under the agrichemical substance heading. “Degradate” in parentheses means that the composition substance to be measured is a degradated product formed during analysis.

ii) The above-mentioned method does not ensure the possibility of simultaneous analyses of every combination of the compounds listed in Table 1. Because interaction between compounds during the analysis may lead to their decomposition or interference, check whether the combination of compounds to be analyzed is suitable to the method.

iii) Gas chromatograph/tandem mass spectrometer (GC/MS/MS) can also be used for analyses.

iv) Sodium phosphate can be used in the preparation of a phosphate buffer.

v) If the volume of sodium chloride to be added (10 grams) is significantly larger than that of the acetonitrile extract, the quantity of salt can be reduced so long as it is sufficiently saturated.

vi) Concentrate solvent to dryness in a nitrogen stream in a moderate manner.

vii) The obtainment of accurately measured values may require use of a matrix-containing standard solution or the standard addition method.

viii) Because limit of quantitation depend on the appratus, and the concentration factor and injected volume of the test solution, it may be necessary to find the optimum testing conditions.

ix) For tea types other than green powdered tea, use the individual analytical methods discussed later for the following agricultural chemicals.

BHC, DDT, Acrinathrin, Acetamiprid, Isoxathion, Imibenconazole, Ethofenprox, Endrin, Chlorpyrifos, Chlorfenapyr, Dicofol, Cyhalothrin, Difenoconazole, Cyfluthrin, Cypermethrin, Dimethoate, Diazinon, Dieldrin, Tetraconazole, Tebufenpyrad, Deltamethrin and Tralomethrin, Trifluralin, Parathion, Parathion-methyl, Halfenprox, Bifenthrin, Pyraclofos, Pyridaben, Pyrifenox, Pirimifos-methyl, Pyrethrins, Fenitrothion, Phenthoate, Fenvalerate, Fenpropathrin, Flucythrinate, Fluvalinate, Prothiofos, Propiconazole, Permethrin, Phosalone, and Miclobutanil.

x) For tea types other than green powdered tea, use the individual analytical methods listed in Column 2 of the following table for the agricultural chemicals listed in Column 1 of the same table.

Column 1	Column 2
XMC	Method for aldicarb, etc.
Tetradifon	Method for BHC, etc.
Profenofos	Method for EPN, etc.
Methidathion	Method for EPN, etc.

11. References

1) Fillion, J. et al, J. AOAC Int, 83: 698-713 (2000)

12. Type

C

Table 1 Multiresidue Method for Agricultural Chemicals by GC/MS (Agricultural Products)

Agricultural Chemicals	Substances to be Analyzed	Retention Index	Monitoring Ions (m/z)				Limit of Measurement (ng)
BHC	α-BHC	1714	***219***	***183***	181		0.010
	β-BHC	1761	***219***	183	181		0.011
	γ-BHC (Lindane)	1779	***219***	183	181		0.011
	δ-BHC	1833	***219***	183	181		0.015
DDT	o,p'-DDT	2295	237	***235***	212	165	0.010
	p,p'-DDD	2289	237	***235***	178	165	0.007
	p,p'-DDE	2196	318	***246***			0.004
	p,p'-DDT	2373	237	***235***	212	165	0.011
EPN	EPN	2484	185	169	***157***		0.032
TCMTB	TCMTB	2162	***180***				0.004
XMC	XMC	1563	***122***				0.001
Acrinathrin	Acrinathrin	2613	289	208	***181***		0.006
Azaconazole	Azaconazole	2216	***217***	173			0.003
Azinphos-methyl	Azinphos-methyl	2572	***160***	132			0.132
Acetamiprid	Acetamiprid	2452	166	***152***			0.021
Acetochlor	Acetochlor	1882	233	223	***146***		0.007
Atrazine	Atrazine	1755	215	***200***			0.002
Anilofos	Anilofos	2512	***226***	125			0.023
Ametryn	Ametryn	1916	***227***	212			0.002
Alachlor	Alachlor	1898	188	***160***			0.002
Aldrin and Dieldrin	Aldrin	1998	293	265	***263***	***261***	0.013
Isazophos	Isazohos	1815	285	***257***	172	***161***	0.005
Isoxadifen-ethyl	Isoxadifen-ethyl	2328	294	***222***	***204***		0.024
Isoxathion	Isoxathion	2234	***313***	285	177	***105***	0.001
Isofenphos	Isofenphos	2064	255	***213***	121		0.001
	Isofenphos oxon	1998	***229***	201			0.002
Isoprocarb	Isoprocarb	1538	***263***	136	125	***121***	0.001
Isoprothiolane	Isoprothiolane	2177	***204***	290	231	***118***	0.008
Iprobenfos	Iprobenfos	1845	246	***204***	91		0.008
Imazamethabenz-methyl	Imazamethabenz-methyl (isomer 1)	2160	256	214	***187***		0.012
	Imazamethabenz-methyl (isomer 2)	2164	256	214	***187***		0.012
Imibenconazole	Imibenconazole	3187	253	250	***125***		0.005
	Imibenconazole debenzylated	2210	270	***235***			0.023
Uniconazole P	Uniconazole P	2193	***234***	165	131		0.004
Esprocarb	Esprocarb	1965	***222***	162			0.001
Ethalfluralin	Ethalfluralin	1648	316	***276***			0.003
Ethion	Ethion	2281	***231***	153			0.004
Edifenphos	Edifenphos	2356	***310***	173			0.019
Etoxazole	Etoxazole	2489	330	***300***	204	***141***	0.000
Ethofenprox	Ehtofenprox	2870	376	183	***163***		0.000
Ethofumesate	Ethofumesate	1953	286	***207***	***161***		0.020
Ethoprophos	Ethoprophos	1641	200	***158***	139		0.011
Etrimfos	Etrimfos	1824	***292***	277	181		0.008
Endosulfan	α-Endosulfan	2152	***241***	***195***			0.018
	β-Endosulfan	2281	***241***	237	***195***		0.034
Endrin	Endrin	2262	***345***	317	281	***263***	0.042
Oxadiazon	Oxadiazon	2189	302	***258***	***175***		0.002
Oxadixyl	Oxadixyl	2280	***163***	132			0.026
Oxyfluorfen	Oxyfluorfen	2198	331	300	***302***	***252***	0.043
Omethoate	Omethoate	1596	***156***	141	110		0.086
Oryzalin	Oryzalin	2667	***317***	***275***			0.055
Cadusafos	Cadusafos	1692	270	***213***	***159***	158	0.015
Cafenstrole	Cafenstrole	2767	188	119	***100***		0.001
Carfentrazone-ethyl	Carfentrazone-ethyl	2327	340	330	***312***		0.002

Agricultural Chemicals	Substances to be Analyzed	Retention Index	Monitoring Ions (m/z)				Limit of Measurement (ng)
Carboxin	Carboxin	2211	235	225	***143***		0.001
Carbofuran	Carbofuran	1743	***164***	149			0.016
	Carbofuran (degradate)	1304	***164***	149			0.005
Quinalphos	Quinalphos	2086	157	156	***146***	***118***	0.019
Quinoxyfen	Quinoxyfen	2347	***237***				0.001
Quinoclamin	Quinoclamin	1968	***207***	***172***			0.024
Quintozene	Quintozene	1759	***295***	***237***			0.005
Kresoxim-methyl	Kresoxim-methyl	2201	206	***116***			0.002
Clomazone	Clomazone	1761	***204***	125			0.001
Chlorthal-dimethyl	Chlorthal-dimethyl	1988	332	***301***			0.000
Chlordane	cis-Chlordane	2148	***373***	272	375		0.000
	trans-Chlordane	2121	***373***	272	375		0.000
Chlorpyrifos	Chlorpyrifos	1982	***314***	***286***	197		0.022
Chlorpyrifos-methyl	Chlorpyrifos-methyl	1885	***286***	125			0.001
Chlorfenapyr	Chlorfenapyr	2222	***408***	247			0.016
Chlorfenvinphos	Chlorfenvinphos (E) α	2048	***323***	***269***	267		0.014
	Chlorfenvinphos (Z) β	2071	***323***	***269***	267		0.009
Chlorpropham	Chlorpropham	1660	***213***	154	127		0.003
Chlorobenzilate	Chlorobenzilate	2261	***251***	139			0.001
Cyanazine	Cyanazine	1987	***225***	***212***			0.016
Cyanophos	Cyanophos	1781	***243***	109			0.001
Diethofencarb	Diethofencarb	1979	***267***	225			0.006
Diclocymet	Diclocymet (isomer 1)	2081	***277***	221			0.015
	Diclocymet (isomer 2)	2114	***277***	221			0.013
Dichlofenthion	Dichlofenthion	1873	***279***	223			0.001
Diclofop-methyl	Diclofop-methyl	2395	***340***	253			0.003
Dicloran	Dicloran	1734	***206***	176			0.023
Dicofol	Dicofol	2539	***251***	139			—
	Dicofol degradate (4,4'-Dichlorobenzophenone)	2018	***250***	***139***			0.008
Cyhalothrin	Cyhalothrin (isomer 1)	2574	449	197	***181***		0.038
	Cyhalothrin (isomer 2)	2597	449	197	***181***		0.012
Cyhalofop-butyl	Cyhalofop-butyl	2581	***357***	***256***			0.010
Diphenamid	Diphenamid	2026	239	***167***			0.001
Difenoconazole	Difenoconazole (isomer 1)	3017	***323***	265			0.005
	Difenoconazole (isomer 2)	3025	***323***	265			0.004
Cyfluthrin	Cyfluthrin (isomer 1)	2775	226	206	***163***		0.114
	Cyfluthrin (isomer 2)	2788	226	206	***163***		0.067
	Cyfluthrin (isomer 3)	2796	226	206	***163***		0.133
	Cyfluthrin (isomer 4)	2801	226	206	***163***		0.074
Diflufenican	Diflufenican	2397	***394***	***266***			0.007
Cyproconazole	Cyproconazole (isomer 1)	2234	***222***	139			0.006
	Cyproconazole (isomer 2)	2238	***222***	139			0.002
Cypermethrin	Cypermethrin (isomer 1)	2828	***181***	163			0.055
	Cypermethrin (isomer 2)	2842	***181***	163			0.040
	Cypermethrin (isomer 3)	2850	***181***	163			0.085
	Cypermethrin (isomer 4)	2855	***181***	163			0.042
Simazine	Simazine	1744	***201***				0.002
Dimethametryn	Dimethametryn	2059	255	***212***			0.001
Dimethylvinphos	Dimethylvinphos (E)	1959	297	***295***			0.008
	Dimethylvinphos (Z)	1986	297	***295***	***204***		0.008
Dimethenamid	Dimethenamid	1875	***230***	154			0.005
Dimethoate	Dimethoate	1736	125	***87***			0.033
Simetryn	Simetryn	1906	***213***	170			0.001
Dimepiperate	Dimepiperate	2093	145	***119***			0.001
Spiroxamine	Spiroxamine (isomer 1)	1896	***100***				0.001
	Spiroxamine (isomer 2)	1949	***100***				0.001
Spirodiclofen	Spirodiclofen	2690	***312***	259			0.021

Agricultural Chemicals	Substances to be Analyzed	Retention Index	Monitoring Ions (m/z)				Limit of Measurement (ng)
Zoxamide	Zoxamide	2428	260	***258***	***187***		0.012
	Zoxamide (degradate)	2094	***242***	***187***			0.054
Terbacil	Terbacil	1816	163	***161***	117		0.013
Diazinon	Diazinon	1791	***304***	***179***	152	137	0.014
Thiobencarb	Thiobencarb	1983	257	125	***100***		0.001
Thiometon	Thiometon	1725	***246***	158	125	***88***	0.009
Thifluzamide	Thifluzamide	2190	***449***	194			0.001
Aldrin and Dieldrin	Dieldrin	2215	277	***263***	***261***		0.023
Tecnazene	Tecnazene	1594	***261***	***203***			0.002
Tetrachlorvinphos	Tetrachlorvinphos	2121	***329***				0.001
Tetraconazole	Tetraconazole	1998	***336***	171			0.001
Tetradifon	Tetradifon	2536	***356***	***159***			0.004
Thenylchlor	Thenylchlor	2384	288	***127***			0.001
Tebuconazole	Tebuconazole	2397	***250***	125			0.006
Tebufenpyrad	Tebufenpyrad	2505	333	***318***			0.002
Tefluthrin	Tefluthrin	1816	383	***197***	***177***		0.003
Demeton-S-methyl	Demeton-S-methyl	1627	***142***	***109***			0.017
Deltamethrin and Tralomethrin	Deltamethrin	3056	***253***	***181***			0.020
Terbutryn	Terbutryn	1945	***226***				0.001
Terbufos	Terbufos	1783	288	***231***	153		0.007
Deltamethrin and Tralomethrin	Tralomethrin	3066	***253***	***181***			0.230
Triadimenol	Triadimenol (isomer 1)	2088	***168***	128	112		0.009
	Triadimenol (isomer 2)	2104	***168***	128	112		0.003
Triadimefon	Triadimefon	1999	***208***				0.006
Triazophos	Triazophos	2310	257	***161***			0.011
Tri-allate	Tri-allate	1827	***268***				0.001
Tricyclazole	Tricyclazole	2185	***189***	162	161		0.045
Tridemorph	Tridemorph	—	***128***				0.031
Tribuphos	Tribuphos	2193	***169***				0.001
Trifluralin	Trifluralin	1663	***306***	***264***			0.008
Trifloxystrobin	Trifloxystrobin	2336	***116***				0.002
Tolclophos-methyl	Tolchlophos-methyl	1899	267	***265***			0.001
Tolfenpyrad	Tolfenpyrad	3106	***383***	***171***			0.032
Napropamide	Napropamide	2165	***271***	***128***	72		0.020
Nitrothal-isopropyl	Nirothal-isopropyl	2009	254	***236***	212		0.003
Norflurazon	Norflurazon	2348	***303***	173	145		0.006
Paclobutrazol	Paclobutrazol	2128	***236***	167	125		0.002
Parathion	Parathion	1994	***291***	261	235		0.007
Parathion-methyl	Parathion-methyl	1896	***263***	233	125		0.005
Halfenprox	Halfenprox	2841	265	***263***	183		0.021
Bioallethrin	Bioallethrin	2076	***123***	79			0.008
Picolinafen	Picolinafen	2483	***376***	***238***			0.013
Bitertanol	Bitertanol (isomer 1)	2695	268	***170***	168		0.001
	Bitertanol (isomer 2)	2710	268	***170***	168		0.006
Bifenox	Bifenox	2515	***341***	310			0.044
Bifenthrin	Bifenthrin	2468	***181***	166			0.001
Piperophos	Piperophos	2486	***320***	140	***84***		0.026
Pyraclofos	Pyrachlofos	2660	***360***	194			0.011
Pyrazophos	Pyrazophos	2622	232	***221***			0.076
Pyraflufen ethyl	Pyraflufen ethyl	2355	***412***	349			0.003
Pyridafenthion	Pyridafenthion	2455	***340***	199	97		0.092
Pyridaben	Pyridaben	2731	309	***147***			0.010
Pyrifenox	Pyrifenox (E)	2122	***262***	187	171		0.003
	Pyrifenox (Z)	2068	***262***	187	171		0.004
Pyributicarb	Pyributicarb	2436	181	***165***	108		0.001
Pyriproxyfen	Pyriproxyfen	2574	226	***136***			0.001
Pyriminobac-methyl	Pyriminobac-methyl (E)	2350	***302***	259	***173***		0.001
	Pyriminobac-methyl (Z)	2255	***302***	256			0.000
Pirimifos-methyl	Pirimifos-methyl	1940	305	***290***			0.001

Agricultural Chemicals	Substances to be Analyzed	Retention Index	Monitoring Ions (m/z)				Limit of Measurement (ng)
Pyrimethanil	Pyrimethanil	1801	199	***198***	183		0.002
Pyrethrin	Pyrethrin I	2314	133	***123***			0.035
	Pyrethrin II	2615	161	***160***			0.117
Pyroquilon	Pyroquilon	1797	***173***	144	130		0.013
Vinclozolin	Vinclozolin	1890	***285***	***187***			0.003
Fipronil	Fipronil	2052	369	***367***	***351***		0.004
Fenamiphos	Fenamiphos	2154	***303***	217	***154***		0.084
Fenarimol	Fenarimol	2629	219	***139***			0.002
Fenitrothion	Fenitrothion	1946	***277***	260			0.004
Fenoxanil	Fenoxanil	2240	***293***	***189***			0.008
Fenothiocarb	Fenothiocarb	2136	***160***	***72***			0.017
Phenothrin	Phenothrin (isomer 1)	2531	***183***	123			0.079
	Phenothrin (isomer 2)	2545	***183***	123			0.030
Fenamidone	Fenamidone	2499	***268***	238			0.019
Fensulfothion	Fensulfothion	2265	308	***293***	156		0.008
Fenthion	Fenthion	1987	***278***	169			0.000
Phenthoate	Phentoate	2078	***274***	246			0.002
Fenvalerate	Fenvalerate (isomer 1)	2959	***419***	***167***	125		0.099
	Fenvalerate (isomer 2)	2989	***419***	***167***	125		0.159
Fenbuconazole	Fenbuconazole	2782	***198***	***129***			0.016
Fenpropathrin	Fenpropathrin	2498	349	265	***181***		0.013
Fenpropimorph	Fenpropimorph	1995	129	***128***	70		0.001
Phthalide	Phthalide	2021	272	***243***			0.005
Butachlor	Butachlor	2129	***176***	160			0.001
Butamifos	Butamifos	2145	***286***	200			0.004
Bupirimate	Bupirimate	2202	***273***	208			0.006
Buprofezin	Buprofezin	2205	172	***105***			0.003
Flamprop-methyl	Flamprop-methyl	2195	276	***105***	77		0.002
Furilazole	Furilazole	1743	***262***	***220***			0.006
Fluacrypyrim	Fluacryppyrim	2289	204	***190***	189	***145***	0.010
Fluquinconazole	Fluquinconazole	2729	***340***	108			0.008
Fludioxonil	Fludioxonil	2169	***248***	154	127		0.012
Flucythrinate	Flucythrinate (isomer 1)	2844	451	***199***	157		0.005
	Flucythrinate (isomer 2)	2871	451	***199***	157		0.007
Fluthiacet-methyl	Fluthiacet-methyl	3244	***403***	84			0.648
Flutolanil	Flutolanil	2161	323	***173***			0.001
Flutriafol	Flutriafol	2157	219	***201***	164	***123***	0.016
Fluvalinate	Fluvalinate (isomer 1)	2964	252	***250***			0.008
	Fluvalinate (isomer 2)	2973	252	***250***			0.009
Flumioxazin	Flumioxazin	2950	***354***	287			0.021
Flumiclorac pentyl	Flumiclorac pentyl	3080	***423***	308			0.010
Fluridone	Fluridone	2903	329	***328***	310		0.011
Pretilachlor	Pretilachlor	2174	262	238	***162***		0.001
Procymidone	Procymidone	2088	***283***	212	96		0.019
Prothiofos	Prothiofos	2170	***309***	267			0.001
Propachlor	Propachlor	1612	176	***120***			0.007
Propazine	Propazine	1759	***229***	***214***	172		0.043
Propanil	Propanil	1876	217	163	***161***		0.013
Propargite	Propargite (isomer 1)	2398	***135***	107			0.044
	Propargite (isomer 2)	2403	173	***135***	107		0.044
Propiconazole	Propiconazole (isomer 1)	2346	***302***	259	256	***173***	0.006
	Propiconazole (isomer 2)	2360	259	***173***			0.005
propyzamide	Propyzamide	1786	175	***173***	145		0.017
Prohydrojasmon	Prohydrojasmon (isomer 1)	1814	184	***153***			0.023
	Prohydrojasmon (isomer 2)	1844	184	***153***			0.196
Profenofos	Profenofos	2184	***339***	337	***139***	97	0.063
Propoxur	Propoxur	1610	152	***110***			0.004
Bromacil	Bromacil	1954	231	***205***			0.002
Prometryn	Prometryn	1919	241	226	***184***		0.017

Agricultural Chemicals	Substances to be Analyzed	Retention Index	Monitoring Ions (m/z)				Limit of Measurement (ng)
Bromobutide	Buromobutide	1887	232	***119***			0.003
Bromopropylate	Bromopropylate	2481	***341***	183			0.004
Bromophos	Bromophos	2026	***331***	125			0.002
Hexaconazole	Hexaconazole	2172	***214***	175			0.002
Hexazinone	Hexazinone	2380	252	***171***	128		0.004
Benalaxyl	Benalaxyl	2334	206	***148***			0.002
Benoxacor	Benoxacor	1853	259	***120***			0.003
Heptachlor	Heptachlor	1922	337	***272***	100		0.001
	Heptachlor-epoxide	2080	***353***	81			0.048
Permethrin	Permethrin (isomer 1)	2711	***183***	163			0.012
	Permethrin (isomer 2)	2728	***183***	163			0.011
Penconazole	Penconazole	2060	***248***	159			0.001
Pendimethalin	Pendimethalin	2046	281	***252***			0.002
Benfluralin	Benfluralin	1668	***292***	264			0.002
Benfuresate	Benfuresate	1871	***256***	***163***			0.003
Phosalone	Phosalone	2555	367	***182***			0.003
Fosthiazate	Fosthiazate (isomer 1)	2027	283	***195***			0.043
	Fosthiazate (isomer 2)	2032	283	***195***			0.047
Phosphamidon	Phosphamidon	1870	***264***	***127***			0.043
Phosmet	Phosmet	2480	161	***160***	133		0.017
Phorate	Phorate	1703	***260***	231	121	***75***	0.008
Malathion	Malathion	1963	***173***	158			0.002
Myclobutanil	Myclobutanil	2198	***179***	152	150		0.015
Metalaxyl (isomer: Mefenoxam)	Metalaxyl (isomer: Mefenoxam)	1915	249	234	220	***206***	0.006
Methidathion	Methidathion	2115	302	***145***	85		0.009
Methoxychlor	Methoxychlor	2495	274	228	***227***	212	0.004
Methoprene	Methoprene	2097	175	153	***111***		0.022
Methominostrobin	Methominostrobin (E)	2169	***238***	196	191		0.005
	Methominostrobin (Z)	2212	***238***	***196***	191	166	0.010
Metolachlor	Meolachlor	1975	238	***162***			0.001
Mevinphos	Mevinphos	1424	192	164	***127***		0.008
Mefenacet	Mefenacet	2588	298	***192***	120		0.002
Mefenpyr-diethyl	Mefenpyr-diethyl	2427	299	271	***253***		0.011
Mepronil	Mepronil	2308	269	***119***			0.001
Monocrotophos	Monocrotophos	1679	192	164	***127***		0.048
Lindane (γ-BHC)	Lindane (γ-BHC)	1779	***219***	183	181		0.011
Lenacil	Lenacil	2359	***153***	136	110		0.002

- The compounds listed in the "Agricultural chemicals" column are arranged according to the Japanese syllabary. The isomers of each chemical are arranged by their retention times.
- Retention indices are obtained based on the retention times of the *n*-alkanes. The figure for each compound is the averaged retention indices obtained from two different organizations.
- Bold italic figures in the "Monitoring Ion" column represent the quantitative ions. The remaining ions are qualitative ions.
- Each limit of mesurement is the value at S/N = 10 obtained using 2 μL of a standard solution injected into a GC/MS. Each limit is the lower of the two values obtained from two different organizations.
- When 2 μL of a fruit or vegetable test solution prepared according to the above method is injected into a GC/MS, 0.08 ng corresponds to 0.01 ppm in the sample.

Multiresidue Method I for Agricultural Chemicals by LC/MS (Agricultural Products)

1. Substances to be analyzed

See Table 2.

2. Apparatus

Liquid chromatograph/mass spectrometer (LC/MS) or Liquid chromatograph/tandem mass spectrometer (LC/MS/MS)

3. Reagents

Use the reagents listed in Section 2 of the General Rules except for the following:

0.5 mol/L Phosphate buffer (pH 7.0) Dissolve 52.7 g of dipotassium hydrogenphosphate (K_2HPO_4) and 30.2 g of potassium dihydrogenphosphate (KH_2PO_4) in about 500 mL of water. Adjust the pH of the solution to 7.0 with 1 mol/L sodium hydroxide or 1 mol/L hydrogen chloride. Add water to make a 1 L solution.

Reference standard of agricultural chemicals: reference standards of known purity

4. Procedure

1) Extraction

i) Grains, beans, nuts and seeds

Add 20 mL of water to 10.0 g of a sample and let stand for 15 minutes.

Add 50 mL of acetonitrile and homogenize the sample. Filter the sample by suction. Add 20 mL of acetonitrile to the residue on the filter paper. Perform homogenization and suction filtration. Mix both filtrates. Add acetonitrile to make a 100 mL solution.

Measure 20 mL of the extracted solution. Add 10 g of sodium chloride and 20 mL of 0.5 mol/L phosphate buffer (pH 7.0). Shake for 10 minutes. Let stand until the solution is clearly separated into layers. Discard the aqueous layer.

Condition octadecylsilanized silica gel mini column (1000 mg) with 10 mL of acetonitrile. Apply the above-mentioned acetonitrile layer to the column. Elute the column with 2 mL of acetonitrile. Collect the entire volume of effluent. Dry the effluent over sodium sulfate (anhydrous) and filter. Concentrate the filtrate to dryness at 40°C or lower. Dissolve the residue in 2 mL of acetonitrile/toluene (3:1).

ii) Fruits, vegetables, herbs, tea and hops

For fruits, vegetables and herbs, weigh out 20.0 g of the sample. For tea and hops, weigh out 5.00 g of the sample and let stand in 20 mL of water for 15 minutes.

Add 50 mL of acetonitrile and homogenize the sample. Filter the sample by suction. Add 20 mL of acetonitrile to the residue on the filter paper, and perform homogenization and suction filtration. Mix both filtrates. Add acetonitrile to the filtrate to make a 100 mL solution.

Measure out 20 mL of the extracted solution. Add 10 g of sodium chloride and 20 mL of 0.5 mol/L phosphate buffer (pH 7.0) and shake. Let stand until the solution is clearly separated into layers. Discard the aqueous layer. Dry the acetonitrile layer over sodium sulfate (anhydrous) and filter. Concentrate the filtrate to dryness at 40°C or lower. Dissolve the residue in 2 mL of acetonitrile/toluene (3:1).

2) Clean-up

Condition a graphite carbon/aminopropylsilanized silica gel layered mini column (500 mg/500 mg) with 10 mL of acetonitrile/toluene (3:1). Apply the solution obtained from the Extraction step to the column and elute the column with 20 mL of acetonitrile/toluene (3:1). Collect the entire volume of effluent. Concentrate the effluent to 1 mL or less at 40°C or lower. Add 10 mL of acetone and concentrate to 1 mL or less at 40°C or lower. Add 5 mL of acetone to the concentrated solution and concentrate to dryness. Dissolve the residue in methanol to make a 4 mL solution. Use this as the test solution

5. Calibration curves

Prepare an acetonitrile solution of the reference standard for each agricultural chemical, etc., and mix them all. Dilute portions of the mixture with methanol to the appropriate concentrations of the reference standards. Inject 5 μL of each diluted portion into an LC/MS or LC/MS/MS. Use peaks in the resulting chromatograms to prepare calibration curves using the peak height or peak area method.

6. Determination

Inject 5 μL of the test solution into an LC/MS or LC/MS/MS for analysis. Determine the content of each agricultural chemical, etc., using LC/MS or LC/MS/MS results and the calibration curve prepared in the above step 5.

7. Confirmation

Perform LC/MS or LC/MS/MS measurement.

8. Measuring conditions

Column: Octadecylsilanized silica gel (particle size: 3 to 3.5 μm, 2 to 2.1 mm I.D. x 150 mm length)
Column temperature: 40°C
Mobile phase: Deliver solution A and B under the conditions described in the following table.
Flow rate: 0.20 mL/min
Solution A: 5 mmol/L Aqueous solution of ammonium acetate
Solution B: 5 mmol/L Methanol solution of ammonium acetate

Time (min)	Solution A (%)	Solution B (%)
0	85	15
1	60	40
3.5	60	40
6	50	50
8	45	55

17.5	5	95
30	5	95
30	85	15

Ionization mode: ESI
Major monitoring ions (*m/z*): See Table 2.
Expected retention times: See Table 2.

9. Limit of quantitation

See Table 2.
Examples of limit of measurement (ng) are listed.

10. Other

1) Test procedure outline

Extract each agricultural chemical, etc., from the samples using acetonitrile. Dry the extracts after salting-out. For fruits and vegetables, clean-up samples with a graphite-carbon/ aminopropylsilanized silica gel layered mini column. For grains, beans, nuts and seeds, clean-up with an octadecylsilanized silica gel mini column, followed by graphite-carbon/aminopropylsilanized silica gel layered mini column. Perform measurements and confirmation by LC/MS or LC/MS/MS.

2) Notes

i) In Table 2, substances that can be analyzed by the above-described method are arranged in the order of the Japanese syllabary, with the exclusion of those comprised of compounds which may be regulated, such as metabolites. When isomers of the same substance have different retention times, their names and times are shown separately in different rows under the agrichemical substance heading.

ii) The above-mentioned method does not ensure the possibility of simultaneous analyses of every combination of the compounds listed in Table 2. Because interaction between compounds during the analysis may lead to their decomposition or interference, check whether the combination of compounds to be analyzed is suitable to the method.

iii) Sodium phosphate can be used in the preparation of a phosphate buffer.

iv) If the volume of sodium chloride to be added (10 grams) is significantly higher than that of the acetonitrile extract, the quantity of salt can be reduced so long as it is sufficiently saturated.

v) Concentrate solvent to dryness in a nitrogen stream in a moderate manner.

vi) High sensitivity of an LC/MS or LC/MS/MS may require further dilution of the test solution with methanol.

vii) Because some agricultural chemicals to be analyzed are unstable, especially in methanol, their measurement must be performed immediately after preparation of the test solution. Prepare fresh solutions for calibration curves. Do not keep test solutions in the autosampler rack of the system at room temperature any longer than necessary.

viii) The obtainment of accurately measured values may require use of a matrix-containing standard solution or the standard addition method.

ix) Because limit of quantitation depend on the apparatus, and the concentration factor and injected volume of the test solution, it may be necessary to find the optimum testing conditions.

11. References

1) Fillion, J. et al, J. AOAC Int, 83: 698-713 (2000)

12. Type

C

Table 2 Multiresidue Method I for Agricultural Chemicals by LC/MS (Agricultural Products)

Agricultural Chemicals	Substances to be Analyzed	Relative Retention Time	LC/MS Monitoring Ions (m/z)								LC/MS/MS Monitoring Ions (m/z)										Limit of Measurement (ng)	
			Positive Mode					Negative Mode			Positive Mode						Negative mode					
			Quantitative		Qualitative			Quantitative	Qualitative		Parent		Daughter (Quantitative)		Daughter (Qualitative)		Parent	Daughter (Quantitative)		Daughter (Qualitative)	LC/MS	LC/ MS/MS
Azamethiphos	Azamethiphos	0.82	***325***		347	215	183				325		***183***		112						0.003	0.002
Azinphos-methyl	Azinphos-methyl	1.05	***318***	***160***	132						318		***159.9***	***76.97***	132						0.005	0.003
Anilofos	Anilofos	1.22	***368***		390	199	157				368		***199.1***		125						0.003	0.001
Abamectin	Abamectin B1a	1.46	***896***	***891***	897	567	305				891		***567.3***	***305.3***	568						0.024	0.026
Isoxaflutole	Isoxaflutole	1.00	***377***	***360***	283	251		***-358***	-359		360		***251***		360	144					0.004	0.003
Iprovalicarb	Iprovalicarb	1.15	***321***		343	119					321		***119***		203						0.002	0.001
Imidacloprid	Imidacloprid	0.53	***256***		209	175					256		***209***	***175***							0.008	0.005
Indoxacarb	Indoxacarb	1.28	***528***		550	203	150				528		***150***		203						0.002	0.004
Oxycarboxin	Oxycarboxin	0.62	***268***		207	175					268		***174.9***		147						0.004	0.001
Oryzalin	Oryzalin	1.17						***-345***	-346	-78							-345	***-281***	***-78***	-147	0.003	0.001
Quizalofop-ethyl	Quizalofop-p-tefuryl	1.30	***429***		431	299					429		***299.1***		85						0.002	0.001
Clonquintocet-mexyl	Clonquintocet-mexyl	1.34	***336***		358	238					336		***238***		192	179					0.014	0.001
Clothianidin	Clothianidin	0.54	***250***		169			***-248***	-58		250		***168.9***		132						0.002	0.002
Chromafennozide	Chromafennozide	1.15	***395***		175						395		***175.1***		339	147					0.002	0.001
Clomeprop	Clomeprop	1.33	***324***		346	120		***-322***	-175		324		***120.3***		203	105					0.004	0.006
Chloridazon	Chloridazon	0.61	***222***		244	104	65				222		***92***	***65***	77						0.004	0.002
Cyazofamid	Cyazofamid	1.18	***325***		140	108					325		***108***		325	261					0.005	0.001
Cyflufenamid	Cyflufenamid	1.25	***413***		435	295	241				413		***295.1***	***241.1***	203						0.005	0.001
Simeconazole	Simeconazole	1.16	***294***		295	135					294		***70***		73						0.002	0.002
Dimethirimol	Dimethirimol	0.96	***210***		232	140	71				210		***71***		140						0.001	0.001
Thiacloprid	Thiacloprid	0.64	***253***		255	126					253		***126.1***		90	73					0.004	0.002
Thiabendazole	Thiabendazole	0.75	***202***		203	175					202		***174.9***		131						0.001	0.001
Thiamethoxam	Thiamethoxam	0.44	***292***	***211***	314						292		***211.1***		181						0.003	0.004
Tralkoxydim	Tralkoxydim (isomer 1)	0.94	***330***		284												-328	***-254***		-66	0.009	0.001
	Tralkoxydim (isomer 2)	1.08	***330***		331	170					330		***137.9***		284		-328	***-254***		-66	0.001	0.0004
Triticonazole	Triticonazole	1.15	***318***		320	70					318		***70***		318	125					0.004	0.003
Tridemorph	Tridemorph (isomer 1)	1.56	***298***	***130***	299						298		***130.1***	***56.97***	98						0.009	0.012
	Tridemorph (isomer 2)	1.57	***298***		299						298		***130.1***		98						0.053	0.105
Naproanilide	Naproanilide	1.19	***292***		293	171					292		***170.9***		120		-290	***-143***		-93	0.003	0.001
Pyrazolynate	Pyrazolynate	1.26	***439***		441	173					439		***91***		229	173					0.004	0.001
Pyriftalid	Pyriftalid	1.05	***319***		320	139					319		***139.1***		179	93					0.001	0.001
Fenoxycarb	Fenoxycarb	1.20	***302***		116						302		***116.2***	***88***							0.005	0.001
Ferimzone	Ferimzone (E)	1.10	***255***		277	132					255		***132***	***91***							0.002	0.001
	Ferimzone (Z)	1.11	***255***		277	132	124				255		***124.2***	***91***	132						0.001	0.001
Phenmedipham	Phenmedipham	1.03	***301***	***136***	168						318	301	***167.9***	***136.1***							0.003	0.001
Butafenacil	Butafenacil	1.15	***492***	***475***	349	331	180				492		***330.9***		180						0.001	0.001
Furathiocarb	Furathiocarb	1.32	***383***		405	252					383		***252.2***		195						0.004	0.001
Benzofenap	Benzofenap	1.31	***431***		433	119	105				431		***105.2***		119						0.003	0.002
Milbemectin	Milbemectin A3	1.43	***546***	***511***	493						551	547	***511.1***	***240***	493	337					0.031	0.012
	Milbemectin A4	1.47	***560***	***525***	507						565	561	***525.2***	***240***	507	337					0.092	0.021
Methoxyfenozide	Methoxyfenozide	1.12						***-367***	-368	-149	369		***148.9***		91		-367	***-149***		-105	0.002	0.001
Lactofen	Lactofen	1.32	***479***		481	344					479		***343.9***		223						0.004	0.002

- The compounds listed in the "Agricultural chemicals" column are arranged according to the Japanese syllabary. The isomers of each chemical are arranged by their retention times.
- Relative retention times are obtained by dividing the retention time of each substance by the retention time of isoxaflutole (15 to 18 minutes). Each relative retention time is the average of values obtained under three to five different conditions (using different instruments with the same column, mobile phases, flow rate, and temperature).
- Bold italic figures in the "Monitoring Ion" column represent quantitative ions. The remaining ions are qualitative ions.
- Each limit of mesurement is a value at S/N = 10 for a standard solution injected into an LC/MS or LC/MS/MS. Each limit is the lowest of the two or three values obtained using two or three different instruments.
- For agricultural chemicals where both positive and negative ions are shown as monitoring ions, the lower value is taken as its limit of mesurement.
- When 5 µL of a fruit or vegetable test solution prepared according to the above method is injected into an LC/MS(/MS), 0.05 ng corresponds to 0.01 ppm in the sample.

Multiresidue Method II for Agricultural Chemicals by LC/MS (Agricultural Products)

1. Substances to be analyzed

See Table 3.

2. Apparatus

Liquid chromatograph/mass spectrometer (LC/MS) or Liquid chromatograph/tandem mass spectrometer (LC/MS/MS)

3. Reagents

Use the reagents listed in Section 2 of the General Rules except for the following:
Reference standard of agricultural chemicals: reference standards of known purity

4. Procedure

1) Extraction

i) Grains, beans, nuts and seeds

Add 20 mL of water to 10.0 g of a sample and let stand for 15 minutes.

Add 50 mL of acetonitrile and homogenize the sample. Filter the sample by suction. Add 20 mL of acetonitrile to the residue on the filter paper. Perform homogenization and suction filtration. Mix both filtrates. Add acetonitrile to make a 100 mL solution.

Measure 20 mL of the extracted solution. Add 10 g of sodium chloride and 20 mL of 0.01 mol/L hydrogen chloride. Shake for 15 minutes. Let stand until the solution is clearly separated into layers. Discard the aqueous layer.

Condition an octadecylsilanized silica gel mini column (1,000 mg) with 10 mL of acetonitrile. Apply the above-mentioned acetonitrile layer to the column. Elute the column with 2 mL of acetonitrile. Collect the entire volume of effluent. Dry the effluent over sodium sulfate (anhydrous) and filter. Concentrate the filtrate to dryness at 40°C or lower. Dissolve the residue in 2 mL of acetone/triethylamine/*n*-hexane (20:0.5:80).

ii) Fruits, vegetables, herbs, tea and hops

For fruits, vegetables and herbs, weigh out 20.0 g of the sample. For tea and hops, weigh out 5.00 g of the sample and let stand in 20 mL of water for 15 minutes.

Add 50 mL of acetonitrile and homogenize the sample. Filter the sample by suction. Add 20 mL of acetonitrile to the residue on the filter paper. Perform homogenization and suction filtration. Mix both filtrates. Add acetonitrile to the filtrate to make a 100 mL solution.

Measure 20 mL of the extracted solution. Add 10 g of sodium chloride and 20 mL of 0.01 mol/L hydrogen chloride and shake. Let stand until the solution is clearly separated into layers. Discard the aqueous layer. Dry the acetonitrile layer over sodium sulfate (anhydrous) and filter.

Concentrate the filtrate to dryness at 40°C or lower. Dissolve the residue in 2 mL of acetone/triethylamine/*n*-hexane (20:0.5:80).

2) Clean-up

Condition a silica gel mini column (500 mg) with 5 mL of methanol, 5 mL of acetone, and 10 mL of *n*-hexane in this order. Apply the solution obtained from the Extraction step above to the column. Elute the column with 10 mL of acetone/triethylamine/*n*-hexane (20:0.5:80), and discard the effluent. Elute the column with 20 mL of acetone/methanol (1:1). Collect the entire volume of effluent. Concentrate the effluent to dryness at 40°C or lower. Dissolve the residue in methanol to make a 4 mL solution. Use this as the test solution.

5. Calibration curves

Prepare an acetonitrile solution of the reference standard of each agricultural chemical, etc., and mix them all. Dilute portions of the mixture with methanol to the appropriate concentrations of the reference standards. Inject 5 μL of each diluted portion into an LC/MS or LC/MS/MS. Use peaks in the resulting chromatograms to prepare calibration curves using the peak height or peak area method.

6. Determination

Inject 5 μL of a test solution into an LC/MS or LC/MS/MS for analysis. Determine the content of each agricultural chemical, etc. using LC/MS or LC/MS/MS results and the calibration curve prepared in the above step 5.

7. Confirmation

Perform LC/MS or LC/MS/MS measurements.

8. Measuring conditions

Column: Octadecylsilanized silica gel (particle size: 3 to 3.5 μm, 2 to 2.1 mm I.D. x 150 mm length)
Column temperature: 40°C
Mobile phase: Deliver liquids A and B under the conditions described in the following table.
Flow rate: 0.20 mL/min
Solution A: 5 mmol/L Aqueous solution of ammonium acetate
Solution B: 5 mmol/L Methanol solution of ammonium acetate

Time (min)	Solution A (%)	Solution B (%)
0	85	15
1	60	40
3.5	60	40
6	50	50
8	45	55
17.5	5	95
30	5	95
30	85	15

Ionization mode: ESI
Major monitoring ions (*m/z*): See Table 3.
Expected retention times: See Table 3.

9. Limit of quantitation

See Table 3.
Examples of limit of measurement (ng) are listed.

10. Other

1) Test procedure outline

Extract each agricultural chemical, etc., from samples using acetonitrile. Dry the extracts after salting-out under acidic conditions. For fruits and vegetables, clean-up samples with a silica gel mini column. For grains, beans, nuts and seeds, clean-up with an octadecylsilanized silica gel mini column, followed by a silica gel mini column. Perform the measurements and confirmation by LC/MS or LC/MS/MS.

2) Notes

i) In Table 3, substances that can be analyzed by the above-described method are arranged in the order of the Japanese syllabary, with the exclusion of those comprised of compounds which may be regulated, such as metabolites. When isomers of the same substance have different retention times, their names and times are shown separately in different rows under the agrichemical substance heading.

ii) The above-mentioned method does not ensure the possibility of simultaneous analyses of every combination of the compounds listed in Table 3. Because interaction between compounds may lead to their degradation or interference during analysis, check whether the combination of compounds to be analyzed is suitable to the method.

iii) If the volume of sodium chloride to be added (10 grams) is significantly higher than that of the acetonitrile extract, the quantity of salt can be reduced so long as it is sufficiently saturated. Because of the high polarity of agricultural chemicals to be analyzed, dissolve sodium chloride in extract by thorough shaking.

iv) Concentrate solvent to dryness in a nitrogen stream in a moderate manner.

v) High sensitivity of the LC/MS or LC/MS/MS may require further dilution of the test solution with methanol.

vi) Because some of the agricultural chemicals to be analyzed are unstable, especially in methanol, their measurement must be performed immediately after preparation of the test solutions. Prepare fresh solutions for calibration curves. Do not keep test solutions in the autosampler rack of the system at room temperature any longer than necessary.

vii) The obtainment of accurately measured values may require use of a matrix-containing standard solution or the standard addition method.

viii) Because limit of quantitation depend on the apparatus, and concentration factor and injected volume of the test solution, it may be necessary to find the optimum testing conditions.

11. References

None.

12. Type

C

Table 3 Multiresidue Method II for Agricultural Chemicals by LC/MS (Agricultural Products)

Agricultural Chemicals	Substances to be Analyzed	Relative Retention Time	LC/MS Monitoring Ions (m/z)				LC/MS/MS Monitoring Ions (m/z)										Limit of Measurement (ng)	
			Positive Mode		Negative Mode		Positive Mode					Negative Mode						
			Quantitative	Qualitative	Quantitative	Qualitative	Parent	Daughter (Quantitative)		Daughter (Qualitative)		Parent	Duaghter (Quantitative)		Daughter (Qualitative)		LC/MS	LC/ MS/MS
MCPB	MCPB	0.97			***-227***	-141						-227	***-141***		-227		0.278	0.012
Ioxynil	Ioxynil	0.73			***-370***	-126						-370	***-127***		-215		0.001	0.003
Acifluorfen	Acifluorfen	1.04			***-360***	-316						-360	***-316***		-195		0.031	0.023
Imazaquin	Imazaquin	0.54	***312***	267			312	***267.3***	***199***	128	86						0.001	0.001
Cloprop	Cloprop	0.64			***-199***	-127						-199	***-127***		-71		0.088	0.023
Cloransulam-methyl	Cloransulam-methyl	0.81	***430***	398			430	***398***	***370.2***	153							0.008	0.006
4-Chlorophenoxyacetic acid	4-Chlorophenoxyacetic acid	0.55			***-185***	-127						-185	***-127***		-185		0.117	0.011
Cyclanilide	Cyclanilide	0.92			***-272***	-160						-272	***-160***		-228		0.005	0.002
Diclosulam	Diclosulam	0.84	***406***	161			406	***161***		378							0.017	0.003
Dichlorprop	Dichlorprop	0.86			***-233***	-161						-233	***-161***		-125		0.057	0.012
Gibberellin	Gibberellin	0.48			***-345***	-143						-345	***-143***		-239	-221	0.066	0.055
Thidiazuron	Thidiazuron	0.84			***-219***	-100	221	***102***		128		-219	***-100***				0.002	0.002
Thifensulfuron-methyl	Thifensulfuron-methyl	0.50	***388***	167			388	***167***		126	56						0.013	0.001
Triclopyr	Triclopyr	0.81			***-254***	-196						-254	***-196***		-218		0.132	0.006
Triflusulfuron-methyl	Triflusulfuron-methyl	0.92	***493***	264			493	***264***		96							0.005	0.001
1-Naphthalenacetic acid	1-Naphthalenacetic acid	0.63			***-185***	-141						-185	***-141***		-185		0.486	0.073
Haloxyfop	Haloxyfop	1.08	***362***	316			362	***316***		288	91						0.010	0.002
Flumetsulam	Flumetsulam	0.44	***326***	129			326	***129***		326	109						0.008	0.005
Fluroxypyr	Fluroxypyr	0.48			***-253***	-195						-253	***-195***		-233		0.421	0.116
Bromoxynil	Bromoxynil	0.57			***-276***	-79						-276	***-81***	***-78.8***	-276		0.002	0.011
Florasuram	Florasuram	0.55	***360***	129			360	***129***		360	82						0.012	0.003
Fomesafen	Fomesafen	1.04			***-437***	-195						-437	***-195***		-316	-222	0.008	0.005
Forchlorfenuron	Forchlorfenuron	1.00	***248***	129			248	***129***		93							0.004	0.001
Mecoprop	Mecoprop (MCPP)	0.85			***-213***	-141						-213	***-141***		-71		0.028	0.005
	Mecoprop (MCPP-P)	0.85			***-213***	-141						-213	***-141***		-71		—	0.036

- The compounds listed in the "Agricultural chemicals" column are arranged accoarding to the Japanese syllabary. The isomers of each chemical are arranged by their retention times.
- Relative retention times are obtained by dividing the retention time of each substance by the retention time of isoxaflutole (15 to 18 minutes). Each relative retention time is the average of values obtained under three to five different conditions (using different instruments with the same column, mobile phases, flow rate, and temperature).
- Bold italic figures in the "Monitoring Ion" column represent quantitative ions. The remaining ions are qualitative ions.
- Each limit of mesurement is a value at S/N = 10 for a standard solution injected into an LC/MS or LC/MS/MS. For LC/MS/MS, the lower of the two values obtained from two types of apparatus was used, and for LC/MS, each value was obtained from one type of apparatus.
- For agricultural chemicals where both positive and negative ions are shown as monitoring ions, the lower value is taken as its limit of mesurement.
- When 5 μL of a fruit or vegetable test solution prepared according to the above method is injected into an LC/MS(/MS), 0.05 ng corresponds to 0.01 ppm in the sample.

Multiresidue Method for Agricultural Chemicals by GC/MS (Animal and Fishery Products)

1. Substances to be analyzed

See Table 4 for muscle, fat, liver, kidney, fish and shellfish.
See Table 5 for milk, eggs and honey.

2. Apparatus

Gas chromatograph/mass spectrometer (GC/MS)

3. Reagents

Use the reagents listed in Section 2 of the General Rules except for the following:
Reference standards of agricultural chemicals: reference standards of known purity

4. Procedure

1) Extraction

i) Muscle, fat, liver, kidney, fish and shellfish

For muscle, liver, kidney, fish and shellfish, weigh out 20.0 g of the sample. For fat, weigh out 5.00 g of the sample.

Add 20 mL of water and homogenize the sample. Add 100 mL of acetone/*n*-hexane (1:2) and perform homogenization again. Centrifuge the sample at 2500 rpm for 5 minutes. Collect the resulting organic layer. Add 50 mL of *n*-hexane to the remaining layer. Perform homogenization and centrifugal separation at 2500 rpm for 5 minutes. Collect the resulting organic layer and add to the first organic layer. Dry the organic layer over sodium sulfate (anhydrous) and filter. Concentrate the filtrate to dryness at 40°C or lower. Weigh the residue, which is recorded as an extracted fat weight. Dissolve the whole or only a part in acetone/cyclohexane (1:4) so that the solution to be applied to the column for gel permeation chromatography (styrene-divinylbenzene copolymer column) includes 5.0 g of the sample. If the extracted fat content of a 5.0-g sample to be applied to the column exceeds 0.5 grams, adjust the volume of the solution to be applied to the column so that it includes 0.50 grams of fat.

ii) Milk, eggs and honey

For milk and eggs, weigh out 20.0 g of the sample. For honey, weigh out 20.0 g of the sample and dissolve in 20 mL of water.

Add 100 mL of acetonitrile and homogenize the sample. Centrifuge at 2500 rpm for 5 minutes. Collect the resulting organic layer. Add 50 mL of acetonitrile to the remainder. Perform homogenization and centrifugal separation at 2500 for 5 minutes. Collect the resulting organic layer and add to the first organic layer. Add 10 g of sodium chloride and shake. Let stand until the solution is clearly separated into layers. Collect the acetonitrile layer. Dry with sodium sulfate (anhydrous) and filter. Concentrate to dryness at 40°C or lower. For milk and eggs, dissolve the residue in acetone/cyclohexane (1:4) so that the solution to be applied to the column for gel

permeation chromatography (styrene-divinylbenzene copolymer column) includes 5.0 g of the sample. For honey, dissolve the residue in acetone/*n*-hexane (1:1) to make a 10 mL solution.

2) Clean-up

i) Muscle, fat, fish, shellfish, milk and eggs

a. Gel permeation chromatography

Centrifuge the solution obtained from the Extraction step above at 3000 rpm for 5 minutes. Collect the resulting supernatant. Apply 5 mL of the supernatant to a column for gel permeation chromatography (styrene-divinylbenzene copolymer column). Elute the column with acetone/cyclohexane (1:4). Collect a volume of solution eluted from the retention time of Acrinathrin to the finish time of Tricyclazole elution. Concentrate the effluent to dryness at 40°C or lower. Dissolve the residue in 2 mL of acetone/*n*-hexane (1:1).

b. Ethylenediamine-*N*-propylsilanized silica gel column chromatography

Condition an ethylenediamine-*N*-propylsilanized silica gel mini column (500 mg) with 10 mL of acetone/*n*-hexane (1:1). Apply the solution obtained from the Gel permeation chromatography step above. Elute the column with 20 mL of acetone/*n*-hexane (1:1). Collect the entire volume of effluent. Concentrate the effluent to dryness at 40°C or lower. Dissolve the residue in acetone/*n*-hexane (1:1) to make a 1 mL solution (0.5 mL solution for fat). Use this as the test solution.

ii) Liver and kidney

a. Gel permeation chromatography

Centrifuge the solution obtained from the Extraction step above at 3,000 rpm for 5 minutes. Collect the resulting supernatant. Apply 5 mL of the liquid to a column for gel permeation chromatography (styrene-divinylbenzene copolymer column). Elute the column with acetone/cyclohexane (1:4). Collect two volumes of effluent: A fraction eluted from the retention time of Acrinathrin to the finish time of Acrinathrin elution (fraction I); and a fraction eluted form the end time of fraction I elution to the finish time of Tricyclazole elution (fraction II).

b. Ethylenediamine-*N*-propylsilanized silica gel column chromatography

Condition an ethylenediamine-*N*-propylsilanized silica gel mini column (500 mg) with 10 mL of acetone/cyclohexane (1:4). Apply fraction I to the column. Elute the column with 5 mL of acetone/cyclohexane (1:4). Collect the entire volume of fraction. Concentrate the fraction to dryness at 40°C or lower. Dissolve the residue in 1 mL of *n*-hexane.

c. Silica gel column chromatography

Condition a silica gel mini column (690 mg) with 10 mL of *n*-hexane. Apply the solution obtained in "b" to the column. Elute the column with 10 mL of *n*-hexane, and discard the effluent. Elute the column with 15 mL of ether/*n*-hexane (1:19). Collect the entire volume of effluent, and add to fraction II obtained in "a." Concentrate the mixture to dryness at 40°C or lower. Dissolve the residue in acetone/*n*-hexane (1:1) to make a 1 mL solution. Use this as the test solution.

iii) Honey

Condition an ethylenediamine-*N*-propylsilanized silica gel mini column (500 mg) with 10 mL of acetone/*n*-hexane (1:1). Apply 2.5 mL of the acetone/*n*-hexane (1:1) solution obtained from the

Extraction step above to the column. Elute the column with 20 mL of acetone/*n*-hexane (1:1). Collect the entire volume of effluent. Concentrate to dryness at 40°C or lower. Dissolve the residue in acetone/*n*-hexane (1:1) to make a 1 mL solution. Use this as the test solution.

5. Calibration curve

Prepare an acetone solution of the reference standard for each of the agricultural chemicals and mix them all. Dilute portions of the mixture with acetone/*n*-hexane (1:1) to the appropriate concentrations of the reference standards. Analyze 2 μL of each diluted portion by GC/MS. Use peaks in the resulting chromatograms to prepare calibration curves using the peak height or peak area method.

6. Determination

Analyze 2 μL of a test solution by GC/MS. Determine the content of each of the agricultural chemicals using GC/MS results and the calibration curve prepared in the above step 5.

7. Confirmation

Perform GC/MS measurements.

8. Measuring conditions

GC/MS
Column: 5% Phenyl-methyl silicon (0.25 mm I.D. x 30 m length x 0.25 μm film thickness)
Column temperature: 50°C (1 min) - 25°C/min heating - 125°C (0 min) - 10°C/min heating - 300°C (10 min)
Injector temperature: 250°C
Carrier gas: Helium
Ionization mode (voltage): EI (70 eV)
Major monitoring ions (*m/z*): See Tables 4 and 5.
Expected retention times: See Tables 4 and 5.

9. Limit of quantitation

See Tables 4 and 5.
Examples of limit of measurement (ng) are listed.

10. Other

1) Test procedure outline

Extract each of the agricultural chemicals from the samples using acetone/*n*-hexane (1:2) (use acetonitrile for milk, eggs and honey). Clean-up the extracts through gel permeation chromatography and ethylenediamine-*N*-propylsilanized silica gel column chromatography. For liver and kidney, clean-up also with silica gel column chromatography, and for honey, omit the gel permeation chromatography clean-up. Perform measurements and confirmation by GC/MS.

2) Notes

i) In Tables 4 and 5, substances that can be analyzed by the above-described method are arranged in the order of the Japanese syllabary, with the exclusion of those substances comprised of compounds which may be regulated, such as metabolites. When isomers of the same substance have different retention times, the isomer names and times are shown separately in different rows under the

agrichemical substance heading. “Degradate” in parentheses means that the compositional substance to be measured is a degradated product formed during analysis.

ii) The above-mentioned method does not ensure the possibility of simultaneous analyses of every combination of the compounds listed in Tables 4 and 5. Because an interaction between compounds during analysis may lead to their decomposition or interfere with the analysis, check whether the combination of compounds to be analyzed is suitable to the method.

iii) Gas chromatograph/tandem mass spectrometer (GC/MS/MS) can also be used for analyses.

iv) If the ratio of sodium chloride to add (10 grams) to the acetonitrile extract is significantly high, the quantity of the salt can be reduced so long as it is sufficiently saturated.

v) Concentrate solvent to dryness in a nitrogen stream in a moderate manner.

vi) Conditions for gel permeation chromatography are as follows:

Column: Styrene-divinylbenzene copolymer column (20 mm I.D. x 300 mm length) connected to a styrene-divinylbenzene copolymer column (as a guard column, 20 mm I.D. x 100 mm length) or the equivalent.

Mobile phase: Acetone/cyclohexane (1:4)

Flow rate: 5 mL/min

Column temperature: 40°C

Injection volume: 5 mL

Measuring wavelength: 254 nm

Fraction collection: Determine the fraction collection range in the following fashion before analysis: Prepare a 5-mg/L acetone/cyclohexane (1:4) solution of a mixture of Acrinathrin and Tricyclazole. Apply 5 mL of the solution to a column for gel permeation chromatography. While eluting with acetone/cyclohexane (1:4), monitor the retention times at 254 nm. Other appropriate methods can be used to determine the retention times, such as a combination of collection of fractions at predetermined times and measurement of both compounds by GC/MS.

a. Collection range for muscle, fat, fish, shellfish, milk and eggs (see Figure 1)
Retention time of acrinathrin to finish time of tricyclazole elution.
(Example) 58 to 165 mL (volume: 107 mL)

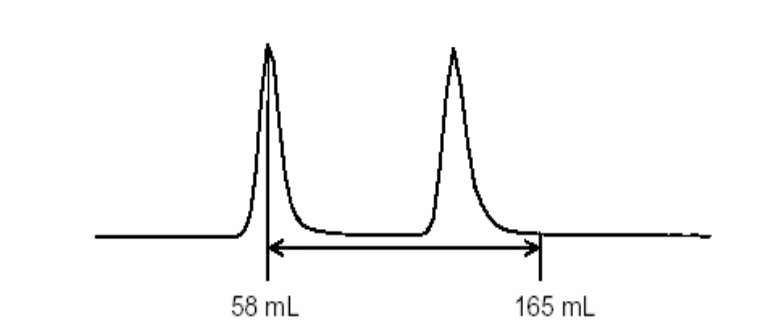

Figure 1 Collection range for muscle, fat, fish, shellfish, milk and eggs

b. Collection range for liver and kidney (see Figure 2)
Fraction I: Retention time of Acrinathrin to finish time of its elution
Fraction II: Finish time of fraction I elution to finish time of Tricyclazole elution
(Example) Fraction I: 58 to 65 mL (volume: 7 mL), Fraction II: 65 to 165 mL (volume: 100 mL)

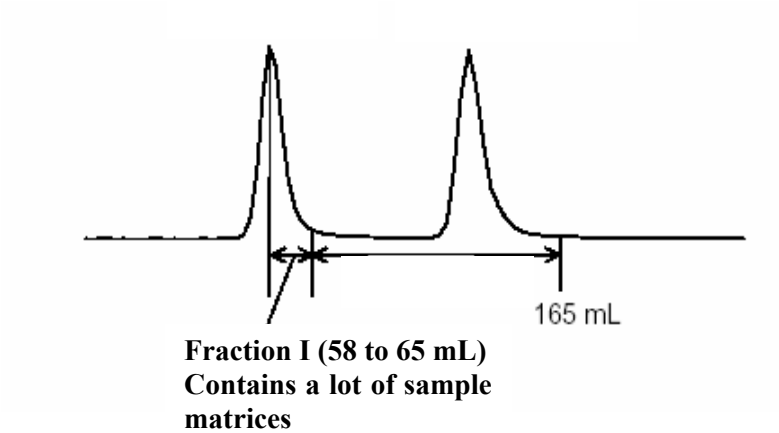

Figure 2 Collection range for liver and kidney

vii) For measurement with a mini column, conduct pretests on the substances to be analyzed of agricultural chemicals under the use conditions to check their elution positions.

viii) For samples with a high fat content, the concentration factors of test solutions are low. If the expected measurement sensitivity is unreachable using such samples, perform the gel permeation chromatography clean-up step and later steps several times, and collect the test solutions. Mix the obtained test solution, and use this mixture as the test solution.

ix) The obtainment of accurately measured values may require use of a matrix-containing standard solution or the standard addition method.

x) Because limit of quantitation depend on the apparatus, and concentration factor and injected volume of the test solution, it may be necessary to determine the optimum test conditions.

11. References

None.

12. Type

C

Table 4 Multiresidue Method for Agricultural Chemicals by GC/MS (Animal and Fishery Products)

Agricultural Chemicals	Substances to be Analyzed	Retention Index	Monitoring Ion (m/z)					Limit of Measurement (ng)
DDT	o,p'-DDT	2289	237	***235***				0.001
	p,p'-DDD	2285	237	***235***				0.001
	p,p'-DDE	2192	***318***	246				0.0005
	p,p'-DDT	2367	237	***235***				0.001
EPTC	EPTC	1360	132	***128***	86			0.002
Azynphos-methyl	Azinphos-methyl	2570	***160***	132				0.006
Atrazine	Atrazine	1755	215	***200***				0.001
Alachlor	Alachlor	1899	237	***188***	160			0.001
Aldrin and Dieldrin	Aldrin	1993	***263***	261				0.003
Allethrin	Allethrin (isomers 1 and 2)	2066	***136***	***123***				0.002
	Allethrin (isomers 3 and 4)	2075	***136***	***123***				0.002
Isoprothiolane	Isoprothiolane	2175	***290***	231	***189***	***162***	118	0.002
Iprodione	Iprodione	2452	316	***314***				0.010
	Iprodione metabilite	2536	***329***	***187***				0.022
Imazalil	Imazalil	2171	***215***	173				0.003
Fenvalerate	Esfenvalerate (isomer 1)	2951	***419***	225	181	***167***		0.059
	Esfenvalerate (isomer 2)	2982	***419***	225	181	***167***		0.003
Ethion	Ethion	2279	384	***231***	153			0.0004
Ethofumesate	Ethofumesate	1951	286	***207***				0.002
Ethoprophos	Ethoprophos	1640	***200***	***158***				0.006
Etridiazole	Etridiazole	1456	213	***211***	183			0.001
Endosulfan	α-Endosulfan	2150	243	***241***	170			0.012
	β-Endosulfan	2277	***241***	195				0.014
	Endosulfan sulphate	2362	274	***272***				0.004
Endrin	Endrin	2255	317	***263***	245			0.005
Oxadiazon	Oxadiazon	2187	344	***258***	***175***			0.001
Oxyfluorfen	Oxyfluorfen	2197	***361***	***252***				0.004
Carboxin	Carboxin	2211	235	***143***	87			0.002
Quinoxyfen	Quinoxyfen	2353	307	***237***				0.001
Quintozene	Quintozene	1764	249	***237***				0.003
Kresoxim-methyl	Kresoxim-methyl	2203	***206***	132	***116***			0.002
Chlordane	cis-Chlordane	2148	375	***373***				0.001
	trans-Chlordane	2121	375	***373***				0.001
	Oxychlordane	2071	389	***387***				0.006
Chlorpyrifos	Chlorpyrifos	1980	316	***314***				0.004
Chlorpyrifos-methyl	Chlorpyrifos-methyl	1885	288	***286***				0.0003
Chlorfenapyr	Chlorfenapyr	2221	406	247	***59***			0.002
Chlorfenvinphos	(E)-Chlorfenvinphos	2046	***323***	***267***				0.009
	(Z)-Chlorfenvinphos	2069	***323***	***267***				0.003
Chloroneb	Chloroneb	1509	208	206	***193***	***191***		0.001
Chlorobenzilate	Chlorobenzilate	2262	253	***251***	139			0.003
Dicofol	Dicofol degradate (4,4'-Dichlorobenzophenone)	2014	250	***139***				0.003
Disulfoton	Disulfoton	1814	274	186	89	***88***		0.001
Cyhalothrin	Cyhalothrin (isomer 1)	2572	***197***	181				0.009
	Cyhalothrin (isomer 2)	2596	***197***	181				0.009
Diphenylamin	Diphenylamine	1634	***169***	168	167			0.0004
Difenoconazole	Difenoconazole (isomer 1)	3019	***323***	265				0.009
	Difenoconazole (isomer 2)	3027	***323***	265				0.007
Cyfluthrin	Cyfluthrin (isomer 1)	2777	226	***206***				0.034
	Cyfluthrin (isomer 2)	2791	226	***206***				0.029
	Cyfluthrin (isomer 3)	2799	226	***206***				0.042
	Cyfluthrin (isomer 4)	2805	226	***206***				0.050
Diflufenican	Diflufenican	2396	394	***266***				0.0002
Cyproconazole	Cyproconazole (isomer 1)	2238	224	***222***				0.008
	Cyproconazole (isomer 2)	2240	224	***222***				0.006

Agricultural Chemicals	Substances to be Analyzed	Retention Index	Monitoring Ion (m/z)					Limit of Measurement (ng)
Cypermethrin	Cypermethrin (isomer 1)	2823	165	***163***	127			0.039
	Cypermethrin (isomer 2)	2837	165	***163***	127			0.025
	Cypermethrin (isomer 3)	2845	165	***163***	127			0.041
	Cypermethrin (isomer 4)	2850	165	***163***	127			0.034
Simazine	Simazine	1748	***201***	186				0.003
Spiroxamine	Spiroxamine (isomer 1)	1896	198	101	***100***			0.002
	Spiroxamine (isomer 2)	1948	198	101	***100***			0.001
Diazinon	Diazinon	1791	304	***179***				0.004
Thiobencarb	Thiobencarb	1985	257	***100***				0.001
Thiometon	Thiometon	1724	125	***88***				0.002
Aldrin and Dieldrin	Dieldrin	2208	277	***263***				0.010
Tetrachlorvinphos	(Z)-Tetrachlorvinphos	2121	331	***329***	109			0.002
Tebuconazole	Tebuconazole	2398	***250***	125				0.005
Tefluthrin	Tefluthrin	1816	197	***177***				0.0004
Deltamethrin and Tralomethrin	Deltamethrin (isomer 1)	3029	***253***	***181***				0.417
	Deltamethrin (isomer 2)	3059	***253***	***181***				0.008
Terbutryn	Terbutryn	1944	241	***226***				0.001
Terbufos	Terbufos	1781	***231***	153				0.002
Triadimenol	Triadimenol	2095	***168***	112				0.010
Triadimefon	Triadimefon	1999	210	***208***	181			0.010
Triazophos	Triazophos	2310	177	***172***	***161***			0.014
Tri-allate	Tri-allate	1827	270	***268***	143			0.003
Trifluralin	Trifluralin	1661	***306***	264				0.001
Parathion	Parathion	1996	***291***	139	87			0.004
Parathion-methyl	Parathion-methyl	1899	***263***	109				0.002
Bioresmethrin	Bioresmethrin (isomer 1)	2401	***171***	***123***				0.223
	Bioresmethrin (isomer 2)	2413	***171***	***123***				0.005
Picolinafen	Picolinafen	2477	***376***	238				0.001
Bitertanol	Bitertanol (isomer 1)	2700	171	***170***	168			0.0004
	Bitertanol (isomer 2)	2714	171	***170***	168			0.002
Bifenthrin	Bifenthrin	2471	***181***	166	165			0.001
Piperonyl butoxide	Piperonyl butoxide	2407	177	***176***	149			0.001
Pyraclofos	Pyraclofos	2664	362	***360***				0.004
Pyridaben	Pyridaben	2732	309	***147***				0.004
Pyriproxyfen	Pyriproxyfen	2578	226	***136***	78			0.002
Pirimicarb	Pirimicarb	1839	238	***166***	72			0.001
Pirimifos-methyl	Pirimifos-methyl	1941	305	***290***				0.001
Pyrethrin	Pyrethrin I	2297	***162***	133	***123***			0.154
	Pyrethrin II	2629	167	***161***	***160***	107		0.258
Vinclozolin	Vinclozolin	1890	287	***285***	212			0.002
Famphur	Famphur	2334	***218***	217				0.005
Fipronil	Fipronil	2049	369	***367***	351	213		0.002
Fenamiphos	Fenamiphos	2152	***303***	288	154			0.009
Fenarimol	Fenarimol	2631	251	***219***				0.007
Fenitrothion	Fenitrothion	1949	***277***	260				0.003
Fenoxaprop-ethyl	Fenoxaprop-ethyl	2667	***361***	288				0.003
Fenobucarb	Fenobucarb	1609	150	***121***				0.001
Fenthion	Fenthion	1990	279	***278***	169			0.001
Fenvalerate	Fenvalerate (isomer 1)	2953	225	***167***				0.006
	Fenvalerate (isomer 2)	2982	225	***167***				0.022
Fenbuconazole	Fenbuconazole	2776	***198***	129				0.004
Fenpropathrin	Fenpropathrin	2495	***181***	125				0.006
Fenpropimorph	Fenpropimorph	1991	303	129	***128***			0.001
Buprofezin	Buprofezin	2204	305	175	***172***	106		0.004
Fluquinconazole	Fluquinconazole	2723	375	342	***340***			0.001
Fludioxonil	Fludioxonil	2169	***248***	154				0.004
Flucythrinate	Flucythrinate (isomer 1)	2847	***199***	157				0.011
	Flucythrinate (isomer 2)	2874	***199***	157				0.017

Agricultural Chemicals	Substances to be Analyzed	Retention Index	Monitoring Ion (m/z)					Limit of Measurement (ng)
Flusilazole	Flusilazole	2202	234	***233***	206			0.001
Flutolanil	Flutolanil	2162	***323***	281	***173***			0.003
Fluridone	Fluridone	2908	329	***328***				0.003
Prochloraz	Prochloraz	2738	310	***180***				0.014
Procymidone	Procymidone	2088	285	***283***				0.003
Propanil	Propanil	1879	217	***163***	***161***			0.013
Propargite	Propargite (isomers 1 and 2)	2402	***350***	173	***135***			0.014
Propiconazole	Propiconazole (isomer 1)	2348	261	***259***				0.007
	Propiconazole (isomer 2)	2362	261	***259***				0.006
Propyzamide	Propyzamide	1789	175	***173***				0.003
Profenofos	Profenofos	2186	339	***337***				0.004
Propetamphos	Propetamphos	1777	***194***	***138***				0.004
Bromopropylate	Bromopropyrate	2487	343	***341***	339			0.005
Hexachlorobenzene	Hexachlorobenzene	1717	286	***284***				0.001
Heptachlor	Heptachlor	1920	337	274	***272***			0.001
	Heptachlor epoxide	2072	***353***	351				0.001
Permethrin	Permethrin (isomer 1)	2706	184	***183***				0.003
	Permethrin (isomer 2)	2723	184	***183***				0.003
Penconazole	Penconazole	2064	***248***	159				0.003
Pendimethalin	Pendimethalin	2047	253	***252***				0.005
Phosmet	Phosmet	2480	161	***160***				0.008
Phorate	Phorate	1700	***260***	231	75			0.010
Malathion	Malathion	1965	***173***	127	125			0.006
Myclobutanil	Myclobutanil	2198	***179***	150				0.006
Methidathion	Methidathion	2113	***145***	85				0.003
Methoxychlor	Methoxychlor	2491	228	***227***				0.002
Methoprene	Methoprene	2097	***191***	153	111	***73***		0.009
Metolachlor	Metolachlor	1977	238	***162***				0.002
Lindane (γ-BHC)	Lindane (γ-BHC)	1775	***219***	183	181			0.005

- The compounds listed in the "Agricultural chemicals" column are arranged according to the Japanese syllabary. The isomers of each chemical are arranged by their retention times.
- Retention indices are obtained based on the retention times of the *n*-alkanes. The figure for each compound is the averaged retention indices obtained from two or three laboratories.
- Bold italic figures in the "Monitoring Ion" column represent the quantitative ions. The remaining ions are qualitative ions.
- Each limit of mesurement is the value at S/N = 10 obtained analyzing 2 μL of a standard solution by GC/MS. Each limit is the lowest of the values obtained from the different laboratories.
- When 2 μL of a test solution prepared according to the above method is injected into a GC/MS, 0.1 ng corresponds to 0.01 ppm for samples other than fat samples[*1] and 0.025 ng for fat samples[*2].

  *1 For a test solution containing 5 g of a sample (final volume of 1 mL)

  *2 For a test solution containing 0.625 g of a sample (containing 0.5 g of fat for an 80% fat content) (final volume of 0.5 mL)

Table 5 Multiresidue Method for Agricultural Chemicals by GC/MS (Animal and Fishery Products)

Agricultural Chemicals	Substances to be Analyzed	Retention Index	Monitoring Ion (m/z)					Limit of Measurement (ng)
DDT	o,p'-DDT	2289	237	***235***				0.001
	p,p'-DDD	2285	237	***235***				0.001
	p,p'-DDE	2192	***318***	246				0.0005
	p,p'-DDT	2367	237	***235***				0.001
Azinphos-methyl	Azinphos-methyl	2570	***160***	132				0.006
Acetamiprid	Acetamiprid	2458	***152***	***126***	90			0.022
Acephate	Acephate	1436	***136***	94				0.003
Azoxystrobin	Azoxystrobin	3083	388	345	***344***			0.002
Atrazine	Atrazine	1755	215	***200***				0.001
Alachlor	Alachlor	1899	237	***188***	160			0.001
Aldicarb	Aldicarb degradate	897	***115***	100				0.012
Aldoxycarb	Aldoxycarb degradate	1131	80	***68***				0.003
Aldrin and Dieldrin	Aldrin	1993	***263***	261				0.003
Allethrin	Allethrin (isomers 1 and 2)	2066	***136***	***123***				0.002
	Allethrin (isomers 3 and 4)	2075	***136***	***123***				0.002
Isofenphos	Isofenphos	2066	255	***213***	121			0.004
	Isofenphos oxon	1998	***229***	201				0.003
Isoprothiolane	Isoprothiolane	2175	***290***	231	***189***	***162***	118	0.002
Iprodione	Iprodione	2452	316	***314***				0.010
Imazalil	Imazalil	2171	***215***	173				0.003
Fenvalerate	Esfenvalerate (isomer 1)	2951	***419***	225	181	***167***		0.059
	Esfenvalerate (isomer 2)	2982	***419***	225	181	***167***		0.003
Ethion	Ethion	2279	384	***231***	153			0.0004
Ethofumesate	Ethofumesate	1951	286	***207***				0.002
Ethoprophos	Ethoprophos	1640	***200***	***158***				0.006
Endosulfan	α-Endosulfan	2150	243	***241***	170			0.012
	β-Endosulfan	2277	***241***	195				0.014
	Endosulfan sulphate	2362	274	***272***				0.004
Endrin	Endrin	2255	317	***263***	245			0.005
Oxadiazon	Oxadiazon	2187	344	***258***	***175***			0.001
Oxyfluorfen	Oxyfluorfen	2197	***361***	***252***				0.004
Omethoate	Omethoate	1596	110	***156***				0.005
Carbaryl	Carbaryl	1912	***144***	115				0.001
Carboxin	Carboxin	2211	235	***143***	87			0.002
Carbofuran	Carbofuran	1742	221	***164***	149			0.001
Quinoxyfen	Quinoxyfen	2353	307	***237***				0.001
Quintozene	Quintozene	1764	249	***237***				0.003
Kresoxim-methyl	Kresoxim-methyl	2203	***206***	132	***116***			0.002
Chlordane	cis-Chlordane	2148	375	***373***				0.001
	trans-Chlordane	2121	375	***373***				0.001
	Oxychlordane	2071	389	***387***				0.006
Chlorpyrifos	Chlorpyrifos	1980	316	***314***				0.004
Chlorpyrifos-methyl	Chlorpyrifos-methyl	1885	288	***286***				0.0003
Chlorfenapyr	Chlorfenapyr	2221	406	247	***59***			0.002
Chlorfenvinphos	(E)-Chlorfenvinphos	2046	***323***	***267***				0.009
	(Z)-Chlorfenvinphos	2069	***323***	***267***				0.003
Chloroneb	Chlorobeb	1509	208	206	***193***	***191***		0.001
Chlorobenzilate	Chlorobenzilate	2262	253	***251***	139			0.003
Dicofol	Dicofol degradate (4,4'-Dichlorobenzophenone)	2014	250	***139***				0.003
Disulfoton	Disulfoton	1814	274	186	89	***88***		0.001
Cyhalothrin	Cyhalothrin (isomer 1)	2572	***197***	181				0.009
	Cyhalothrin (isomer 2)	2596	***197***	181				0.009
Diphenylamine	Diphenylamine	1634	***169***	168	167			0.0004
Difenoconazole	Difenoconazole (isomer 1)	3019	***323***	265				0.009
	Difenoconazole (isomer 2)	3027	***323***	265				0.007

Agricultural Chemicals	Substances to be Analyzed	Retention Index	Monitoring Ion (m/z)					Limit of Measurement (ng)
Cyfluthrin	Cyfluthrin (isomer 1)	2777	226	***206***				0.034
	Cyfluthrin (isomer 2)	2791	226	***206***				0.029
	Cyfluthrin (isomer 3)	2799	226	***206***				0.042
	Cyfluthrin (isomer 4)	2805	226	***206***				0.050
Diflufenican	Diflufenican	2396	394	***266***				0.0002
Cyproconazole	Cyproconazole (isomer 1)	2238	224	***222***				0.008
	Cyproconazole (isomer 2)	2240	224	***222***				0.006
Cypermethrin	Cypermethrin (isomer 1)	2823	165	***163***	127			0.039
	Cypermethrin (isomer 2)	2837	165	***163***	127			0.025
	Cypermethrin (isomer 3)	2845	165	***163***	127			0.041
	Cypermethrin (isomer 4)	2850	165	***163***	127			0.034
Simazine	Simazine	1748	***201***	186				0.003
Dimethoate	Dimethoate	1733	125	93	***87***			0.005
Spiroxamine	Spiroxamine (isomer 1)	1896	198	101	***100***			0.002
	Spiroxamine (isomer 2)	1948	198	101	***100***			0.001
Diazinon	Diazinon	1791	304	***179***				0.004
Thiabendazole	Thiabendazole	2091	***201***	174				0.002
Thiobencarb	Thiobencarb	1985	257	***100***				0.001
Thiometon	Thiometon	1724	125	***88***				0.002
Aldrin and Dieldrin	Dieldrin	2208	277	***263***				0.010
Tetrachlorvinphos	(Z)-Tetrachlorvinphos	2121	331	***329***	109			0.002
Tebuconazole	Tebuconazole	2398	***250***	125				0.005
Tefluthrin	Tefluthrin	1816	197	***177***				0.0004
Deltamethrin and Tralomethrin	Deltamethrin (isomer 1)	3029	***253***	***181***				0.417
	Deltamethrin (isomer 2)	3059	***253***	***181***				0.008
Terbutryn	Terbutryn	1944	241	***226***				0.001
Terbufos	Terbufos	1781	***231***	153				0.002
Triadimenol	Triadimenol	2095	***168***	112				0.010
Triadimefon	Triadimefon	1999	210	***208***	181			0.010
Triazophos	Triazophos	2310	177	***172***	***161***			0.014
Tri-allate	Tri-allate	1827	270	***268***	143			0.003
Trifluralin	Trifluralin	1661	***306***	264				0.001
Norflurazon	Norflurazon	2339	305	***303***	145			0.005
Parathion	Parathion	1996	***291***	139	87			0.004
Parathion-methyl	Parathion-methyl	1899	***263***	109				0.002
Bioresmethrin	Bioresmethrin (isomer 1)	2401	***171***	***123***				0.223
	Bioresmethrin (isomer 2)	2413	***171***	***123***				0.005
Picolinafen	Picolinafen	2477	***376***	238				0.001
Bitertanol	Bitertanol (isomer 1)	2700	171	***170***	168			0.0004
	Bitertanol (isomer 2)	2714	171	***170***	168			0.002
Bifenthrin	Bifenthrin	2471	***181***	166	165			0.001
Piperonyl butoxide	Piperonyl butoxide	2407	177	***176***	149			0.001
Pyridaben	Pyridaben	2732	309	***147***				0.004
Pyriproxyfen	Pyriproxyfen	2578	226	***136***	78			0.002
Pirimicarb	Pirimicarb	1839	238	***166***	72			0.001
Pirimifos-methyl	Pirimifos-methyl	1941	305	***290***				0.001
Pyrethrin	Pyrethrin I	2297	***162***	133	***123***			0.154
	Pyrethrin II	2629	167	***161***	***160***	107		0.258
Vinclozolin	Vinclozolin	1890	287	***285***	212			0.002
Famphur	Famphur	2334	***218***	217				0.005
Fipronil	Fipronil	2049	369	***367***	351	213		0.002
Fenamiphos	Fenamiphos	2152	***303***	288	154			0.009
Fenarimol	Fenarimol	2631	251	***219***				0.007
Fenitrothion	Fenitrothion	1949	***277***	260				0.003
Fenoxaprop-ethyl	Fenoxaprop-ethyl	2667	***361***	288				0.003
Fenobucarb	Fenobucarb	1609	150	***121***				0.001
Fenthion	Fenthion	1990	279	***278***	169			0.001

Agricultural Chemicals	Substances to be Analyzed	Retention Index	Monitoring Ion (m/z)					Limit of Measurement (ng)
envalerate	Fenvalerate (isomer 1)	2953	225	***167***				0.006
	Fenvalerate (isomer 2)	2982	225	***167***				0.022
Fenbuconazole	Fenbuconazole	2776	***198***	129				0.004
Fenpropathrin	Fenpropathrin	2495	***181***	125				0.006
Fenpropimorph	Fenpropimorph	1991	303	129	***128***			0.001
Buprofezin	Buprofezin	2204	305	175	***172***	106		0.004
Fluquinconazole	Fluquinconazole	2723	375	342	***340***			0.001
Fludioxonil	Fludioxonil	2169	***248***	154				0.004
Flucythrinate	Flucythrinate (isomer 1)	2847	***199***	157				0.011
	Flucythrinate (isomer 2)	2874	***199***	157				0.017
Flusilazole	Flusilazole	2202	234	***233***	206			0.001
Flutolanil	Flutolanil	2162	***323***	281	***173***			0.003
Flutriafol	Flutriafol	2152	***219***	164				0.010
Fluvalinate	Fluvalinate (isomer 1)	2966	252	***250***				0.004
	Fluvalinate (isomer 2)	2976	252	***250***				0.004
Fluridone	Fluridone	2908	329	***328***				0.003
Prochloraz	Prochloraz	2738	310	***180***				0.014
Procymidone	Procymidone	2088	285	***283***				0.003
Propanil	Propanil	1879	217	***163***	***161***			0.013
Propargite	Propargite (isomers 1 and 2)	2402	***350***	173	***135***			0.014
Propiconazole	Propiconazole (isomer 1)	2348	261	***259***				0.007
	Propiconazole (isomer 2)	2362	261	***259***				0.006
Propyzamide	Propyzamide	1789	175	***173***				0.003
Profenofos	Profenofos	2186	339	***337***				0.004
Propetamphos	Propetamphos	1777	***194***	***138***				0.004
Propoxur	Propoxur	1612	152	***110***				0.002
Bromopropylate	Bromopropylate	2487	343	***341***	339			0.005
Hexazinone	Hexazinone	2381	172	***171***				0.005
Heptachlor	Heptachlor	1920	337	274	***272***			0.001
Heptachlor	Heptachlor epoxide	2072	***353***	351				0.001
Permethrin	Permethrin (isomer 1)	2706	184	***183***				0.003
	Permethrin (isomer 2)	2723	184	***183***				0.003
Penconazole	Penconazole	2064	***248***	159				0.003
Bendiocarb	Bendiocarb	1674	***166***	***151***				0.003
Pendimethalin	Pendimethalin	2047	253	***252***				0.005
Phosmet	Phosmet	2480	161	***160***				0.008
Phorate	Phorate	1700	***260***	231	75			0.010
Malathion	Malathion	1965	***173***	127	125			0.006
Myclobutanil	Myclobutanil	2198	***179***	150				0.006
Methamidophos	Methamidophos	1230	141	***94***				0.004
Metalaxyl	Metalaxyl	1916	***249***	234	***206***	132		0.006
Methidathion	Methidathion	2113	***145***	85				0.003
Methoxychlor	Methoxychlor	2491	228	***227***				0.002
Methoprene	Methoprene	2097	***191***	153	111	***73***		0.009
Metolachlor	Metolachlor	1977	238	***162***				0.002
Metribuzin	Metribuzin	1888	199	***198***	144			0.003
Lindane (γ-BHC)	Lindane (γ-BHC)	1775	***219***	183	181			0.005

- The coumpounds listed in the "Agricultural chemicals" column are arranged according to the Japanese syllabary. The isomers of each chemical are arranged by their retention times.
- Retention indices are obtained based on the retention times of the *n*-alkanes. The figure of each compound is the averaged retention indices obtained from two or three laboratories.
- Bold italic figures in the" Monitoring Ion" column represent the quantitative ions. The remaining ions are qualitative ions.
- Each limit of mesurement is the value at S/N = 10 obtained by analyzing 2 μL of a standard solution by GC/MS. Each limit is the lowest of the values obtained from different laboratories.
- When 2 μL of a test solution prepared according to the above method is injected into a GC/MS[*1], 0.1 ng corresponds to 0.01 ppm in a sample.

  *1 For a test solution containing 5 g of a sample (final volume of 1 mL)

Multiresidue Method I for Veterinary Drugs, Etc. by HPLC (Animal and Fishery Products)

1. Substances to be analyzed

See Table 6.

2. Apparatus

High performance liquid chromatograph equipped with a diode array detector (HPLC-DAD), High performance liquid chromatograph equipped with a fluorescence detector (HPLC-FL), or Liquid chromatograph/mass spectrometer (LC/MS)

3. Reagents

Use the reagents listed in Section 2 of the General Rules except for the following.
Acetonitrile: Acetonitrile for liquid chromatography
Water: Water for liquid chromatography
Reference standards of veterinary drugs, etc.: reference standards of known purity

4. Procedure

Weigh out 5.00 g of the sample. Add 30 mL of acetonitrile, 20 mL of acetonitrile saturated *n*-hexane, and 10 g of sodium sulfate (anhydrous). Homogenize the mixture. Centrifuge at 3000 rpm for 5 minutes. Collect the resulting organic layer. Separate and collect the acetonitrile layer from the organic layer. Add the remaining *n*-hexane layer to the remainder. Add 20 mL of acetonitrile and vigorously shake. Centrifuge at 3000 rpm for 5 minutes, and remove the resulting *n*-hexane layer. Mix the resulting acetonitrile layer with the first acetonitrile layer. Add 10 mL of *n*-propanol to the mixture. Concentrate the mixture to dryness at 40°C or lower. Dissolve the residue in 1.0 mL of acetonitrile/water (4:6). Place a 0.5-mL layer of acetonitrile saturated hexane on the solution. Centrifuge at 3000 rpm for 5 minutes, and use the resulting acetonitrile-water layer as the test solution.

5. Calibration curve

Prepare a methanol solution of the reference standard for each of the veterinary medicines, etc. Dilute the solutions with acetontrile/water (4:6) to the appropriate concentration of the reference standards. Analyze 10 μL of each solution by HPLC. Use peaks in the resulting chromatograms to prepare a calibration curve using the peak height or peak area method.

6. Determination

Analyze 10 μL of the test solution by HPLC. Determine the content of each of the veterinary medicines, etc. using peaks in the resulting chromatograms and the calibration curve obtained in the above step 5.

7. Confirmation

Perform LC/MS or LC/MS/MS measurements.

8. Measuring conditions

Detection: See table 6.

Column: Octadecylsilanized silica gel (3.0 mm I.D. x 150 mm length, particle size: 3 μm)
Column temperature: 40°C
Mobile phase: Gradient of the acetonitrile/0.05% trifluoroacetic acid concentration from a ratio of 1:99 to 1:0 over 35 minutes, and hold the 1:0 ratio for the next 5 minutes. For LC/MS measurement by ESI (-), 0.1% formic acid is used instead of 0.05% trifluoroacetic acid.
Measuring conditions: See Table 6.

9. Limit of quantitation

See Table 6.

10. Other

1) Test procedure outline

Extract each of the veterinary medicines, etc., from samples using acetonitrile. Remove fat and fat-soluble foreign substances using *n*-hexane, and water and water soluble foreign substances using sodium sulfate (anhydrous). Perform measurements using HPLC-DAD, HPLC-FL, or LC/MS.

2) Notes

i) In Table 6, those substances that can be analyzed by the above-described method are arranged in the order of the Japanese syllabary, with the exception of those substances comprised of compounds which may be regulated, such as metabolites.

ii) The above-mentioned method does not ensure the possibility of simultaneous analyses of every combination of the compounds listed in Table 6. Because an interaction between compounds during analysis may lead to their decomposition or interfere with the analysis, check whether the combination of compounds to be analyzed is suitable to the method.

iii) Because some of the substances in Table 6 are readily susceptible to air oxidation or light degradation, perform all procedures quickly and away from direct sunlight.

iv) If a reference standard is not very soluble in methanol, dissolve the standard in a small volume of *N, N*-dimethylformamide before dilution with methanol.

v) Concentrate solvent to dryness in a nitrogen stream in a moderate manner.

vi) The high sensitivity of LC/MS or LC/MS/MS may require additional dilution of the test solution with the HPLC mobile phase.

vii) If the absolute calibration method does not provide the expected accuracy or precision, the accuracy or precision can be corrected using the stable isotope-labeled internal standard method or the standard addition method.

viii) Because limit of quantitation depend on the apparatus, and concentration factor and injected volume of the test solution, it may be necessary to determine the optimum test conditions.

11. References

1) Mitsunori Murayama et al., J. Food Hyg. Soc. Japan, 32, 155-160 (1991).
2) "Food Hygiene Inspection Guidelines (for Veterinary Drugs and Feed Additives)," edited by the Ministry of Health, Labour and Welfare, pp 26-43, Japan Food Hygiene Association (2003).

12. Type

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Table 6 Multiresidue Method I for Veterinary Drugs, Etc. by HPLC (Animal and Fishery Products)

Veterinary Drugs, Etc.	Substances to be Analyzed	Measuring Wavelength (nm)	Monitoring Ions (m/z)	Limit of Quantitation (mg/kg)
Aklomide	Aklomide		199	0.5
Azaperone	Azaperone		328	0.01
2-Acetylamino-5-nitrothiazole	2-Acetylamino-5-nitrothiazole		186	0.01
Allethrin	Allethrin		303	0.01
Amprolium	Amprolium	245	243	0.01
Ethopabate	Ethopabate		238	0.01
Eprinomectin	Eprinomectin B1a		914	0.03
Emamectin benzoate	Emamectin B1a		886	0.003
Erythromycin	Erythromycin		716	0.01
Enrofloxacin	Enrofloxacin		360	0.005
Oxacillin	Oxacillin		402	0.4
Oxolinic acid	Oxolinic acid	260	262	0.01
Ofloxacin	Ofloxacin		362	0.01
Olaquindox	Olaquindox	260	264	0.01
Ormetoprim	Ormetoprim	230	275	0.02
Oleandomycin	Oleandomycin		688	0.01
Xylazine	Xylazine		221	0.001
Clenbuterol	Clenbuterol		277	0.001
Cloxacillin	Cloxacillin		436	0.1
Clopidol	Clopidol	230	192	0.01
Clorsulon	Clorsulon		380	0.01
Chlorhexidine	Chlorhexidine		506	0.01
Sarafloxacin	Sarafloxacin		386	0.01
Diaveridin	Diaveridin		261	0.02
Diclazuril	Diclazuril	275	382	0.01
Dicyclanil	Dicyclanil		191	0.01
Diflubenzuron	Diflubenzuron		311	0.03
Sulfaquinoxaline	Sulfaquinoxaline	270	301	0.01
Sulfaguanidine	Sulfaguanidine		215	0.01
Sulfachlorpyridazine	Sulfachlorpyridazine		285	0.01
Sulfadiazine	Sulfadiazine		251	0.01
Sulfadimidine	Sulfadimidine	270	279	0.01
Sulfadimethoxine	Sulfadimethoxine	275	311	0.01
Sulfacetamide	Sulfacetamide		215	0.01
Sulfathiazole	Sulfathiazole		256	0.01
Sulfadoxine	Sulfadoxine		311	0.01
Sulfanitran	Sulfanitran		336	0.01
Sulfapyridine	Sulfapyridine		250	0.01
Sulfabenzamide	Sulfabenzamide		277	0.01
Sulfamethoxazole	Sulfamethoxazole		254	0.01
Sulfamethoxypyridazine	Sulfamethoxypyridazine		281	0.01
Sulfamerazine	Sulfamerazine	270	265	0.01
Sulfamonomethoxine	Sulfamonomethoxine	275	281	0.01
Tylosine	Tylosine		916	0.01
Danofloxacin	Danofloxacin		358	0.01
Thiabendazole	Thiabendazole	300	202	0.01
	5-Hydroxythiabendazole	300	218	0.01
Tiamulin	Tiamulin		494	0.05
Thiamphenicol	Thiamphenicol	225	354*	0.01
Tilmicosin	Tilmicosin	235	870	0.05 (muscle, fat and offal), 0.01 (milk)
Dexamethasone	Dexamethasone		393	0.01
Temephos	Temephos		467	0.05

Veterinary Drugs, Etc.	Substances to be Analyzed	Measuring Wavelength (nm)	Monitoring Ions (m/z)	Limit of Quantitation (mg/kg)
Trichlorphon	Trichlorphon		258	0.1
Tripelennamine	Tripelennamine		256	0.002-0.02
Trimethoprim	Trimethoprim	230	291	0.01
Tolfenamic acid	Tolfenamic acid		261	0.005
Trenbolone acetate	α-Trenbolone (Liver)	340	271	0.002
	β-Trenbolone (Muscle)	340	271	0.002
Nafcillin	Nafcillin		447	0.01
Nalidixic acid	Nalidixic acid	260	233	0.01
Nitroxynil	Nitroxynil		291	0.05
Halofuginone	Halofuginone	245		0.01
Nicarbazin	N,N'-Bis(4-nitrophenyl)urea	350	303	0.02
Hydrocortisone	Hydrocortisone		405	0.01
Pyrantel	Pyrantel		207	0.01
Pyrimethamine	Pyrimethamine	230	249	0.02
Famphur	Famphur		326	0.02
Phenoxymethylpenicillin	Phynoxymethylpenicillin		383	0.02
Fenobucarb	Fenobucarb		208	0.01
Flunixin	Flunixin		297	0.005
Flubendazole	Flubendazole	315	314	0.01
Prednisolone	Prednisolone		361	0.002
Brotizolam	Brotizolam		395	0.0005
5-Propylsulphonyl-1H-benzimidazole-2-amine	5-Propylsulphonyl-1H-benzimidazole-2-amine	300	240	0.01
Florfenicol	Florfenicol		356	0.01
Marbofloxacin	Marbofloxacin		363	0.01
Miloxacin	Miloxacin		264	0.01
Methylprednisolone	Methylprednisolone		375	0.01
Mebendazole	Mebendazole		296	0.01
Monensin	Monensin		679	0.001
Morantel	Morantel		221	0.01
Lasalocid	Lasalocid		613	0.01
Rifaximin	Rifaximin		786	0.01
Lincomycin	Lincomycin		407	0.05
Levamisole	Levamisole	220	205	0.01
Robenidine	Robenidine		334	0.01

- The compounds listed in the “Veterinary Drugs, Etc.” column are arranged according to the Japanese syllabary.
- Wavelengths are measured by a high performance liquid chromatograph equipped with a UV detector or a diode array detector.
- High performance liquid chromatography with a fluorescence detector (ex 300 nm, and em 370 nm) can be used to measure 5-Propylsulphonyl-1H-benzimidazole-2-amine and Thiabendazole.
- Ions are monitored with an ESI positive mode by LC/MS (except Thiamphenicol, which is monitored with an ESI negative mode).

Multiresidue Method II for Veterinary Drugs, Etc. by HPLC (Animal and Fishery Products)

1. Substances to be analyzed

See Table 7.

2. Apparatus

A high performance liquid chromatograph equipped with a diode array detector (HPLC-DAD), or high performance liquid chromatograph equipped with an electrochemical detector (HPLC-ECD), or liquid chromatograph/mass spectrometer (LC/MS)

3. Reagents

Use those reagents listed in Section 2 of the General Rules except for the following.

Acetonitrile: Acetonitrile for liquid chromatography

Water: Water for liquid chromatography

Methanol: Methanol for liquid chromatography

Phosphate buffer (pH 3.0)

Solution 1: Dissolve 27.2 g of monopotassium phosphate in a small amount of water. Add water to make a 1,000 mL solution.

Solurion 2: Dissolve 2.31 g of phosphoric acid in a small amount of water. Add water to make a 100 mL solution.

Mix Solution 1 and 2 and adjust the pH to 3.0.

Phosphate buffer (pH 5.0)

Solution 1: Dissolve 27.2 g of monopotassium phosphate in a small amount of water. Add water to make a 1,000 mL solution.

Solution 2: Dissolve 3.48 g of dipotassium phosphate in a small amount of water. Add water to make a 100 mL solution.

Mix liquids 1 and 2 and adjust the pH to 5.0.

Reference standards of veterinary drugs, etc.: reference standards of known purity

4. Procedure

1) Extraction

i) Muscle, liver, kidney, other edible offal, and milk

Weigh out 5.00 g of the sample. Add 30 mL of a 95% aqueous solution of acetonitrile. Homogenize the mixture, and centrifuge at 2,500 rpm for 5 minutes. Collect the resulting acetonitrile layer. Add 30 mL of a 95% aqueous solution of acetonitrile to the remainder and vigorously shake. Centrifuge at 2500 rpm for 5 minutes. Collect the resulting acetonitrile layer, and add to the first layer.

ii) Fat

Weigh out 5.00 g of the sample. Add 30 mL of a 95% aqueous solution of acetonitrile and 30 mL of *n*-hexane. Homogenize the mixture, and centrifuge at 2,500 rpm for 5 minutes. Collect the resulting acetonitrile layer. Add 30 mL of a 95% aqueous solution of acetonitrile to the remainder

and vigorously shake. Centrifuge at 2,500 rpm for 5 minutes, collect the resulting acetonitrile layer, and add to the first layer.

2) Clean-up

i) Synthetic magnesium silicate column chromatography

Suspend 8 g of synthetic magnesium silicate for column chromatography in acetonitrile. Place the suspension into a chromatograph tube with an inner diameter of 15 mm and a length of 300 mm. Let the actonitrile run until a small volume is left on top of the magnesium silicate layer. Condition the column with 100 mL of acetonitrile. Apply the solution obtained from the Extraction step above to the column. Elute the column with 30 mL of acetonitrile. Collect the entire volume of effluent. Add 100 mL of *n*-hexane to the effluent, and vigorously shake for 3 minutes with a shaking machine. Let stand until the solution is clearly separated into layers. Collect the resulting acetonitrile layer, and concentrate to dryness at 40°C or lower. Dissolve the residue in 4 mL of a phosphate buffer (pH 5.0). Add 6 mL of water.

ii) Octadecylsilanized silica gel column chromatography

Condition an octadecylsilanized silica gel mini column (360 mg) with 10 mL of methanol, 10 mL of water, and 2 mL of a phosphate buffer (pH 5.0) in this order. Load the solution obtained in the last step of the Clean-up step i) above to the column. Elute the column with 5 mL of phosphate buffer (pH 5.0), and discard the effluent. Elute the column with 10 mL of a 40% aqueous solution of methanol. Collect the entire volume of effluent. Elute the column with 10 mL of a 70% aqueous solution of acetonitrile. Collect the entire volume of effluent. Concentrate each effluent to dryness separately at 40°C or lower. Dissolve the residue of the effluent of the 40% aqueous solution of methanol in 2.0 mL of a 5% aqueous solution of methanol. Dissolve the residue of the effluent of the 70% aqueous solution of acetonitrile in 2.0 mL of a 35% aqueous solution of methanol. Use these solutions as test solutions.

5. Calibration curve

Prepare a methanol solution of the reference standard for each of the veterinary medicines, etc. For solutions of veterinary substances, etc., designated as “A” in the C18 Fraction column of Table 7, dilute with a 5% aqueous solution of methanol to the appropriate concentration, and for those designated as “B” in the same column, dilute with a 35% aqueous solution of methanol to the appropriate concentration to prepare the standard solutions for calibration curves. Analyze 200 μL of each solution by HPLC. Use peaks in the resulting chromatograms to prepare a calibration curve using the peak height or peak area method.

6. Determination

Analyze 200 μL of the test solution by HPLC. Determine the content of each of the veterinary medicines, etc., using HPLC results and the calibration curve prepared in the above step 5.

7. Confirmation

Use LC/MS or LC/MS/MS.

8. Measuring conditions

Detection: See Table 7.

Column: Octadecylsilanized silica gel (4.6 mm I.D. x 250 mm length; particle size of 5 μm)

Column temperature: 40°C

Mobile phases:

HPLC-DAD: Gradient of the acetonitrile/water/phosphate buffer (pH 3.0) concentration from 1:18:1 to 14:5:1 over 30 minutes. Hold the ratio of 14:5:1 for the next 10 minutes.

HPLC-ECD: Acetonitrile/0.085 mol/L monopotassium phosphate (2:3)

Detecting conditions: See Table 7.

9. Limit of quantitation

See Table 7.

10. Other

1) Test procedure outline

Extract each of the veterinary medicines, etc., from the samples with a 95% aqueous solution of methanol. Clean-up the extracts through synthetic magnesium silicate column chromatography. Remove fat and fat-soluble foreign substances using *n*-hexane. Clean-up through octadecylsilanized silica gel column chromatography. Perform the measurements using HPLC-DAD or HPLC-ECD.

2) Notes

i) In Table 7, substances that can analyzed by the above-described method are arranged in the order of the Japanese syllabary, with the exception of those substances comprised of compounds which may be regulated, such as metabolites.

ii) The above-mentioned method does not ensure the possibility of simultaneous analyses of every combination of the compounds listed in Table 7. Because an interaction between compounds during analysis may lead to their decomposition or interfere with the analysis, check whether the combination of compounds to be analyzed is suitable to the method.

iii) Because some of the substances in Table 7 are readily susceptible to air oxidation or light degradation, perform all procedures quickly and away from direct sunlight.

iv) If a reference standard is not very soluble in methanol, dissolve the standard in a small volume of *N, N*-dimethylformamide before dilution with methanol.

v) Concentrate solvent to dryness in a nitrogen stream in a moderate manner.

vi) The high sensitivity of LC/MS or LC/MS/MS may require additional dilution of the test solution with the HPLC mobile phase.

vii) For two types of test solutions obtained by octadecylsilanized silica gel column chromatography, Table 7 lists the fractions in which they are expected to be eluted. Because elution behavior depends on the lot and storage conditions of the octadecylsilanized silica gel mini column, check whether the elution behavior is correct using reference standards.

viii) Because limit of quantitation depend on the apparatus, and concentration factor and injected volume of the test solution, it may be necessary to determine the optimum test conditions.

11. References

1) Hisaya Terada, et al., Journal of Nagoya City Public Health Research Institute, 35, 101-105 (1989).

12. Type

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Table 7 Multiresidue Method II for Veterinary Drugs, Etc. by HPLC (Animal and Fishery Products)

Veterinary Drugs, Etc.	Substances to be Analyzed	Measuring Wavelength (nm)	Fraction C18	Li,mit of Quantitation (mg/kg)
Ethopabate	Ethopabate	280	A	0.01
Oxibendazole	Oxibendazole	300	B	0.01
Ormethoprim	Ormethoprim	280	A	0.02
Closantel	Closantel	230	B	0.05
Clopidol	Clopidol	280	A	0.01
Melengestrol acetate	Melengestrol acetate	300	B	0.001
Diclazuril	Diclazuril	300	B	0.01
Sulfaquinoxaline	Sulfaquinoxaline	270	A	0.01
Sulfachlorpyridazine	Sulfachlorpyridazine	270	A	0.01
Sulfadiazine	Sulfadiazine	270	A	0.01
Sulfadimidine	Sulfadimidine	270	A	0.01
Sulfadimethoxine	Sulfadimethoxine	270	A	0.01
Sulfathiazole	Sulfathiazole	270	A	0.01
Sulfadoxine	Sulfadoxine	270	A	0.01
Sulfanitran	Sulfanitran	270	A	0.01
Sulfapyridine	Sulfapyridine	270	A	0.01
Sulfabenzamide	Sulfabenzamide	270	A	0.01
Sulfamethoxazole	Sulfamethoxazole	270	A	0.01
Sulfamethoxypyridazine	Sulfamethoxypyridazine	270	A	0.01
Sulfamerazine	Sulfamerazine	270	A	0.01
Sulfamonomethoxine	Sulfamonomethoxine	270	A	0.01
Zeranol	Zeranol	*	B	0.0005
Thiabendazole	Thiabendazole	320	A	0.01
	5-Hydroxythiabendazole	320	A	0.01
Thiamphenicol	Thiamphenicol	230	A	0.01
Trimethoprim	Trimethoprim	280	A	0.02
Trenbolone acetate	α-Trenbolone (Liver)	350	B	0.002
	β-Trenbolone (Mscle)	350	B	0.002
Novobiocin	Novobiocin	300	B	0.01
Nicarbazin	N,N'-Bis(4-nitrophenyl)urea	300	B	0.01
Flubendazole	Flubendazole	300	B	0.002
5-Propylsulphonyl-1H-benzimidazole-2-amine	5-Propylsulphonyl-1H-benzimidazole-2-amine	280	A	0.01
Levamisole	Levamisole	230	A	0.002

- The compounds in the "Veterinary Drugs, Etc." column are arranged according to the Japanese syllabary.
- Wavelengths are measured by a high performance liquid chromatograph equipped with a UV detector or a diode array detector.
- Zeranol is measured with high performance liquid chromatography equipped with an electrochemical detector (Eg 850 mV, E1 500 mV and E2 750 mV).
- In the "C18 Fraction" column, A represents a 40% methanol-water fraction and B represents a 70% acetonitrile fraction.