

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 Compositional 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 outermost shell for a large sample, and homogenize..
- (12) For eggs, use only the white and yolk of the sample. Mix thoroughly, and homogenize the mixture (except if a maximum residue limit in the white or yolk 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 ethylsilanized 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 μ L 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 $\mu\text{mol}/\text{min}$ 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 2×10^{-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 2×10^{-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 $\mu\text{mol}/\text{min}$ 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 benzenethiolate

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 μ L 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.

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 μ L 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.

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/aminopropylsilylated 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/ aminopropylsilylated silica gel layered mini column. For grains, beans, nuts and seeds, clean-up with an octadecylsilylated silica gel mini column, followed by a graphite-carbon/aminopropylsilylated 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 agricultural substance heading. “Degradate” in parentheses means that the composition substance to be measured is a degraded 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 apparatus, 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, Difenconazole, 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	Isazophos	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
Ethalfuralin	Ethalfuralin	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	Ethofenprox	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
Quinoxifen	Quinoxifen	2347	237				0.001
Quinoclamín	Quinoclamín	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	Tolclophos-methyl	1899	267	265			0.001
Tolfenpyrad	Tolfenpyrad	3106	383	171			0.032
Napropamide	Napropamide	2165	271	128	72		0.020
Nitrothal-isopropyl	Nitrothal-isopropyl	2009	254	236	212		0.003
Norflurazon	Norflurazon	2348	303	173	145		0.006
Paclbutrazol	Paclbutrazol	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
Pyraclufos	Pyraclufos	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	Phenthoate	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	Fluacrypyrim	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 measurement is the value at S/N = 10 obtained using 2 μ L 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 μ L 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 octadecylsilylated 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/aminopropylsilylated 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: Octadecylsilylated 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 agricultural 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			LC/MS	LC/MS/MS					
			Quantitative	Qualitative		Quantitative	Qualitative		Parent	Daughter (Quantitative)	Daughter (Qualitative)	Parent	Daughter (Quantitative)	Daughter (Qualitative)							
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					360	251		360	144				0.004	0.003	
lprovalicarb	lprovalicarb	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																		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						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					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															0.009	0.001
	Tralkoxydim (isomer 2)	1.08	330		331	170					330	137.9		284						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					0.003	0.001	
Pyrazolynate	Pyrazolynate	1.26	439		441	173					439	91		229	173				0.004	0.001	
Pyrifthalid	Pyrifthalid	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	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	
Benzo fenap	Benzo fenap	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												91					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 measurement 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 measurement.
- 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 octadecylsilylated 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 agricultural 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			LC/MS	LC/MS/MS				
			Quantitative	Qualitative	Quantitative	Qualitative	Parent	Daughter (Quantitative)	Daughter (Qualitative)	Parent	Daughter (Quantitative)	Daughter (Qualitative)						
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
Triflusaluron-methyl	Triflusaluron-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
Haloxifop	Haloxifop	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 (MCP)	0.85			-213	-141						-213	-141		-71		0.028	0.005
	Mecoprop (MCP-P)	0.85			-213	-141						-213	-141		-71		—	0.036

- 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 measurement 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 measurement.
- 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*-propylsilylated silica gel column chromatography

Condition an ethylenediamine-*N*-propylsilylated 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 from the end time of fraction I elution to the finish time of Tricyclazole elution (fraction II).

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

Condition an ethylenediamine-*N*-propylsilylated 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*-propylsilylated 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

agricultural substance heading. “Degradate” in parentheses means that the compositional substance to be measured is a degraded 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)

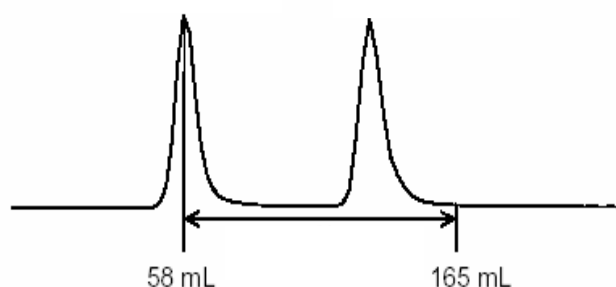


Figure 1 Collection range for muscle, fat, fish, shellfish, milk and eggs

- 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)

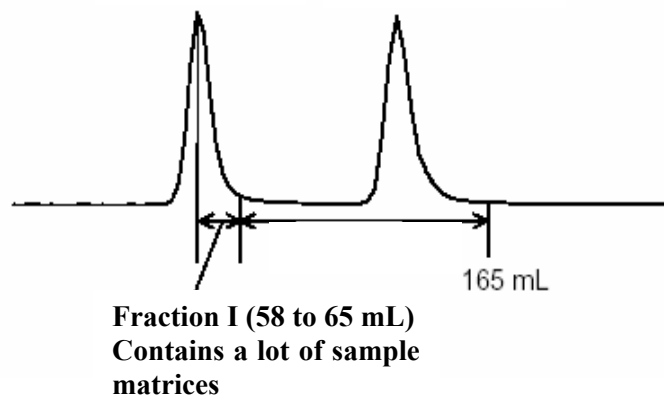


Figure 2 Collection range for liver and kidney

- 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
Azinphos-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 metabolite	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
Quinoxifen	Quinoxifen	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
Pyraclufos	Pyraclufos	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	Bromopropylate	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 measurement is the value at S/N = 10 obtained analyzing 2 μ L of a standard solution by GC/MS. Each limit is the lowest of the values obtained from the different laboratories.
- When 2 μ L 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
Quinoxifen	Quinoxifen	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
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 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 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 measurement is the value at S/N = 10 obtained by analyzing 2 μ L of a standard solution by GC/MS. Each limit is the lowest of the values obtained from different laboratories.
- When 2 μ L 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 acetonitrile/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: Octadecylsilylated 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
Olaquinox	Olaquinox	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.

Solution 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 acetonitrile 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) Octadecylsilylated silica gel column chromatography

Condition an octadecylsilylated 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: Octadecylsilylated silica gel (4.6 mm I.D. x 250 mm length; particle size of 5 μ m)

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 octadecylsilylated silica gel column chromatography. Perform the measurements using HPLC-DAD or HPLC-ECD.

2) Notes

- i) In Table 7, 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 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 octadecylsilylated 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 octadecylsilylated silica gel mini column, check whether the elution behavior is correct using reference standards.
- viii) Because limit of quantitation depends 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.