Multi-residue Method II for Agricultural Chemicals by LC-MS (Animal and Fishery Products)

1. Analytes

See Table.

2. Instrument

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

3. Reagents

Use the reagents listed in Section 3 of the General Rules, except the following.

Styrene-divinylbenzene copolymer column: Stainless tube of 12 mm in inside diameter and 100-300 mm in length packed with styrene-divinylbenzene copolymer prepared for gel permeation chromatography (hard-type gel, 16 μm in particle diameter, 30 Å in pore size), or other cartridge with equal separation characteristics.

Reference standards of each agricultural chemical: Agricultural chemicals which clearly shows its purity. (If the analytical method for each agricultural chemical specifies its purity, use the solution having the purity. if not specified, the solution contains not less than 95 % of the analyte is recommended to use for this method.)

4. Procedure

1) Extraction

Weigh 10.0 g (5.00 g for fat) of sample. Add 15 mL (10 mL for watery sample like milk or egg) of 2 vol% acetic acid to the sample, homogenize, add 60 mL of acetone/*n*-hexane (2:3, v/v), homogenize again. Centrifuge at 3,000 rpm for 5 minutes, and filter the organic layer and the aqueous layer with suction. Add 40 mL of acetone/*n*-hexane (2:3, v/v) to the residue, homogenize, and filter all the residue with suction. Combine the resulting filtrates and concentrate to not more than 20 mL (about 18 mL is desirable) at below 40°C. Dissolve the residue in 5-7 g of sodium chloride.

2) Clean-up

i) Except for honey

a) Porous diatomaceous earth column chromatography

Transfer all the solution obtained in **1)** to a porous diatomaceous earth column (to hold 20 mL of solution). Let stand the column 10 minutes, elute with 150 mL of ethyl acetate, concentrate the eluate at below 40°C, and remove the solvent. Weigh the residue, dissolve in acetone/cyclohexane (3:17, v/v) to make exactly 20 mL, and use this solution as the extract.

b) Gel permeation chromatography

Centrifuge the extract obtained in a) at 3,000 rpm for 10 minutes, transfer 2 mL of the supernatant solution to the gel permeation chromatography column (styrene-divinylbenzene copolymer column), and elute with acetone/cyclohexane (3:17,

v/v). Collect the fraction eluted from the retention time of acrinathrin to the finish time of tricyclazole elution, concentrate at below 40°C, and remove the solvent. Dissolve the residue in 2 mL of acetone.

c) Trimethylaminopropylsilanized silica gel column and ethylenediamine-*N*-propylsilanized silica gel column chromatography

Connect an ethylenediamine-*N*-propylsilanized silica gel cartridge (500 mg) to the bottom of a trimethylaminopropylsilanized silica gel cartridge (500 mg), add 10 mL of acetone, and discard the effluent. Transfer the solution obtained in b) to the cartridge, elute with 18 mL of acetone, collect the total eluate, concentrate the eluate at below 40°C, and remove the solvent. Dissolve the residue in methanol to make exactly 5 mL (2.5 mL for fat), and use this solution as the test solution.

ii) Honey

a) Porous diatomaceous earth column chromatography

Transfer all the solution obtained in **1)** to a porous diatomaceous earth cartridge (to hold 20 mL of solution). Let stand for 10 minutes, elute with 150 mL of ethyl acetate, concentrate the eluate at below 40°C, and remove the solvent. Dissolve the residue in acetone to make exactly 20 mL.

b) Trimethylaminopropylsilanized silica gel column and ethylenediamine-*N*-propylsilanized silica gel column chromatography

Connect an ethylenediamine-*N*-propylsilanized silica gel cartridge (500 mg) to the bottom of a trimethylaminopropylsilanized silica gel cartridge (500 mg), add 10 mL of acetone, and discard the effluent. Transfer 2 mL of the solution obtained in **a)** to the cartridge, elute with 18 mL of acetone, collect the total eluate, concentrate the eluate at below 40°C, and remove the solvent. Dissolve the residue in methanol to make exactly 5 mL, and use this solution as the test solution.

5. Calibration curve

Prepare standard solutions (acetonitrile) of each agricultural chemical. Mix them, prepare solutions (methanol) of several concentration, inject each solution to LC-MS/MS, and make calibration curves by peak-height or peak-area method. When the test solution is prepared following the above procedure, the sample containing 0.01 mg/kg of each agricultural chemical gives the test solution of 0.002 mg/L in concentration.

6. Quantification

Inject the test solution to LC-MS/MS, and calculate the concentration of each agricultural chemical from the calibration curves made in **5**.

7. Confirmation

Confirm using LC-MS/MS.

8. Measurement conditions

Example

Column: Octadecylsilanized silica gel, 2.0 mm in inside diameter, 150 mm in length and 3 μm in

particle diameter

Column temperature: 40°C

Mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: 10 mmol/L ammonium acetate solution

Mobile phase B: acetonitrile

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	90	10
20	5	95
35	5	95
35	90	10

Flow rate: 0.2 mL/min

Ionization mode: ESI (+ or -)

Major monitoring ions (*m/z*): See Table.

Injection volume: 3 µm

Expected retention time: See Table.

9. Limit of quantification

See Table.

10. Explanatory note

1) Outline of analytical method

The method consists of extraction of each agricultural chemical from sample with acetone/*n*-hexane (2:3, v/v) under acidic condition with acetic acid, clean-up with a porous diatomaceous earth column, gel permeation chromatography and the connected column of a trimethylaminopropylsilanized silica gel cartridge and an ethylenediamine-*N*-propylsilanized silica gel cartridge (omit a gel permeation chromatography for honey), and quantification and confirmation using LC-MS/MS.

2) Notes

i) This analytical method is intended for agricultural chemicals which logPow is more than approximately -0.8 (ex. methamidophos) and are non-acidic.

ii) Table shows analytes which are applicable to this method in the order of the Japanese syllabary. Agricultural chemicals could include chemicals like metabolites which are inapplicable to this method. Isomers having different retention time are listed separately in “Analytes”.

iii) This method does not ensure all simultaneous analysis using analytes listed in Table. In advance, confirm the interaction by the intended combination of analytes does not interfere decomposition and measurement.

iv) In suction filtration, use filter aid (diatomaceous earth). In the first extraction, leaving the residue (solids) in a centrifuge tube after centrifugation, filter only the organic layer and the

aqueous layer as much as possible with suction. If the residue dropped on the filter aid, try to take only the residue as possible after suction filtration, and back it to the centrifuge tube. In the second extraction, not performing centrifugation, filter all the amount with suction. If no residue was left after centrifugation in the first extraction, the second extraction is not needed. Wash the centrifuge tube with 40 mL of acetone/*n*-hexane (2:3, v/v), and filter the washing with suction.

v) In the case of fat of cow, honey and so on, it may congeal if the extract is left a while after concentration. Even if not congealed, the extract may increase its viscosity and not uniformly dispersed on the surface of the diatom earth when applied to a porous diatomaceous earth column. This may lower the current speed of the eluate or stuff the column up. So procedure after concentration should be performed as quickly as possible. If congealed, melt the extract using approximately 40°C of a water bath and apply to the column promptly.

vi) When dissolve the extract obtained by concentration in sodium chloride (5-7 g), it needs to be completely saturated. For example, use of ultrasonic washing machine may help the saturation. If the amount of sodium chloride is too much for addition, it may be reduced as far as it is enough for saturation.

vii) After applying the extract to a porous diatomaceous earth column, wash the recovery flask with 20 mL of ethyl acetate two times, and wash again with 110 mL of ethyl acetate. If insoluble material was remained in the recovery flask, washing by ultrasonic washing machine with appropriate addition of anhydrous sodium sulfate will help the cleaning. Anhydrous sodium sulfate is useful to disperse the material.

viii) In elution using a porous diatomaceous earth column, the current speed should be adjusted 4-5 mL/min. For the adjustment, use of stop valve (made from materials with a little possibility of pollution such as teflon) attached to the mouth of the column is recommended.

ix) When it is clear that the residue after porous diatomaceous earth column chromatography is not more than 2 g, there is no need to weigh the residue.

x) When the residue after porous diatomaceous earth column chromatography is more than 2 g, perform the following procedure. Dilute the extract with acetone/cyclohexane (3:17, v/v) by a dilution ratio using integral value so that the residue amount which apply to a gel permeation chromatography column will be about not more than 0.2 g. Repeat the procedure which centrifuge the extract at 3,000 rpm for 10 minutes, transfer 2 mL of the supernatant solution to a gel permeation chromatography column, and elute the eluate as same times as the multiple number for the dilution, and combine the eluates. For example, fat sample leave about 3.5 g of residue. In this case, dilute two times, perform clean-up by gel permeation chromatography two times, and combine the eluates.

xi) An example of the condition for gel permeation chromatography is shown below.

Column: Styrene-divinylbenzene copolymer column (12 mm in inside diameter, 300 mm in

length, hard-type gel, 16 μm in particle diameter and 30 Å in pore size) connected with styrene-divinylbenzene copolymer column (12 mm in inside diameter, 100 mm in length hard-type gel, 16 μm in particle diameter and 30 Å in pore size) as a guard column, or other column with equal characteristics.

Mobile phase: Acetone/cyclohexane (3:17, v/v)

Current speed: 3 mL/min

Column temperature: 45°C

Injection volume: 2 mL

Collection range:[*1] From retention time of acrinathrin to finish time of tricyclazole elution

Confirmation of collection range: Determine in advance by preparing a standard solution of acrinathrin/tricyclazole (5 mg/L[*2]) in mobile phase, transferring to the gel permeation chromatography column, and monitoring[*3] the retention times at 254 mm.

*1 If clean-up was insufficient, set the range from the middle of each retention time of acrinathrin and fluvalinate to finish time of tricyclazole elution as the collection range. When confirm the collection range, acrinathrin and fluvalinate should be measured separately because their peaks cannot be detected separately.

*2 The concentration of each standard solution may be changed depending on the sensitivity of the instrument.

*3 Other appropriate methods, for example, taking eluates with proper intervals and measuring by each fraction can be used.

xii) Concentration and complete removal of the solvent should be performed gently in nitrogen stream.

xiii) Before measurement using cartridges, perform pretest on elution of each agricultural chemical and confirm the elution position under the use condition.

xiv) To obtain accurate measured values, matrix-containing standard solution or standard addition method may be required.

xv) Limit of quantification differs by the instrument to use, and the measuring condition. Consider optimum condition appropriately.

11. References

None

12. Type

C

Table. Multi-residue Method II for Agricultural Chemicals by LC-MS (Animal and Fishery Products)

Agricultural Chemicals	Analytes	Relative retention time(RRT	Major monitoring ions (*m* /z)					Limit of quantification （mg/kg）
Azamethiphos	Azamethiphos	0.74	+325→183	+325→112				0.01
Acetamiprid	Acetamiprid	0.59	+223→126	+223→99	+223→73			0.01
Acephate	Acephate	0.21	+184→143	+184→101	+184→95			0.01
Azoxystrobin	Azoxystrobin	1.00	+404→372	+404→344				0.01
Aldoxycarb	Aldoxycarb	0.38	+240→148	+240→86	+223→148	+223→86	+223→76	0.01
Dipropyl Isocinchomeronate	Dipropyl Isocinchomeronate	1.00	+252→210	+252→164	+252→140			0.004
Imazalil	Imazalil	1.04	+297→201	+297→159	+297→69			0.01
Imidacloprid	Imidacloprid	0.54	+256→209	+256→175				0.01
Indoxacarb	Indoxacarb (*S* , *R*)	1.21	+528→218	+528→203	+528→150			0.01
Ethopabate	Ethopabate	0.64	+238→206	+238→136	-236→192	-236→162		0.01
Oxamyl	Oxamyl	0.38	+237→90	+237→72				0.01
Oxydemeton-methyl	Oxydemeton-methyl	0.35	+247→169	+247→109				0.01
Omethoate	Omethoate	0.25	+214→183	+214→125				0.01
Carbaryl	Carbaryl	0.83	+202→145	+202→127				0.01
Carpropamid	Carpropamid	1.15	+334→196	+334→139	+334→103			0.01
Carbendazim	Carbendazim	0.54	+192→160	+192→132				0.01
Coumafos/Coumaphos	Coumafos/Coumaphos	1.19	+363→307	+363→227				0.01
Clothianidin	Clothianidin	0.52	+250→169	+250→132	-248→166	-248→58		0.01
Chromafenozide	Chromafenozide	1.09	+395→339	+395→175	-393→149	-393→105		0.01
Chlorantraniliprole	Chlorantraniliprole	0.92	+484→453	+484→286	+482→451	+482→284		0.01
Chloroxuron	Chloroxuron	0.98	+291→164	+291→75	+291→72	+291→46		0.01
Diuron	Diuron	0.86	+233→160	+233→72	+233→46			0.01
Dimethenamid	Dimethenamid（*RS* racemate)	0.98	+276→244	+276→168				0.01
Dimethoate	Dimethoate	0.58	+230→199	+230→171	+230→125			0.01
Dimethomorph	Dimethomorph (*E* ,*Z*)	0.92	+388→301	+388→165				0.01
Thiacloprid	Thiacloprid	0.65	+253→126	+253→99	+253→90			0.01
Thiabendazole	Thiabendazole	0.63	+202→175	+202→131				0.01*
Thiamethoxam	Thiamethoxam	0.46	+292→211	+292→281				0.01
Tebuthiuron	Tebuthiuron	0.65	+229→172	+229→116				0.01
Tebufenozide	Tebufenozide	1.09	+353→297	+353→133	-351→149	-351→105		0.01*
Trifloxystrobin	Trifloxystrobin	1.24	+409→186	+409→145				0.01*
Buquinolate	Buquinolate	1.00	+362→316	+362→204				0.01
Parbendazole	Parbendazole	0.91	+248→216	+248→173	+248→145			0.01
Bitertanol	Bitertanol	1.05	+338→269	+338→99	+338→70			0.01
Pyridalyl	Pyridalyl	1.53	+492→183	+492→109	+490→164	+490→109		0.01
Ferimzone	Ferimzone (*E*)	0.91	+255→132	+255→91				0.01
Ferimzone	Ferimzone (*Z*)	0.86	+255→132	+255→91				0.01
Fenamidone	Fenamidone	1.00	+312→236	+312→92				0.01
Fentrazamide	Fentrazamide	1.17	+350→197	+350→154				0.01
Fenpyroximate	Fenpyroximate (*E*)	1.35	+422→366	+422→138	+422→135			0.005
Fenpyroximate	Fenpyroximate (*Z*)	1.28	+422→366	+422→138				0.005
Fluazifop-p-butyl	Fluazifop-p-butyl	1.30	+384→328	+384→282				0.01
Flubendazole	Flubendazole	0.78	+314→282	+314→123				0.01
Flumiclorac pentyl	Flumiclorac pentyl	1.28	+441→354	+441→308	+424→354	+424→308		0.01
Propachlor	Propachlor	0.90	+212→170	+212→94				0.01
Propoxur	Propoxur	0.79	+210→168	+210→111	+210→93			0.01
Bendiocarb	Bendiocarb	0.79	+224→167	+224→109				0.01
Methomyl	Methomyl	0.42	+163→106	+163→88				0.01
Metalaxyl	Metalaxyl	0.84	+280→220	+280→192				0.01
Metominostrobin	Metominostrobin (*E*)	0.90	+285→196	+285→194				0.01

1) The analytes are listed in the order of the Japanese syllabary. Agricultural chemicals may include chemicals like metabolites which are inapplicable to this method. Isomers having different retention time are listed separately in “Analytes”.

2) Relative retention time (RRT) is the relative value for retention time of Isoxaflutole, and shows the average of values which obtained from laboratories.

3）The figures in "Major monitoring ions" shows [precursor ion → product ion] in LC-MS/MS measurement, and the code before the figures (+ or -) means ionization mode (ESI (+) or ESI (-)) in ESI measurement. Each ion is listed in descending order.

4）We described 0.01 mg/kg (or the minimum additive concentration) as the limit of quantification when S/N ratio at the peak of an analyte in at least one sample in recovery test which conducted with fortification level 0.01 ppm (or minimum fortification level) was not less than 10. When recovery test with additive concentration 0.01 ppm was not existed: we conducted recovery test using matrix-containing standard solution, and when the S/N ratio at peak of an analyte which corresponded to 0.01 mg/kg in sample got value which is not less than 10 at least in one sample, we assumed the limit of quantification was 0.01 mg/kg and described with "*".