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

1. Analytes

See Table.

2. Applicable foods

Animal and Fishery Products

3. Instrument

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

4. Reagents

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

20 mmol/L Ammonium acetate solution (pH 4.5): Weigh 1.54 g of ammonium acetate, dissolve the sample in about 950 mL of water, adjust the pH to 4.5 with acetic acid, and add water to make a 1 L solution.

Reference standards of agricultural chemicals: Reference standards of known purities for each agricultural chemical. (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.)

5. Procedure

1) Extraction

i) Except for honey

Weigh sample exactly, add ethanol/water (1:1, w/w), homogenize, and then take the sample equivalent to 10.0 g. Add 100 mL of acetone and 1 mL of acetic acid to the sample, and homogenize. Centrifuge at 3,000 rpm for 5 minutes, and collect the organic layer. Add 10 mL of ethanol/water (1:1, w/w) to the residue, stir, add 50 mL of acetone and 1 mL of acetic acid, and homogenize. Centrifuge as described above, collect the organic layer, combine with the first organic layer, and add acetone to make exactly 200 mL. Take exactly 10 mL of the solution, and concentrate to about 1 mL at below 40ºC. Add 30 mL of *n*-hexane, and extract with shaking three times with 30 mL each of acetonitrile saturated with *n*-hexane. Combine the resulting filtrates, concentrate the filtrate at below 40°C, and remove the solvent. Dissolve 2 mL of acetonitrile/20 mmol/L ammonium acetate (pH 4.5), (9:1, w/w) to the residue.

ii) Honey

Weigh sample exactly, add ethanol/water (1:1, w/w), homogenize, and then take the sample equivalent to 10.0 g. Add 100 mL of acetone and 1 mL of acetic acid to the sample, and homogenize. Centrifuge at 3,000 rpm for 5 minutes, and collect the organic layer. Add 10

mL of ethanol/water (1:1, w/w) to the residue, stir, add 50 mL of acetone and 1 mL of acetic acid, and homogenize. Centrifuge as described above, collect the organic layer, combine with the first organic layer, and add acetone to make exactly 200 mL. Take exactly 10 mL of the solution, and remove the solvent at below 40ºC. Dissolve 2 mL of acetonitrile/20 mmol/L ammonium acetate (pH 4.5), (9:1, v/v) to the residue.

2) Clean-up

i) Octadecylsilanized silica gel column chromatography

Add 5 mL each of acetonitrile and acetonitrile/20 mmol/L ammonium acetate (pH 4.5), (9:1, v/v) to a octadecylsilanized silica gel cartridge (1,000 mg), and discard the effluent. Transfer the solution obtained in **1)** to the cartridge, elute with 15 mL of acetonitrile/20 mmol/L ammonium acetate (pH 4.5), (9:1, v/v), collect the total eluate including load solution. Concentrate the eluate at below 40°C, and remove the solvent. Dissolve the residue in 2 mL of ethyl acetate/toluene/methanol (2:1:2, v/v/v).

ii) Chromatography of graphitized carbon black column and silica gel column

Add 15 mL of ethyl acetate/toluene/methanol (2:1:2, v/v/v) to a graphitized carbon black cartridge (250 mg), and discard the effluent. Add 10 mL ethyl acetate/trimethylamine/ toluene/methanol (40:1:20:40, v/v/v/v) and 5 mL of ethyl acetate/toluene/methanol (2:1:2, v/v/v) to a silica gel cartridge (500 mg) sequentially, and discard the effluents. Connect the silica gel cartridge to the lower part of graphitized carbon black cartridge, transfer the solution obtained in **i)** to the cartridge, elute with 5 mL of ethyl acetate/toluene/methanol (2:1:2, v/v/v). Detach the graphitized carbon black cartridge, elute with 5 mL of ethyl acetate/toluene/methanol (2:1:2, v/v/v) to the silica gel cartridge, collect the total eluate including load solution. Concentrate the eluate at below 40°C and remove the solvent. Dissolve the residue in acetonitrile/20 mmol/L ammonium acetate (pH 4.5) to make exactly 1 mL, and use this solution as the test solution.

6. Calibration curve

Dissolve reference standard of each agricultural chemical in the appropriate solvent respectively, and prepare stock standard solutions. Mix these stock standard solutions appropriately, prepare solutions, acetonitrile/20 mmol/L ammonium acetate (pH 4.5), (1:1, v/v) of several concentrations, inject each standard solution to LC-MS/MS, and make a calibration curve by peak-height or peak-area method.

7. Quantification

Inject the test solution to LC-MS/MS, and calculate the concentration of each agricultural chemical from the calibration curves made in **6**.

8. Confirmation

Confirm using LC-MS/MS.

9. Measurement conditions

Column: Octadecylsilanized silica gel, 3.0 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: 20 mmol/L ammonium acetate solution (pH 4.5)

Mobile phase B: Acetonitrile

Time (min)	Mobile phase A (%)	Mobile phase B: (%)
0.0	99	1
5.0	99	1
35.0	0	100
40.0	0	100

Ionization mode: ESI(+), ESI(-)

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

Injection volume: 5 μL

Expected retention time: See Table.

10. Limit of quantification

See Table.

11. Explanatory note

1) Outline of analytical method

The method consists of extraction of each agricultural chemical from sample with acetone acidified with acetic acid, clean-up with acetonitrile/hexane partitioning (omitted for honey), octadecylsilanized silica gel cartridge, graphitized carbon black cartridge and silica gel cartridge, and quantification and confirmation using LC-MS/MS.

2) Notes

i) Table list the analytes for which this method is applicable in the order they appear in the Japanese syllabary. Note that the maximum residue limits (MRLs) defined for some agricultural chemicals include not only the parent compounds, but also their metabolites or other transformation products, which are inapplicable to this method. Isomers with different retention times are listed as separate “Analytes”.

ii) This method does not ensure simultaneous analysis of all of the analytes listed in the Table. In advance, confirm that degradation or interference does not occur as the result of

interaction between the target analytes.

iii) Some analytes listed in the Table reduce with time under analysis operation, all procedure should be performed promptly.

iv) Use reference standards of agricultural chemicals in the highest purity, if possible.

v) If the suspended solids is found in the acetone extract after fixing the volume, centrifuge, and the supernatant can be used next measurement.

vi) Concentration and complete removal of the solvent should be performed under a gentle stream of nitrogen.

vii) Before using cartridges for clean-up, confirm the elution position of each agricultural chemical.

viii) Dilution of test solution, Matrix-matched calibration or standard addition may be required to obtain accurate measurement results.

ix) Because the limit of quantification differs depending on the instrument used, the concentration rate of the test solution, and the injection volume, it may be necessary to optimize the conditions.

12. Reference

None

13. Type

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

Veterinary Drugs	Analytes[*1]	Relative retantion time (RRT)[*2]	Monitoring ions (*m/z*)[*3]					Limit of quantification (mg/kg)
Dipropyl Isocinchomeronat	Dipropyl Isocinchomeronat	1.00	+252→210	+252→192	+252→164			0.004
Isoprothiolane	Isoprothiolane	1.05	+291→231	+291→189	+291→145			0.01
Ethopabate	Ethopabate	0.74	+238→206	+238→136	-236→192	-236→132		0.01
Ormetoprim	Ormetoprim	0.56	+275→259	+275→231	+275→123	+275→81		0.01
Oleandomycin	Oleandomycin	0.72	+689→544	+689→158	+688→544	+688→158		0.01
Carbetamide	Carbetamide	0.75	+237→192	+237→118				0.01
Xylazine	Xylazine	0.62	+221→164	+221→90				0.01
Cloxacillin	Cloxacillin	0.74	+436→220	+436→178	+436→150			0.01
Ketoprofen	Ketoprofen	0.83	+255→209	+255→105	+255→77			0.01
Melengestrol acetate	Melengestrol acetate	1.10	+397→337	+397→279				0.01
Dicloxacillin	Dicloxacillin	0.79	+470→254	+470→212	+470→184			0.01
Sulfadiazine	Sulfadiazine	0.51	+251→156	+251→92				0.01*
Sulfanitran	Sulfanitran	0.83	-334→136	-334→133				0.01
Thiabendazole	Thiabendazole	0.70	+202→175	+202→131				0.01*
Tiamulin	Tiamulin	0.83	+494→192	+494→119				0.01
Thiamphenicol	Thiamphenicol	0.56	-354→290	-354→185	-354→79			0.01
Tilmicosin	Tilmicosin	0.70	+870→174	+870→88				0.01
Temephos	Temephos	1.13	+467→419	+467→405	+467→125			0.01
Triclabendazole	Triclabendazole	1.10	+361→346	+361→274	+359→344	+359→274		0.01
Tripelennamine	Tripelennamine	0.72	+256→211	+256→119	+256→91			0.01
Trimethoprim	Trimethoprim	0.54	+291→275	+291→230	+291→123			0.01
Tolfenamic acid	Tolfenamic acid	0.99	+262→209	-260→216				0.01
Nitroxinil	Nitroxinil	0.66	-289→162	-289→127				0.01
Pyrimethamine	Pyrimethamine	0.69	+249→233	+249→198	+249→177			0.01
Famphur	Famphur	0.97	+326→281	+326→217	+326→93			0.01*
Praziquantel	Praziquantel	0.91	+313→203	+313→83				0.01
Flunixin	Flunixin	0.82	+297→279	+297→264	+297→109	-295→251	-295→231	0.01
Flubendazole	Flubendazole	0.84	+314→282	+314→123	+314→95			0.01
Propoxur	Propoxur	0.83	+210→168	+210→111				0.01
Florfenicol	Florfenicol	0.68	-356→336	-356→185				0.01
Menbutone	Menbutone	0.83	+259→241	+259→185	+259→159	+259→127		0.01
Ractopamine	Ractopamine	0.58	+302→164	+302→121	+302→107			0.01
Levamisole	Levamisole	0.51	+205→178	+205→91				0.01
Warfarin	Warfarin	0.91	+309→251	+309→163	+309→121			0.001

1) The analytes, which are applicable to the testing methods, are listed in the order of the Japanese syllabary. Note that the maximum residue limits (MRLs) defined for some agricultural chemicals include not only the parent compounds, but also their metabolites or other transofrmation products, which are inapplicable to the testing methods. Isomers with different RRTs are listed in the column of analytes separately.

2) Relative retantion time (RRT) is the relative value to one in Isoxaflutole (retention time: 25-26 min.). The RRT above show the average values obtained in laboratories.

3) The figures in "Monitoring ions" show [precursor ion → product ion] in LC-MS/MS measurement, and the code before the figures means ionization mode in ESI (-) or ESI (+) measurement. Each ion are listed in decending order.

4) In a spike-and-recovery assessment with 0.01 ppm of analytes (or minimum concentration of analytes) , the values of limit of quantification were set to be 0.01 mg/kg (or minimum concentration of analytes) when a peak value of S/N was 10 or more from at least one food commodity. When there were no results obtained with 0.01 ppm of analytes, matrix spike standard solutions were used. If a peak value of S/N was 10 or more from at least one food commodity with the matrix spike standard solution, the estimated value of limit of quatification was set to be 0.01 mg/kg and expressed with "*" in the table.