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

1. Analytes

See Table 4.

2. Instrument

Liquid chromatograph-mass spectrometer (LC-MS)

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

3. Reagents

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

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

4. Procedure

1) Extraction

i) Grains, beans, nuts and seeds

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

Add 50 mL of acetonitrile, homogenize, and filter with suction. Add 20 mL of acetonitrile to the residue on the filter paper, homogenize, and filter with suction. Combine the resulting filtrates, and add acetonitrile to make exactly 100 mL.

Take a 20 mL aliquot of the extract, add 10 g of sodium chloride and 20 mL of 0.01 mol/L hydrochloric acid, and shake for 15 minutes. Let stand, and discard the separated aqueous layer.

Add 10 mL of acetonitrile to an octadecylsilanized silica gel cartridge (1,000 mg) and discard the effluent. Transfer the acetonitrile layer to the cartridge, elute with 2 mL of acetonitrile, collect the total eluates, dehydrate with anhydrous sodium sulfate, and filter out the anhydrous sodium sulfate. Concentrate the filtrate at below 40°C and remove the solvent. Dissolve the residue in 2 mL of acetone/triethylamine/*n*-hexane (20:0.5:80, v/v/v).

ii) Fruits, vegetables, herbs, tea and hops

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

Add 50 mL of acetonitrile, homogenize, and filter with suction. Add 20 mL of acetonitrile to the residue on the filter paper, homogenize, and filter with suction. Combine the resulting filtrates, and add acetonitrile to make exactly 100 mL.

Take a 20 mL aliquot of the extract, add 10 g of sodium chloride and 20 mL of 0.01 mol/L hydrochloric acid, and shake. Let stand, and discard the separated aqueous layer.

Dehydrate the acetonitrile layer with anhydrous sodium sulfate, and filter out the anhydrous sodium sulfate. Concentrate the filtrate at below 40°C and remove the solvent. Dissolve the residue in 2 mL of acetone/triethylamine/*n*-hexane (20:0.5:80, v/v/v).

2) Clean-up

Add 5 mL each of methanol and acetone to a silica gel cartridge (500 mg) sequentially, and discard the effluents. Add 10 mL of *n*-hexane and discard the effluents. Transfer the solution obtained in 1) to the cartridge, add acetone/triethylamine/*n*-hexane (20:0.5:80, v/v/v) and discard the effluents. Wash the container which had the solution obtained in 1) with 2 mL of acetone/methanol (1:1, v/v), transfer the washing to the silica gel cartridge, elute with 18 mL of acetone/methanol (1:1, v/v), concentrate the eluate at below 40°C and remove the solvent. Dissolve the residue in methanol to make exactly 4 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 concentrations. Inject 5 μL of each standard solution to LC-MS or LC-MS/MS, and make calibration curves by peak-height or peak-area method.

6. Quantification

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

7. Confirmation

Confirm using LC-MS or LC-MS/MS.

8. Measurement conditions

Column: Octadecylsilanized silica gel, 2-2.1 mm in inside diameter. 150 mm in length and 3-3.5 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.

Flow rate: 0.2 mL/min

Mobile phase A: 5 mmol/L ammonium acetate solution

Mobile phase B: 5 mmol/L ammonium acetate-methanol solution

Time (min)	Mobile phase A (%)	Mobile phase 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 4.

Expected retention time: See Table 4.

9. Limit of quantification

See Table 4.

Note that the table shows examples of limits of measurement (ng), not limits of quantification.

10. Explanatory note

1) Outline of analytical method

The method consists of extraction of each agricultural chemical from sample with acetonitrile, salting out under acidic condition, dehydration, clean-up with an octadecylsilanized silica gel cartridge for grains, beans, nuts and seeds, (omit for fruits, vegetables, herbs, tea and hops), clean-up with a silica gel cartridge, and quantification and confirmation using LC-MS or LC-MS/MS.

2) Notes

i) Table 4 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 4. In advance, confirm that degradation or interference does not occur as the result of interaction between the target analytes.

iii) If the quantity of sodium chloride (10 g) is too large to add to the acetonitrile extract, it may be reduced so long as saturation is achieved. Because the agricultural chemicals have highly polar character, shake the extracts thoroughly to dissolve sodium chloride.

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

v) Because some agricultural chemicals are slightly soluble in acetone/triethylamine/*n*-hexane (20:0.5:80, v/v/v), wash with eluting solvent, (2 mL of acetone/methanol (1:1, v/v)) after washing operation in clean-up with a silica gel cartridge.

vi) Depending on the sensitivity of the LC-MS or LC-MS/MS, it may be necessary to dilute the test solution with methanol.

vii) Because some agricultural chemicals are particularly unstable in methanol, LC-MS/MS analysis should be performed immediately after preparation of a test solution. The standard solutions used to determine the calibration curves should be prepared just prior to use. Do not leave the test solutions in the autosampler rack at room temperature for a long time.

viii) 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.

11. References

None

12. Type

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Table 4. Multi-residue Method II for Agricultural Chemicals by LC-MS (Agricultural Products)

Agricultural Chemicals	Analytes	RRT	Monitoring ions for LC-MS (m/z)					Monitoring ions for LC/MS/MS (m/z)												Limit of measurement (ng)	
			Positive mode		Negative mode			Positive mode						Negative mode							
			Quant.	Conf.	Quant.	Conf.		Precursor		Product (quantification)		Product (confirmation)		Precursor	Product (quantification)		Product (confirmation)		LC-MS	LC-MS/MS
2,4-D	2,4-D	0.73			***-161***		-163							-219	***-161***		-125		0.025	0.15
MCPA	MCPA	0.73			***-141***		-199							-199	***-141***		-199		0.007	0.118
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
Azimsulfuron	Azimsulfuron	0.52			***-423***		-424	425		***182***		139							0.116	0.005
Iodosulfuron methyl	Iodosulfuron methyl	0.72	***508***	509				508		***167***		508							0.011	0.004
Imazaquin	Imazaquin	0.54	***312***	267				312		***267***	***199***	128	86						0.001	0.001
Imazosulfuron	Imazosulfuron	0.54			***-411***	-413		415		***156***		78		-411	***-229***		-154	-153	0.017	0.079
Ethametsulfuron-methyl	Ethametsulfuron-methyl	0.66	***411***	412				411		***196***		168							0.001	0.003
Ethoxysulfuron	Ethoxysulfuron	0.83	***399***	400				399		***261***		218							0.002	0.002
Clodinafop acid	Clodinafop acid	0.9			***-310***		-238	312		***266***		238							0.007	0.016
Clofencet	Clofencet	0.6	***279***	261				279		***261***		166							0.004	0.024
Cloprop	Cloprop	0.64			***-199***	-127								-199	***-127***		-71		0.088	0.023
Cloransulam-methyl	Cloransulam-methyl	0.81	***430***	398				430		***398***	***370***	153							0.008	0.006
Chlorimuron-ethyl	Chlorimuron-ethyl	0.8			***-413***		-415	415		***186***		83							0.018	0.003
Chlorsulfuron	Chlorsulfuron	0.55	***358***	360				358		***141***		167							0.008	0.005
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
Cyclosulfamuron	Cyclosulfamuron	0.99	***422***	444				422		***261***	***139***	218	69	-420	***-265***		-78		0.008	0.002
Diclomezine	Diclomezine	1.21	***255***	257				257	255	***140***	***89***	158	75	-253	***-182***		-40		0.255	0.087
Dichlorprop	Dichlorprop	0.86			***-233***	-161								-233	***-161***		-125		0.057	0.012
Cinosulfuron	Cinosulfuron	0.48	***414***	436				414		***183***		157	83	-412	***-154***		-66		0.011	0.002
Gibberellin	Gibberellin	0.48			***-345***	-143								-345	***-143***		-239	-221	0.066	0.055
Sulfentrazone	Sulfentrazone	0.85			***-387***		-385	387		***307***		146							0.017	0.146
Sulfosulfuron	Sulfosulfuron	0.59			***-469***		-470	471		***211***		261							0.001	0.007
Thidiazuron	Thidiazuron	0.84			***-219***	-100		221		***102***		128		-219	***-100***				0.002	0.002
Thifensulfuron-methyl	Thifensulfuron-methyl	0.5	***388***	167				388		***167***		126	56						0.013	0.001
Triasulfuron	Triasulfuron	0.62	***402***	404				402		***167***		141							0.008	0.009
Triclopyr	Triclopyr	0.81			***-254***	-196								-254	***-196***		-218		0.132	0.006
Triflusulfuron-methyl	Triflusulfuron-methyl	0.92	***493***	264				493		***264***		96							0.005	0.001
Trifloxysulfuron	Trifloxysulfuron	0.72			***-436***		-437	438		***182***		257							0.007	0.003
Tribenuron-methyl	Tribenuron-methyl	0.56	***396***	418				396		***181***	***155***	364		-394	***-153***		-55		0.058	0.003
Naptalam	Naptalam	0.66			***-290***	-246		292		***144***		149		-290	***-246***		-142		0.02	0.016
1-Naphthaleneacetic acid	1-Naphthaleneacetic acid	0.63			***-185***	-141								-185	***-141***		-185		0.486	0.073

Haloxyfop	Haloxyfop	1.08	***362***	316				362		***316***		288	91						0.01	0.002
Halosulfuron methyl	Halosulfuron methyl	0.69			***-433***	-435		435		***182***		83		-433	***-252***		-154	-78	0.02	0.005
Pyrazosulfuron-ethyl	Pyrazosulfuron-ethyl	0.66	***415***	437				415		***182***	***181***	139	83	-413	***-154***		-232		0.029	0.002
Fenhexamid	Fenhexamid	1.18	***302***	304				302		***97***	***96***	55		-300	***-264***		-249		0.037	0.005
Flazasulfuron	Flazasulfuron	0.54	***408***	430				408		***182***	***181***	227	139	-406	***-154***		-251		0.011	0.001
Primisulfuron-methyl	Primisulfuron-methyl	0.93			***-467***			491		***264***		250		-467	***-226***	***-225***	-175	-126	0.009	0.008
Fluazifop-butyl	Fluazifop acid	0.91	***328***	350				328		***283***	***282***	255	254	-326	***-254***		-206		0.027	0.023
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
Prosulfuron	Prosulfuron	0.84			***-418***			420		***167***		141		-418	***-139***		-138	-107	0.009	0.006
Propoxycarbazone-sodium	Propoxycarbazone	0.55	***399***	416				421	399	***180***	***115***	264	134	-397	***-156***		-113		0.047	0.007
Bromoxynil	Bromoxynil	0.57			***-276***	-79								-276	***-81***	***-78.8***	-276		0.002	0.011
Florasulam	Florasulam	0.55	***360***	129				360		***129***		360	82						0.012	0.003
Penoxsulam	Penoxsulam	0.73			***-482***			484		***195***		164							0.01	-
Bensulfuron-methyl	Bensulfuron-methyl	0.91	***411***	433				433	411	***179***	***148***	278	181	-409	***-154***		-254		0.009	0.004
Fomesafen	Fomesafen	1.04			***-437***	-195								-437	***-195***		-316	-222	0.008	0.005
Foramsulfuron	Foramsulfuron	0.51	***453***	475				453		***182***		254	83	-451	***-296***		-267		0.068	0.005
Forchlorfenuron	Forchlorfenuron	1	***248***	129				248		***129***		93							0.004	0.001
Mecoprop	Mecoprop (MCPP)	0.85			***-213***	-141								-213	***-141***		-71		0.028	0.005
	Mecoprop (MCPP-P)	0.85			***-213***	-141								-213	***-141***		-71		―	0.036
Mesosulfuron-methyl	Mesosulfuron-methyl	0.59	***504***	526				504		***182***		306	83	-502	***-267***		-347		0.066	0.003
Metosulam	Metosulam	0.73	***418***	420				420	418	***176***	***174***	189	140	-416	***-179***		-66		0.017	0.004
Metsulfuron-methyl	Metsulfuron-methyl	0.43	***382***	404				382		***168***	***167***	198	57	-380	***-139***		-107		0.015	0.047

・The compounds are listed in the order of the Japanese syllabary.

・Relative retention time (RRT) is the relative value when isoxaflutole (retention time: 15-18 min.) is 1. The RRT above shows the average values obtained from two to five measurement environments (which have same measurement conditions such as a type of the column, mobile phase, flow rate, temperature, but different instruments).

・Monitoring ions in bold italic font are for quantification (Quant.); the others are for confirmation (Conf.).

・The limit of measurement is the value at S/N=10 when a standard solution is injected into an LC-MS or LC-MS/MS. The limits of measurement were obtained from one or two laboratories, and the lower value was adopted.

・For the limit of measurement of the agricultural chemicals which show both positive ion and negative ion as a monitoring ion, the lower value is adopted as the limit of measurement.

・When 5μL of a test solution for fruits and vegetables prepared by the described method is injected into an LC-MS(/MS), 0.05 ng corresponds to 0.01 ppm.