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

1. Analytes

See Table 1.

2. Instrument

Gas chromatograph/mass spectrometer (GC-MS)

3. Reagents

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

0.5 mol/L Phosphate buffer (pH 7.0): Weigh 52.7 g of dipotassium hydrogenphosphate (K_2HPO_4) and 30.2 g of potassium dihydrogenphosphate (KH_2PO_4), dissolve in about 500 mL of water, adjust the pH to 7.0 with 1 mol/L sodium hydroxide or 1 mol/L hydrochloric acid, and add water to make a 1 L solution.

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 0.5 mol/L phosphate buffer (pH 7.0), and shake for 10 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 acetonitrile/toluene (3:1, 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 0.5 mol/L phosphate buffer (pH 7.0), 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 acetonitrile/toluene (3:1, v/v).

2) Clean-up

Add 10 mL of acetonitrile/toluene (3:1, v/v) to a graphite carbon/aminopropylsilanized silica gel layered cartridge (500 mg/500 mg) and discard the effluent. Transfer the solution obtained in 1) to the cartridge, elute with 20 mL of acetonitrile/toluene (3:1, v/v), collect the total eluate, and concentrate to below 1 mL at below 40°C. Add 10 mL of acetone and concentrate to below 1 mL at below 40°C, add 5 mL of acetone, and remove the solvent. Add acetone/*n*-hexane (1:1, v/v) to the residue to make exactly 1 mL, and use this solution as the test solution.

5. Calibration curve

Prepare standard solutions (acetone) of each agricultural chemical. Mix them, prepare solutions (acetone/*n*-hexane (1:1, v/v)) of several concentrations. Inject 2 µL of each standard solution to GC-MS, and make calibration curves by peak-height or peak-area method.

6. Quantification

Inject 2 µL of the test solution to GC-MS, and calculate the concentration of each agricultural chemical from the calibration curves made in **5**.

7. Confirmation

Confirm using GC-MS.

8. Measurement conditions

GC-MS

Column: 5% Phenyl-methyl silicon, 0.25 mm in inside diameter. 30 m in length and 0.25 µm in film thickness

Column temperature: 50°C (1 min) - 25°C/min heating - 125°C (0 min) -10°C/min heating - 300°C (10 min)

Inlet temperature: 250°C

Carrier gas: Helium

Ionization mode (voltage): EI (70 eV)

Major monitoring ions (*m/z*): See Table 1.

Expected retention time: See Table 1.

9. Limit of quantification

See Table 1.

Note that the table show 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, dehydration by salting out, 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 graphite carbon/aminopropylsilanized silica gel layered cartridge, and quantification and confirmation using GC-MS.

2) Notes

i) Table 1 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”. “Degradation product” in parentheses means that the analyte is the degraded product which is formed during analysis.

ii) This method does not ensure simultaneous analysis of all of the analytes listed in the Table 1. In advance, confirm that degradation or interference does not occur as the result of interaction between the target analytes.

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

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

v) 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.

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

vii) Matrix-matched calibration or standard addition may be required to obtain accurate measurement results.

viii) 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.

ix) To test agricultural chemicals, listed in Column 1 of the following table, in tea except powdered tea, use the individual analytical method described in Column 2 of the same line.

Column 1	Column 2
BHC	Method for BHC, etc.
DDT	Method for BHC, etc.
XMC	Method for Aldicarb, etc.
Acrinathrin	Method for Acrinathrin, etc.
Acetamiprid	Method for Acetamiprid
Aldrin/Dieldrin	Method for BHC, etc.
Isoxathion	Method for EPN, etc.[*1]
Imibenconazole	Method for Imibenconazole
Ethion	Method for EPN, etc.
Ethofenprox	Method for Ethofenprox
Endrin	Method for BHC, etc.
Chlorpyrifos	Method for EPN, etc.
Chlorfenapyr	Method for Chlorfenapyr, etc.
Dicofol	Method for BHC, etc.
Cyhalothrin	Method for Acrinathrin, etc.
Difenoconazole	Method for Cafenstrole, etc.
Cyfluthrin	Method for Acrinathrin, etc.
Cypermethrin	Method for Acrinathrin, etc.
Dimethoate	Method for EPN, etc.
Diazinon	Method for EPN, etc.
Tetraconazole	Method for Cafenstrole, etc.
Tetradifon	Method for BHC, etc.
Tebufenpyrad	Method for Alachlor, etc.
Deltamethrin/Tralomethrin	Method for Acrinathrin, etc.
Trifluralin	Method for BHC, etc.
Parathion	Method for EPN, etc.
Parathion-methyl	Method for EPN, etc.
Halfenprox	Method for BHC, etc.
Bifenthrin	Method for Acrinathrin, etc.
Pyraclofos	Method for EPN, etc.
Pyridaben	Method for Pyridaben
Pyrifenox	Method for Pyrifenox
Pirimifos-methyl	Method for EPN, etc.
Pyrethrins	Method for Acrinathrin, etc.
Fenitrothion	Method for EPN, etc.
Phenthoate	Method for EPN, etc.

Fenvalerate	Method for Acrinathrin, etc.
Fenpropathrin	Method for BHC, etc.
Flucythrinate	Method for Acrinathrin, etc.
Fluvalinate	Method for Acrinathrin, etc.
Prothiofos	Method for EPN, etc.
Propiconazole	Method for Cafenstrole, etc.
Profenofos	Method for EPN, etc.
Permethrin	Method for Acrinathrin, etc.
Phosalone	Method for EPN, etc.
Miclobutanil	Method for Miclobutanil
Methidathion	Method for EPN, etc.

*1: For isoxathion in tea except powdered tea, perform the test according to “a” in Method for EPN, etc.

11. Reference

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

12. Type

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Table 1. Multi-residue Method for Agricultural Chemicals by GC-MS (Agricultural Products)

Agricultural chemicals	Analytes	Retention index	Monitoring ions (m/z)					Limit of measurement (mg/kg)
BHC	β-BHC	1757	219*	183	181*			0.01
	δ-BHC	1829	219*	183	181*			0.01
γ-BHC	γ-BHC	1776	219*	183	181*			0.01**
Acetochlor	Acetochlor	1882	223*	162	146*			0.01
Aldicarb/Aldoxycarb	Aldicarb (degradation product)	899	115*	100*				0.01**
Isoprocarb	Isoprocarb	1538	136*	121*				0.01
Imazalil	Imazalil	2173	215*	173*				0.01**
Imibenconazole	Imibenconazole debenzylated	2216	270	235*				0.01
Esfenvalerate	Esfenvalerate (isomer 1)	2953	419*	167*	125			0.01**
	Esfenvalerate (isomer 2)	2983	419*	167*	125			
Esprocarb	Esprocarb	1968	222*	162*	91*	71		0.01
Ethalfluralin	Ethalfluralin	1647	316	276*	292			0.01
Ethychlozate	Ethychlozate	2073	238*	165*				0.01
Endosulfan	Endosulfan sulfate	2364	422*	387*	272*	239*	229	0.01**
Oxadiazon	Oxadiazon	2188	344*	302*	258	175*		0.01
Oxadixyl	Oxadixyl	2283	163*	132				0.01**
2-Phenylphenol	2-Phenylphenol	1531	170*	141	115*			0.01
Quizalofop-ethyl	Quizalofop-ethyl	2856	372*	244*				0.01
Quintozene	Quintozene	1766	295*	249	237*	214	142	0.01
Chlozolinate	Chlozolinate	2060	331*	259				0.01**
Clomazone	Clomazone	1760	204*	127	125*			0.01**
Chlorthal-dimethyl	Chlorthal-dimethyl	1990	332	301*	299			0.01
Chlordane	*cis*-Chlordane	2150	375	373*	272	237		0.01**
	trans-Chlordane	2123	375	373	272	267		0.01**
	Oxychlordane	2073	389	387*	237	185	115*	0.01
Chlorbufam	Chlorbufam	1752	223*	164	129			0.01
Chloroneb	Chloroneb	1511	208*	193*				0.01
2,6-Diisopropylnaphthalene	2,6-Diisopropylnaphthalene	1739	212*	197*	155			0.01
Diclocymet	Diclocymet (isomer 1)	2085	277*	173				0.01
	Diclocymet (isomer 2)	2118	277*	173				
Diclofop-methyl	Diclofop-methyl	2400	340*	253				0.01
Disulfoton	Disulfoton	1815	274*	142	88*			0.01**
Diphenylamine	Diphenylamine	1635	169*	168*	167	77		0.01
Cyprodinil	Cyprodinil	2051	225	224*	210			0.01
Dimethenamid	Dimethenamid (*RS*)	1879	230*	154				0.01
Dimethomorph	Dimethomorph (isomer 1)	3107	387*	301*				0.01
	Dimethomorph (isomer 2)	3149	387*	301*				
Dimepiperate	Dimepiperate	2094	145*	119*				0.01
Di-allate	Di-allate (isomer 1)	1698	236	234*	128	86		0.01
	Di-allate (isomer 1)	1716	236	234*	128	86		
Thiobencarb	Thiobencarb	1985	257	100*	125	72		0.01
Thiometon	Thiometon	1724	246*	158	125	88*	60*	0.01
Tefluthrin	Tefluthrin	1816	383	197	177*			0.01
Triadimefon	Triadimefon	2002	210	208*	181			0.01**
Tricyclazole	Tricyclazole	2182	189*	162*	161			0.01**
Triflumizole	Triflumizole metabolite	1757	201*	167*				0.01**
Nitrothal-isopropyl	Nitrothal-isopropyl	2007	254*	236*	212	194		0.01
Piperonyl butoxide	Piperonyl butoxide	2413	176*	149*				0.01**
Pyributicarb	Pyributicarb	2438	181	165*	108	93		0.01
Pyriproxyfen	Pyriproxyfen	2582	226*	136*				0.01
Pyroquilon	Pyroquilon	1801	229*	214	173*	130		0.01

Vinclozolin	Vinclozolin	1893	285*	212	198	187	178	0.01
Fenoxaprop-ethyl	Fenoxaprop-ethyl	2675	361*	288*				0.01**
Fenobucarb	Fenobucarb	1610	150*	121*				0.01
Fenpropimorph	Fenpropimorph	1995	303	129	128*			0.01**
Furametpyr	Furametpyr	2529	298*	157				0.01**
Fluridone	Fluridone	2908	328*	310*				0.01
Propachlor	Propachlor	1613	176*	169	136	120*		0.01
Propazine	Propazine	1762	229*	214*	201*	167		0.01
Propanil	Propanil	1874	217*	161*				0.01
Propyzamide	Propyzamide	1787	255*	240	175	173*	145	0.01**
Prometryn	Prometryn	1921	226	184*	241			0.01
Bromobutide	Bromobutide metabolite (deBr-Bromobutide)	1694	233*	120	119*			0.01
Bromophos-ethyl	Bromophos-ethyl	2114	359*	303*				0.01
Hexaconazole	Hexaconazole	2170	214*	175				0.01**
Benoxacor	Benoxacor	1856	259*	120*				0.01
Heptachlor	Heptachlor	1921	337*	272*	237	100		0.01
	Heptachlor epoxide (isomer A)	2082	353*	253*	217*	183*	81	0.01
	Heptachlor epoxide (isomer B)	2074	357	353*	263	253	81	0.01
Penconazole	Penconazole	2062	248*	161*	159			0.01**
Pendimethalin	Pendimethalin	2048	281*	252*	220	162	16	0.01**
Benfuresate	Benfuresate	1876	256*	163*				0.01
Boscalid	Boscalid	2834	342*	140*				0.01
Mecarbam	Mecarbam	2072	329*	296*	159	131*	97*	0.01**
Metalaxyl/ Mefenoxam	Mefenoxam	1917	249+	206				0.01**
Methoxychlor	Methoxychlor	2496	227*	212*				0.01
Metolachlor	*S* -Metolachlor	1975	238*	162*				0.01
Metribuzin	Metribuzin	1890	198*	144*				0.01
Resmethrin	Resmethrin (isomer 1)	2400	171*	143	123*			0.01**
	Resmethrin (isomer 2)	2415	171*	143	123*			0.01**

- The analytes are listed in the order of the Japanese syllabary, and the isomers are listed by their retention times. 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.
- Relative retention time (RRT) is the relative value when *n*-alkane is 1. The Retention index above shows the average values obtained from laboratories.
- Ions used for quantification in laboratories was indicated with “*”.
- Limit of quantification was regarded as 0.01 mg/kg when the *S/N* of analyte peak obtained from a recovery test at a spiked level of 0.01 ppm was ≥ 10 in at least one sample. For analyte which was not performed a recovery test at a spiked level of 0.01 ppm, the limit of quantification was estimated to be 0.01 mg/kg, when the *S/N* of the analyte peak of matrix-matched standard corresponding to 0.01 mg/kg in the sample was ≥ 10 in at least one sample, and indicated this in the table with “**”.