LC TOF-MS

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Original Article
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Multi-residue screening method of acidic pesticides in agricultural products by liquid chromatography/ time of flight mass spectrometry

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A simple and rapid screening method of 95 acidic pesticides in agricultural products was developed. As acidic pesticides adsorb on the primary secondary amine mini-column with fatty acids which become interfering peaks during GC/MS analysis, they were not included in multi-residue analysis entailing PSA clean-up. In this study, the intermediate extracted solutions before PSA clean-up were analyzed by LC/TOF-MS. Accurate mass measurement of TOF-MS enabled the detection of molecular ions and fragment ions with high selectivity. Mean recoveries of 95 pesticides added to 6 agricultural products at 0.1 μg/g were 49–127% with RSD <20%. Limits of quantitation were 0.01–0.02 μg/g for 95 pesticides. The method was applied to 140 samples, and 2,4-D from lemon and orange, fluazifop from baby kidney bean, dichlorprop from apple were detected, respectively, at 0.02–0.03 μg/g below MRLs. The proposed method showed good sensitivity for 95 acidic pesticides and enabled rapid screening in combination with our multi-residue method targeted to 520 pesticides. © Pesticide Science Society of Japan

Keywords: acidic pesticide, multi-residue, agricultural product, screening method, LC/TOF-MS.

Introduction

In Japan, the Positive List System was introduced for the regulation of agricultural chemical residues in foods on May 29, 2006. Maximum residue limits (MRLs) were set for 799 substances, including feed additives and veterinary drugs, by the addition of many provisional MRLs.[1] Among them, MRLs for pesticides were 586, and increased to 600 until June 2009.[2] As some MRLs are set for the sum of several pesticides and their metabolites, the number of compounds we have to aim to analyze as the target of regulatory monitoring is supposed to be more than 700.

We have been developing multi-residue analytical methods by gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS), and more than 500 pesticides are simultaneously extracted from foods and purified with octadecylsilyl (ODS) and primary secondary amine (PSA) mini-columns.[3,4] PSA is effective to remove

fatty acids which generate large interfering peaks during GC/MS analysis,[5] but it also retains pesticides containing acidic functions, such as carboxyl, phenol, sulfonyl, *etc.* So these pesticides were not included in our multi-residue analysis.

In the Director Notice of the Department of Food Safety, the Ministry of Health, Labour and Welfare of Japan showed two clean-up procedures for multi-residue analysis in agricultural products: one is with an aminopropyl/graphite carbon black (NH_2/GCB) mini-column for 338 pesticides analyzed by GC/MS and LC/MS; the other is with a silica-gel mini-column for 58 acidic pesticides analyzed by LC/MS;[6] however, it is difficult for a few restricted inspectors to complete all procedures in the routine analysis of many samples and a simple screening method would be preferable. Anastassiades suggested analyzing phenoxyalcanoic acids without clean-up by liquid chromatography/tandem mass spectrometry (LC/MS/MS) in negative ion mode in his multi-residue method, named QuEChERS.[7] LC/MS/MS gives high sensitivity in the multiple reaction monitoring mode and enables sample dilution. On the other hand, liquid chromatography/ time-of-flight mass spectrometry (LC/TOF-MS) gives accurate mass information and enables the detection of an unlimited

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number of compounds with high selectivity in full-scan acqui-sition mode.[8]

In this study, we investigated the use of intermediate ex-tracted solutions before PSA clean-up of our multi-residue method for the analysis of acidic pesticides in agricultural products by LC/TOF-MS. Dispersive solid-phase extraction (D-SPE) with GCB is a simple and rapid clean-up procedure to adsorb the pigments,[7–9] especially chlorophyll, which could be removed by PSA clean-up[5]; however, GCB also ad-sorbs pesticides with a planar structure.[10] We evaluated the effect of GCB clean-up on LC/TOF-MS analysis and the re-covery of pesticides. As a result, it was clarified that 95 pesti-cides retained on PSA were detected quantitatively without remarkable interfering peaks only by solvent exchange. Screening these pesticides would be performed in a series of multi-residue analyses of other pesticides.

Materials and Methods

1. Chemicals

Acetone, acetonitrile, and anhydrous sodium sulfate (Wako Pure Chemical Ind., Japan), n-hexane and sodium chloride (Kanto Chemical, Japan) were of pesticide analysis grade. Acetonitrile (Wako) used for LC/TOF-MS was of liquid chro-matography grade, and others were of analytical grade. As an internal standard (IS) solution, triphenylphosphate (TPP) (Wako) and 1-ethyl-3-phenylurea (EPU) (Frinton Laborato-ries, USA) were mixed at each 5 and 10 μg/ml with acetone-n-hexane (1:4).

ODS: Isolute C18 (endcapped), 1 g (Biotage, Sweden) was conditioned with 10ml acetonitrile and 10ml water; GCB: Su-pelclean ENVI-Carb 120/400 (Sigma-Aldrich, USA) was used without conditioning.

Pesticides and their metabolites were obtained from Wako, Kanto, Sigma-Aldrich, Hayashi Pure Chemical Ind. (Japan) and Dr. Ehrenstorfer (Germany). Individual stock standard so-lutions (250 μg/ml) were prepared with acetone. Mixed stan-dard solutions each containing 20–30 compounds at 10 μg/ml were prepared from stock solutions with acetone. Fortification standard solution containing 95 compounds at 2.5 μg/ml was prepared from mixed solutions with acetone. Solvent standard solutions for LC/TOF-MS analysis were freshly prepared by evaporating 0.1–1.0 ml of fortification standard and 0.25 ml IS solution under a gentle stream of nitrogen, and dissolving with 2.5 ml acetonitrile. Matrix-matched standard solutions were prepared by evaporating 0.4 ml blank sample extracts and dissolving with 0.2 ml each concentration of solvent stan-dard solutions.

2. LC/TOF-MS analysis

The Agilent 1200 series LC system (consisting of a binary pump, vacuum degasser, column oven, and autosampler) was connected to a time-of-flight mass spectrometer Agilent 6210 MSD TOF (Agilent Technologies, USA) equipped with an electrospray interface (ESI) operating in both the positive

and negative ion mode. The LC conditions were as follows: column, Ascentis C18 (100 mm, 3.0 mm, 3 μm) (Sigma-Aldrich); guard column, Inertsil ODS3 (10 mm, 3.0 mm, 3 μm) (GL Sciences, Japan); mobile phase, CH_3CN–10 mM CH_3COONH_4 [(15:85)→(95:5)]/16 min+(95:5) 9 min; flow rate, 0.5 ml/min; column temp., 40°C; sample cooler, 15°C. According to the injector program, 4 μl of the sample extract was mixed with 16 μl water, and then 20 μl was in-jected in each run. The MS parameters were as follows: capil-lary voltage, 4000 V (positive), 3500 V (negative); nebulizer gas, 50 psi; drying gas, 10 L/min (350°C); fragmentor voltage (FV), 100 V and 250 V. LC/TOF-MS accurate mass spectra were recorded across the range of m/z 50–1050 (1 cycle/sec). Internal mass calibration was performed automatically using a dual-nebulizer ESI with an automated calibrant delivery sys-tem. Purine ($C_5H_4N_4$, m/z 121.050873 (positive), 119.036320 (negative)) and HP-0921 (hexakis-(1H,1H,3H-tetrafluoropen-toxy)-phosphazene, $C_{18}H_{18}O_6N_3P_3F_{24}$, m/z 922.009798 (posi-tive), 1033.988109 (negative, trifluoroacetate adduct)) were used for internal reference masses. Data were processed with Agilent Mass Hunter software (version B02.00).

3. Sample preparation

All agricultural products were collected at local markets in Hyogo prefecture. We confirmed that the samples used as blanks or fortifications were pesticide-free with this proposed method. About 500 g sample was chopped in a MK-K58 food processor (Panasonic, Japan) for more than 1 min to obtain thoroughly mixed homogenates.

To 25 g portions of chopped samples (20 ml water was added in case of low-moisture content samples, and 3–5 g sodium acetate was added to citrus fruits for neutralization), 0.25 ml IS solution was added except for the blank sample, and 1 ml fortification standard solution was added to give a final concentration of 0.1 μg/g for the recovery test. After standing for 30 min, the sample was extracted with 60 ml ace-tonitrile by a HF93 homogenizer (SMT, Japan) for 3 min and then filtered. The filtrates were cleaned up through an ODS (1 g) mini-column. Acetonitrile was separated by salting-out with 10 ml of 2 M phosphate buffer/saturated brine solution (pH 7) and 6 g sodium chloride, and then 36 ml acetonitrile layer was collected. After evaporation to dryness, the residue was adjusted to 3 ml with acetone-n-hexane (1:1). A 2 ml aliquot was purified with PSA (200 mg) mini-column for multi-residue analysis of 520 pesticides by GC/MS and LC/MS.[3,4] In this study, the residual 0.4 ml was evaporated to dryness and dissolved with 0.2 ml acetonitrile for LC/TOF-MS analysis. A 0.2 ml aliquot of the final acetonitrile solution corresponds to 2 g sample matrix. Fig. 1 summarizes the pro-cedure.

4. Clean-up with GCB

To investigate the effect of GCB, clean-up by D-SPE was per-formed before solvent exchange; that is, 5 mg GCB was

Acetonitrile extraction

| Sample 25 g + Acetonitrile 60 ml

ODS (1 g) mini-column clean-up

Salting out

| Evaporate 36 ml of acetonitrile layer
| Adjust to 3 ml with acetone/hexane (1:1)

(2 ml)   (1 ml)
PSA (200 mg)
    mini-column clean-up   Solvent exchange
    | Adjust to 2 ml with   (Evaporate 0.4 ml
    | acetone/hexane (1:4)   Dissolve with
    | 0.2 ml acetonitrile)
    | Solvent
    | exchange
GC/MS   LC/MS   LC/TOF-MS
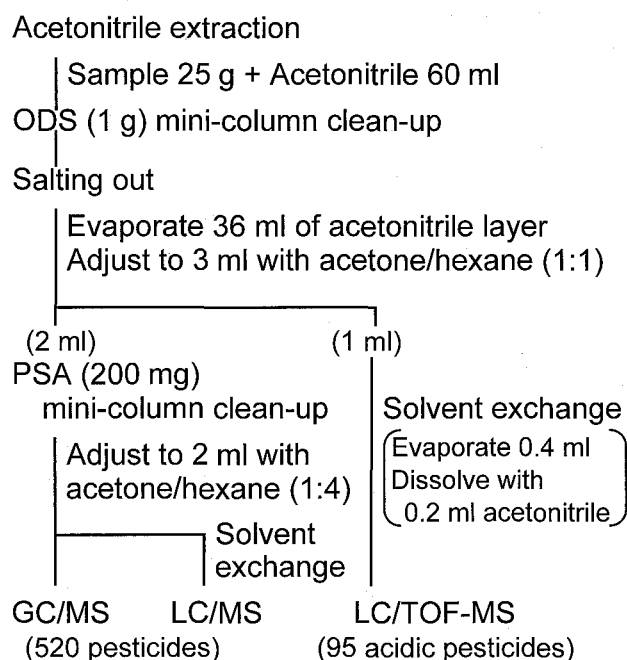(520 pesticides)   (95 acidic pesticides)

Fig. 1. Sample preparation method for multi-residue analysis

added to the residual acetone-n-hexane (1:1) solution (*ca.* 1 ml). The mixture was shaken for 30 sec, mixed on a TM-105 Vortex mixer (Thermonics, Japan) for 30 sec, and then centrifuged for 10 min at 3000 rpm. A 0.4 ml supernatant sample was evaporated to dryness and dissolved with 0.2 ml acetonitrile.

Results and Discussion

1. LC/TOF-MS analysis

The LC conditions were same as those previously set[3,4] for multi-residue analysis by single quadrupole LC/MS. A column of 3.0 mm diameter was chosen to load high amounts of sample solution. For the final sample solution, composition close to the initial mobile phase is preferable. Otherwise peaks of early-eluted polar compounds become broad or split-shape; however, water-rich solvent such as 20% acetonitrile in water (v/v) could not dissolve sample matrix well so low-polar compounds with a long retention time (RT) gradually precipitated with matrix while waiting for the order of injection. To solve this problem, we prepared the final sample solution with acetonitrile and diluted with water just before automatic injection by adopting an injector program. After injection, the loop was on-line and could be cleaned up by mobile phase. To achieve high sensitivity, each sample was injected 2 times in positive and negative ion mode operated separately. During one analysis, MS data were acquired for both FVs set at 100 V to detect mainly protonated or deprotonated molecules and at 250 V for fragment ions. As TOF-MS is highly vacuumed, high FV (250 V) was required compared with quadrupole MS (200 V).

Table 1 shows the qualitative parameters of the studied pes-

ticides. Among 95 pesticides, 43 were in the sulfonyl group, 29 in the carboxyl group, 11 in the hydroxy group, and 12 others. The details, including molecular formulas, are presented in Supplemental Table 1. Some pesticides were not fragmented even with FV 250 V. The relative mass error for each compound was kept within 5 ppm with a real-time reference mass correction system. Target compounds were automatically found according to the database of RT and molecular formula. In addition, target ion monitored at FV 100 V and qualifier ion at FV 250 V were extracted with a range m/z ±0.01 at RT ±1 min. The ratio of the peak area detected on each chromatogram and mass spectra of these peaks, including the isotopic pattern, made it easy to confirm the positive analytes. The peak area of the target ion was used for quantitation and the limits of detection (LODs) presented in Table 1 were equal to the peak height of 150 counts. In positive ion mode, both EPU and TPP used for IS were detected. The ratio of these 2 peaks was effective to estimate the matrix effect. As EPU with short RT easily suppressed its ionization, TPP usually played the role of IS.

2. Sample preparation

In our procedure shown in Fig. 1, acetonitrile content for both extraction and ODS clean-up is maintained between 70 and 75% according to the California Department of Food and Agriculture (CDFA) method.[11] Non-polar co-extractives were effectively removed by ODS clean-up. Pigments were considerably removed by this step, but remained in pre-PSA solutions. With the addition of 5 mg GCB, as described in *4. Clean-up with GCB*, chlorophyll was removed from pre-PSA solutions, although some carotinoides remained for green and yellow vegetables, as recommended by Anastassiades.[7]

To investigate the matrix effect on LC/TOF-MS analysis, matrix-matched standard solutions were prepared as described in the section, *1. Chemicals*. Suppression of ionization was observed for compounds with short RT in positive mode such as pyridate metabolite, flumetsulam, cyromazine, *etc.* Peak areas of those compounds were decreased to almost half by the coexistence of matrix, for which there was no remarkable difference between with/without GCB clean-up. The difference was also not observed on the chromatograms.

Some structurally planar pesticides retained on PSA were included in the target of this study, and they had good affinity to the planar structure of GCB. The recovery of these pesticides was reduced by GCB clean-up, as shown in Table 2. From the above results, we decided not to adopt GCB clean-up and applied pre-PSA solutions to LC/TOF-MS analysis only by solvent exchange.

3. Recovery test

The recovery tests were performed for 6 agricultural products (brown rice, spinach, lemon, lettuce, sweet pepper and Japanese pear) at a level of 0.1 μg/g. The data are summarized in Table 1. The test for each product was conducted on a differ-

Table 1.   LC/TOF-MS qualitative parameters and recovery data for pesticides

Pesticide	RT[a] (min)	Monitor ion (m/z)		LOD[b] (ng)	Mean recovery (%)[c]		Mean RSD (%)[c]
		Target	Qualifier		Solvent st.[d]	Matrix st.[e]	
⟨Positive ionization mode⟩							
Asulam	1.14	248.0700	253.0253	0.04	35.0	49.2	16.3
Imazethapyr	1.35	290.1499	290.1499	0.01	42.5	58.4	12.5
Cyromazine	1.37	167.1040	167.1040	0.01	49.8	85.7	12.6
Pyridate met.	2.04	207.0320	207.0320	0.02	51.7	94.4	8.6
Benzobicyclon met.	2.47	372.0667	355.0401	0.17	81.4	98.7	10.7
Nicosulfuron	2.72	411.1081	182.0560	0.01	70.3	72.1	8.9
Diflufenzopyr	2.75	162.0662	162.0662	0.15	84.7	91.7	8.1
Imazaquin	2.90	312.1343	312.1343	0.01	55.5	72.3	7.0
Flumetsulam	2.98	326.0518	129.0385	0.06	82.2	105.7	5.7
Thifensulfuron-methyl	3.03	388.0380	167.0564	0.09	106.4	86.3	5.6
Metsulfuron-methyl	3.34	382.0816	167.0564	0.07	130.1	93.7	4.3
Tepraloxydim met.	3.48	358.1416	266.1387	0.15	78.9	89.2	7.5
Chlorsulfuron	3.96	358.0371	141.0771	0.06	135.9	89.2	3.8
Rimsulfuron	4.18	432.0642	182.0560	0.08	148.9	88.3	5.3
Azimsulfuron	4.32	425.1099	182.0560	0.05	87.8	84.6	4.4
Foramsulfuron	4.57	453.1187	182.0560	0.10	102.9	86.0	6.5
Trinexapac-ethyl	4.58	253.1071	179.0703	0.06	87.0	97.8	9.9
Clofencet	4.72	279.0531	261.0425	0.03	40.9	53.3	8.8
Pyrasulfotole	4.95	363.0621	250.9984	0.05	94.9	66.7	10.8
Mesotrione	4.98	293.0478	315.0298	0.29	65.0	73.0	7.7
Sulfosulfuron	5.07	471.0751	261.0288	0.06	102.7	92.5	4.6
Cinosulfuron	5.10	414.1078	183.0513	0.02	125.6	125.8	5.1
Flucarbazone	5.10	397.0424	130.0611	0.29	105.6	87.8	7.4
Florasulam	5.24	360.0373	129.0385	0.04	111.8	96.5	6.7
Imazosulfuron	5.25	413.0429	156.0768	0.01	85.6	105.8	8.6
Propoxycarbazone	5.28	399.0969	116.0455	0.12	133.5	87.1	6.9
Flazasulfuron	5.33	408.0584	182.0560	0.01	93.4	89.2	9.2
Clethodim sulfone	5.51	392.1293	300.1264	0.02	120.1	106.1	6.6
Triasulfuron	5.55	402.0633	141.0771	0.02	136.2	97.5	4.4
Naptalam	5.63	292.0968	144.0808	0.06	80.9	72.1	7.6
Ethametsulfuron-methyl	5.82	411.1081	196.0829	0.01	90.1	84.8	4.6
Iodosulfuron-methyl	5.86	507.9782	167.0564	0.03	114.5	85.6	4.6
Pyrithiobac	5.86	327.0201	309.0095	0.02	77.1	84.3	5.8
Pyrazosulfuron-ethyl	5.92	415.1030	182.0560	0.02	91.3	85.8	5.0
Tribenuron-methyl	6.08	396.0972	155.0927	0.01	94.3	82.1	5.3
Mesosulfuron-methyl	6.28	504.0853	504.0853	0.02	139.8	98.0	3.6
IS (Ethylphenylurea)	6.28	165.1022	94.0651	0.01			
Halosulfuron-methyl	6.32	435.0484	182.0560	0.02	91.5	100.9	9.3
Benzylaminopurine	6.34	226.1087	91.0542	0.01	51.7	70.2	10.8
Bispyribac	6.76	431.1197	413.1092	0.01	114.0	95.0	5.0
Trifloxysulfuron	6.82	438.0690	182.0560	0.01	111.2	95.6	6.5

Table 1.   (Continued)

Pesticide	RT[a] (min)	Monitor ion (m/z)		LOD[b] (ng)	Mean recovery (%)[c]		Mean RSD (%)[c]
		Target	Qualifier		Solvent st.[d]	Matrix st.[e]	
Warfarin	6.85	309.1121	251.0703	0.01	84.6	92.7	7.5
Chlorimuron-ethyl	7.00	415.0474	186.0065	0.02	108.6	94.3	6.8
Metosulam	7.01	418.0138	174.9950	0.01	113.3	94.5	6.1
Penoxsulam	7.03	484.0709	484.0709	0.01	108.8	94.3	5.0
Ethoxysulfuron	7.07	399.0969	261.0288	0.01	85.7	114.4	3.5
Cloransulam-methyl	7.15	430.0383	398.0121	0.02	122.0	106.1	8.3
Diclosulam	7.28	405.9938	160.9794	0.03	113.4	94.5	7.5
Imazamox-methyl	7.56	320.1605	320.1605	0.14	77.3	92.2	7.5
Bensulfuron-methyl	8.52	411.0969	182.0560	0.01	99.2	87.1	4.4
Triflusulfuron-methyl	8.64	493.1112	264.0703	0.01	117.8	96.1	4.9
Sulfentrazone	8.89	404.0157	386.9891	0.03	102.7	93.5	4.8
Cyclosulfamuron	9.87	422.1129	261.0288	0.01	106.0	87.0	5.1
Diclomezine	11.07	255.0086	255.0086	0.03	84.5	87.9	9.7
Fenhexamid	12.17	302.0709	302.0709	0.02	80.2	94.9	6.0
Brodifacoum	12.28	523.0903	523.0903	0.02	85.5	91.4	9.8
Pinoxaden	13.09	401.2435	317.1860	0.01	52.5	66.0	8.5
IS (Triphenylphosphate)	14.30	327.0781	327.0781	0.01			
⟨Negative ionization mode⟩							
Cyflumetofen met.	1.36	189.0169	145.0271	0.23	58.5	55.2	7.7
4-CPA	3.95	185.0011	126.9956	0.34	53.0	67.8	17.1
Bentazone	3.95	239.0496	239.0496	0.04	101.0	100.4	9.2
Cloprop	4.35	199.0167	126.9956	0.10	84.7	78.1	10.0
Spiromesifen met.	4.83	271.1340	271.1340	0.01	87.6	77.8	5.6
Bromoxynil	5.11	273.8509	275.8488	0.02	107.8	94.4	10.3
DNOC	5.32	197.0204	197.0204	0.01	88.2	78.1	10.3
MCPA	5.46	199.0167	141.0113	0.06	80.1	66.2	10.7
2,4-D	5.54	218.9621	160.9566	0.11	74.7	74.3	9.2
Mecoprop	5.89	213.0324	141.0113	0.09	90.4	79.6	8.1
Dichlorprop	6.02	232.9778	160.9566	0.07	90.4	75.9	8.1
IS (Ethylphenylurea)	6.28	223.1088	163.0877	0.01			
Ioxynil	6.30	369.8231	126.9050	0.02	97.4	99.6	6.8
2,4,5-T	6.49	252.9232	194.9177	0.06	84.4	81.5	12.4
DADK	6.52	168.0779	168.0779	0.17	110.2	93.0	8.6
Clomeprop acid	6.78	246.9934	174.9723	0.09	106.0	79.4	8.9
Pindone	6.78	229.0870	229.0870	0.24	82.3	58.2	9.8
Clodinafop acid	6.80	310.0288	238.0077	0.17	101.2	106.4	7.8
Fenoprop	6.85	266.9388	194.9177	0.04	95.4	90.8	9.8
DA	6.89	198.0707	198.0707	0.02	107.1	99.0	7.1
Prosulfuron	6.99	418.0802	139.0625	0.05	110.8	107.8	5.4
Fluazifop	7.05	326.0646	254.0434	0.08	106.2	90.8	6.5
Quizalfop	7.43	343.0491	271.0280	0.11	86.2	88.7	9.6
2,4-DB	7.59	160.9566	160.9566	0.10	108.3	88.9	7.0

Table 1. (Continued)

| Pesticide | RT[a] (min) | Monitor ion (m/z) | | LOD[b] (ng) | Mean recovery (%)[c] | | Mean RSD (%)[c] |
		Target	Qualifier		Solvent st.[d]	Matrix st.[e]	
MCPB	7.69	227.0480	141.0113	0.17	105.3	95.7	8.9
Fenoxaprop	7.82	332.0331	260.0120	0.12	107.3	86.7	11.2
Thidiazuron	7.98	219.0346	70.9835	0.01	95.2	76.5	10.8
Haloxyfop	8.17	360.0256	288.0045	0.09	91.9	96.6	11.1
Dinoseb	8.30	239.0673	239.0673	0.01	95.5	79.1	5.9
Fenoxaprop met.	8.30	167.9858	132.0091	0.03	106.2	93.9	10.0
Primisulfuron-methyl	8.40	467.0290	176.0277	0.04	184.4	126.8	6.5
Acifluorfen	8.42	359.9892	315.9994	0.06	99.2	96.7	8.0
Dinoterb	8.70	239.0673	239.0673	0.01	97.8	90.8	8.5
Tecloftalam	9.01	399.8430	401.8400	0.06	85.3	77.6	13.6
Fomesafen	9.07	436.9827	436.9827	0.02	130.9	120.4	6.0
Pentachlorophenol	9.10	262.8397	264.8368	0.04	88.6	76.2	9.0
Forchlorfenuron	9.74	246.0440	127.0068	0.01	66.8	65.6	12.7
Flusulfamide	11.15	412.9383	412.9383	0.01	106.8	109.0	5.8
Dinocap	11.78	295.1299	295.1299	0.05	153.1	97.4	9.4
Fluazinam	14.57	462.9441	462.9441	0.01	104.2	94.2	3.5

[a] Retention time. [b] Limit of detection defined by peak filter set at 150 counts of peak height, 0.04 ng is equivalent to 0.001 μg/g in foods. [c] The mean value of the recovery tests for 6 agricultural products fortified at 0.1 μg/g (brown rice ($n=3$), spinach ($n=3$), lemon ($n=3$), lettuce ($n=5$), sweet pepper ($n=5$), and Japanese pear ($n=5$)). [d] Recovery data calculated for solvent standard. [e] Recovery data calculated for matrix-matched standard. met.: metabolite. IS: Internal standard.

ent day and details for each are shown in Supplemental Table 2.

The mean recoveries of 95 pesticides ranged 35–184% for solvent standard, asulam in positive mode showed low recovery and primisulfuron-methyl and dinocap in negative mode showed high recovery, which was improved in the range of 49–127% by calculating the matrix-matched standard, and 82 pesticides showed satisfactory values between 70 and 120%. RSDs were below 10% for 78 pesticides and below 20% for all. For individual samples, imazethapyr, nicosulfuron,

mesotrione, and naptalam showed poor recoveries in lemon.

Although the official multi-residue method adopts separation of the water phase by salting-out at acidic pH for acidic pesticides and at pH 7.0 for others,[6] we performed salting-out at pH 7.0 for all pesticides. It was not the best condition for acidic pesticides, and more polar compounds, such as endothal, imazapyr, imazapic, *etc.*, were not included in the target of this method. For the multi-residue method, Lee[11] recommended salting-out at pH 7.0, but currently salting out at weak-acidic pH has been adopted by Anastassiades[7] and Okihashi.[12] If salting-out at pH 5–5.5 was adopted after verifying the applicability for the target pesticides in our multi-residue method, recovery of acidic pesticides would be improved.

This method is considered useful for the screening method, even though it is difficult to prepare matrix-matched standard solutions for all kinds of samples in routine analysis. At the limits of quantitation (LOQs,) defined by the signal to noise ratio (S/N≥10), 0.01 μg/g was available for most pesticides, except for 0.02 μg/g for mesotrione, flucarbazone, 4-CPA, pindone and cyflumetofen metabolite.

Table 2. Effect of GCB clean-up on recovery of pesticides with planar structure added to spinach

| Pesticide (0.1 μg/g added) | with GCB | | without GCB | |
	Recovery (%)[a]	RSD (%)	Recovery (%)[a]	RSD (%)
Cyromazine	20.0	21.7	45.6	12.6
Benzylaminopurine	28.1	34.3	63.1	7.9
Diclomezine	56.1	22.0	109.6	12.0
Thidiazuron	56.1	19.4	97.7	10.2

[a] Recoveries were calculated for solvent standard ($n=3$).

4. Monitoring results

We applied this method to routine analysis of FY 2008. Among 140 agricultural products (80 domestic and 60 imported), 3 pesticides were detected from 4 samples (Table 3).

Table 3.   Targeted pesticide residues found in agricultural products during FY2008

Sample[a]	No. analyzed	No. detected	Producing district	Pesticide	Residue (μg/g)	MRL (μg/g)[b]
Lemon	5	1	America	2,4-D	0.03	2
Orange	5	1	South Africa	2,4-D	0.02	2
Baby kidney bean (frozen)	5	1	China	Fluazifop	0.02	0.1
Apple	1	1	Japan (Hyogo)	Dichlorprop	0.02	3

[a] Positive samples among 39 kinds of 80 domestic samples and 22 kinds of 60 imported samples. [b] Maximum Residue Limit under the Japanese Food Sanitation Law.

Fig. 2 shows, as an example, the extracted ion chromatograms and mass spectra for fluazifop detected from baby kidney bean extracts. The exact coincidence between the sample and the standard was observed on m/z to three decimal places. During 1-year analysis, a decline of the sensitivity and resolution caused by damage to the column and instrument was not observed.

Residues shown in Table 3 were low compared with MRLs. For acidic pesticides, MRLs are often set for the sum of several compounds. In 2,4-D, total analysis of 2,4-D, ester derivatives, amine salts, etc. is required by hydrolysis and butyl esterification to specify the violation of MRLs. So the purpose of the multi-residue analysis is rapid screening rather than precise determination. If the total residue of 2,4-D analyzed by this method and ester derivatives analyzed by GC/MS in our multi-residue method is high, reexamination by the official method, notified individually[6] would be performed.

The proposed method showed good sensitivity for 95 acidic pesticides and allowed for rapid screening in combination with our multi-residue method. The time and labor were con-siderably reduced by comparing with the official multi-residue method using a silica-gel mini-column. The whole procedure covers more than 600 pesticides and is applicable to most agricultural products. Conventional LC/MS/MS will provide more sensitive analysis for acidic pesticides and enable sample dilution, but information is limited for target pesticides. On the other hand, the number of pesticides found and confirmed by LC/TOF-MS can be increased unlimitedly, even after analysis. The analytical data of LC/TOF-MS were used not only for acidic pesticides but also for the confirmation of pesticides usually determined after PSA clean-up.

Supplemental Tables 1 and 2 are available in the online publication at http://www.jstage.jst.go.jp/browse/jpestics/.

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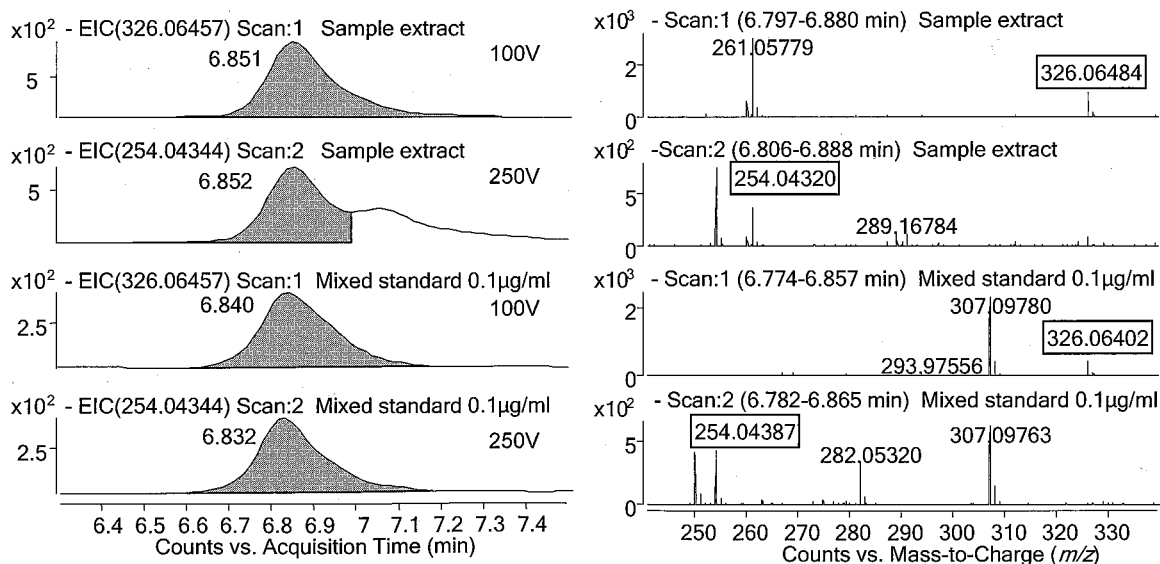2) Notification of Ministry of Health, Labour and Welfare of Japan, No. 325, Jun. 4, (2009).

Fig. 2.   Extracted ion chromatograms and mass spectra for fluazifop detected from baby kidney bean extracts.

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のように蚊の防除プログラムの正否に影響するかをよりよく理解するため，4種類の殺幼虫剤（テメホス，フェンチオン，*Bacillus thuringiensis israilensis*（*Bti*）および *Bacillus sphaericus*（*Bs*））と4種類の殺成虫剤（DDT，マラチオン，デルタメトリンおよびペルメトリン）に対する7ケ所から採集された蚊種の殺虫剤抵抗性を調べた．殺成虫剤は，DDTを除き登録されており，いずれもトルコの蚊防除プログラムで非常によく使われている．Birecik種，Viransehir種，Mersin種，Ankara種および Antalya種に，高いテメホス抵抗性が見つかった．また，Birecik種，Mersin種，Cankiri種および Antalya種にフェンチオン抵抗性が見つかった．ほかの殺幼虫剤（*Bti* と *Bs*）に対する抵抗性またはテメホスとフェンチオンに対する抵抗性種の抵抗性は10倍以下であった．すべての種において，DDTの診断用量に対する死亡率が低かった．Birecik種と Viransehir種の死亡率は30％以下であった．その他の殺成虫剤の診断用量死亡率は大きく変動（マラチオンは Antalya種で65.8％，Birecik種で97.5％；デルタメトリンは Hatay種で97.5％）したが，Ankara種と Antalya種は使用したすべての殺成虫剤に対して抵抗性を示した．その他の種は WHO の分類で要監視種目に分類されている．診断用量実験の結果では，トルコで過去30年間 DDT は使用されていなかったにも関わらず，すべての群種に DDT 抵抗性が存在することを明らかにした．我々の結果から，*Bti* と *Bs* が幼虫の駆除に有効であること，ペルメトリンとデルタメトリンは成虫の駆除に有効であることが示された．

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LC/TOF-MS を用いた農産物中の酸性農薬等の多成分スクリーニング分析法

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LC/TOF-MS を用いて，農産物中に残留する酸性農薬等95種を迅速にスクリーニング分析する方法を開発した．酸性農薬等は，GC/MS 分析において妨害となる脂肪酸を除去するために用いられる PSA に吸着するため，多成分一斉分析法の対象に加えることができなかった．そこで，多成分分析法において，PSA 精製前の抽出液の一部を分取し，溶媒置換したものを LC/TOF-MS で測定した．精密質量を測定することにより，分子イオンおよびフラグメントイオンを共存成分から分離し，選択的に検出することができた．6種類の農産物について，添加濃度 0.1 μg/g で回収実験を行った結果，平均回収率は49〜127％，RSD は20％未満であり，定量限界値は 0.01〜0.02 μg/g であった．農産物140検体のモニタリング検査に本法を適用したところ，2,4-D がレモンおよびオレンジから，フルアジホップが未成熟いんげんから，ジクロルプロップがりんごから，それぞれ 0.02〜0.03 μg/g で検出されたが，いずれも基準値未満であった．本分析法は，農薬520種を対象とした既存の多成分一斉分析法と組み合わせて，酸性農薬等95種に対しても，高感度かつ迅速なスクリーニング分析を可能にした．

光異性化および代謝分解を考慮した水田における農薬挙動予測モデル（PADDY）の開発：除草剤ピリミノバックメチルへの適用

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水稲用除草剤ピリミノバックメチルを対象とし，水田における光異性化および代謝分解を考慮した挙動予測が行えるように PADDY モデルの改良を行った．田面水中におけるピリミノバックメチル *E* 体と *Z* 体間の異性化は，紫外線（B 領域）により進行する可逆的反応として表現した．また，土壌中における主要な代謝分解物の生成・消失を逐次の一次反応として表現した．改良したモデルの検証を行うため，水田ライシメータや水田圃場におけるピリミノバックメチルおよび主要な代謝分解物の消長を実測した．改良型 PADDY モデルは，*E* 体と *Z* 体間の光異性化反応を考慮することにより，田面水および土壌中におけるピリミノバックメチルの挙動を精度よく予測することができた．また，主要な代謝分解物についても予測できることが示された．

短報

殺虫剤毒性の評価における新規高速大量スクリーニング法（HTS 法）とボトルアッセイ法の比較

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殺虫剤の毒性は，一般的に WHO テスト法やボトルアッセイ法で評価されている．最近，高速大量スクリーニング法（HTS 法）が開発され，蚊の挙動や死滅に対する殺虫剤効果の評価に使われるようになってきた．我々は，HTS 法とボトルアッセイ法を比較して，ネッタイシマカ Aedes aegypti に対する殺虫剤の毒性を評価した．二つの方法は等価であることが分かった．異なった主な点は：1）HTS 法よりボトルアッセイ法において蚊の反応時間は短かった；2）蚊のノックダウンと死滅はより低い用量で発生した．これらの結果は，各種媒介生物群の挙動反応に対する様々な化学物質の効果評価手法の指針となるであろう．

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