Original Paper

Multi-Residue Analysis of Pesticides in Agricultural Products by Liquid Chromatography Time-of-Flight Mass Spectrometry

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The applicability of liquid chromatography time-of-flight mass spectrometry (LC-TOF-MS) for determining pesticide residues in agricultural products was investigated. TOF-MS conditions for monitoring target ions, together with their fragment ions, were carefully optimized. The developed LC-TOF-MS method was evaluated for 154 pesticides in soybean and spinach by using matrix-matched standards. No significant matrix effect was observed for most of the tested pesticides at a concentration level of 0.01 mg/kg, where the limits of quantification were less than 0.01 mg/kg for 145 of the 154 pesticides ($S/N>10$). In addition, no significant interference was observed in the chromatograms of the blank extracts. These results indicate that LC-TOF-MS determination may become a powerful tool for multi-residue analysis of pesticides in agricultural products.

Key words: liquid chromatography time-of-flight mass spectrometry (LC-TOF-MS); pesticide residue; agricultural product; multi-residue analysis

Introduction

In 2006, Japan introduced a "positive list system" for the regulation of residual agricultural chemicals (*i.e.*, pesticides, feed additives, and veterinary drugs) in foods. The system prohibits the distribution of foods containing agricultural chemicals above their maximum residue limits (MRLs) or, if the MRL has not been established, a uniform limit of 0.01 mg/kg. So far, the Ministry of Health, Labour and Welfare of Japan has set MRLs for approximately 820 agricultural chemicals. Therefore, there is an urgent need for a rapid and reliable multi-residue method for the determination of hundreds of agricultural chemicals in complex food matrices.

The use of liquid or gas chromatography coupled with quadrupole mass spectrometry (LC-MS/MS or GC-MS/MS) in the selective reaction monitoring (SRM) mode has become the dominant technique in pesticide residue analysis, achieving both high sensitivity and selectivity. The technique is, however, limited in the number of compounds that can be monitored in a single run, and it only provides information on the target compounds, and neglects non-target or unknown compounds.

Recently, time-of-flight mass spectrometry (TOF-MS) techniques such as LC-TOF-MS[1)–6)] and GC-TOF-MS[7), 8)], have been applied to pesticide residue analysis in foods. The main advantage of the TOF-MS method is that there is theoretically no limitation in the number of compounds that can be analyzed simultaneously[1), 9)]. Moreover, there is no need to optimize monitor ions, cone voltages, or collision energies for each target compound separately, as is necessary for quadrupole tandem mass spectrometry in the SRM mode. Further, since full scan spectra are recorded, non-target or unknown compounds can be monitored simultaneously with the target compounds. On the other hand, in TOF-MS measurement, MS parameters do need to be optimized to cover a wide range of target compounds.

In this study, an LC-TOF-MS method for multi-residue analysis of pesticides in agricultural products was carefully optimized and evaluated for 154 pesticides in soybean and spinach by using matrix-matched standards. The results show the potential of LC-TOF-MS for determining pesticide residues in agricultural products.

Materials and Methods

1. *Chemicals and reagents*

Pesticide residue analysis grade acetonitrile and toluene, and LC-MS grade methanol and water were purchased from Kanto Chemical Co. (Japan). Pesticide residue analysis grade anhydrous sodium sulfate and sodium chloride, and JIS special grade ammonium acetate, dipotassium hydrogenphosphate and potassium dihydrogenphosphate were from Wako Pure Chemical Industries, Ltd. (Japan). Water used for preparing the test solutions was purified with a distillation apparatus NZJ-2DSYW (Fujiwara Scientific Co., Japan). Leucine-enkephalin for the lock-mass internal calibration was purchased from Sigma-Aldrich (Germany).

Phosphate buffer (0.5 mol/L, pH 7.0) was prepared as follows: dipotassium hydrogen phosphate (52.7 g) and potassium dihydrogen phosphate (30.2 g) were dissolved

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in 500 mL of water, and adjusted to pH 7.0 by adding either 1 mol/L sodium hydroxide or 1 mol/L hydrochloric acid, and the final volume of the solution was made up to 1 L with water.

A total of 10 pesticide standards were used for optimization of the TOF-MS parameters. Azoxystrobin, MCPA, propoxycarbazone sodium, and iodosulfuron methyl sodium were purchased from Dr. Ehrenstorfer GmbH (Germany); methomyl and acifluorfen, from Riedel-de Haën (Germany); hexythiazox, from Kanto Chemical Co.; simeconazole and 2,4-D, from Wako Pure Chemical Industries, Ltd.; and abamectin (avermectin B1a/avermectin B1b (92.3 : 6.9)), from Hayashi Pure Chemical Industries (Japan). Individual stock solutions (1 mg/mL) of the analytical standards were prepared in either acetonitrile or methanol, depending on their solubility. Working solutions were prepared by dilution of the stock solutions with methanol.

The pesticide standard mixtures (PL2005 pesticide LC/MS Mix4–10, Table 1) were purchased from Hayashi Pure Chemical Industries, and were used for optimizing the LC-TOF-MS method.

2. Samples

Food samples were purchased from a market in Tokyo (Japan). Spinach was chopped using a food processor (Grindomix GM200, Retsch GmbH, Germany), and soybean was ground into small particles and passed through a 425 μm pore-size standard sieve using a centrifugal mill (Ultra Centrifugal Mill ZM 200, Retsch GmbH).

3. Sample preparation

Test solutions of spinach and soybean were prepared via Japanese official method "Multiresidue Method I for Agricultural Chemicals by LC-MS (Agricultural Products)" (Shoku-An No. 1129002, November 29, 2005), except for a change in final volume, as follows: For spinach, a 20.0 g sample was weighed in a 250-mL glass tube. For soybean, a 10.0 g sample was weighed, and allowed to swell in 20 mL of water for 30 min. Both samples were then extracted with 50 mL of acetonitrile by using a homogenizer (PT 10–35 GT, Kinematica, Switzerland). The homogenate was filtered under vacuum through a pad of Celite (Celite 545, Wako Pure Chemical Industries, Ltd.), and the residue was re-homogenized with 20 mL of acetonitrile and filtered. The volume of the combined extract was made up to 100 mL with acetonitrile. A 20-mL aliquot of the extract was added to a 100-mL separating funnel containing 10 g of sodium chloride and 20 mL of phosphate buffer (0.5 mol/L, pH 7.0), and the funnel was shaken vigorously for 10 min. The acetonitrile layer was loaded on an ODS column (Mega Bond Elut C18, 1,000 mg, Agilent Technologies, USA) preconditioned with 10 mL acetonitrile, and eluted with an additional 2 mL of acetonitrile. The combined eluate was concentrated to approximately 2 mL with a rotary evaporator below 40℃, and evaporated to dryness under a stream of nitrogen.

The residue was subsequently re-dissolved in 2 mL of acetonitrile–toluene (3 : 1), loaded on a tandem graphite carbon/aminopropylsilanized silica gel column (InertSep GC/NH_2, 500 mg/500 mg, GL Sciences, Japan) preconditioned with 10 mL of acetonitrile–toluene (3 : 1), and eluted with an additional 20 mL of acetonitrile–toluene (3 : 1). The combined eluate was concentrated to approximately 2 mL with a rotary evaporator below 40℃, then evaporated to dryness under a stream of nitrogen, and the residue was redissolved in methanol (4 mL for spinach, and 2 mL for soybean).

4. LC-TOF-MS determination

4.1 LC conditions

LC-TOF-MS determinations were carried out using an Ultra-Performance LC system ACQUITY UPLC and time-of-flight mass spectrometer LCT Premier (Waters, USA). Chromatographic separation was performed on an ODS column (ACQUITY UPLC BEH C18, 100 mm × 2.1 mm, i.d.: 1.7 μm, Waters) by gradient elution with 10 mmol/L ammonium formate (A) and methanol (B). The gradient used for evaluation of matrix effects was as follows: 0 min (A : B=95 : 5) → 10 min (A : B=5 : 95) → 15 min (A : B=5 : 95) → 15.1 min (A : B=0 : 100) → 25 min (A : B=0 : 100). The flow rate was set at 0.30 mL/min at a column temperature of 40℃. The injection volume was 3 μL.

4.2 MS conditions for optimization of TOF-MS parameters

The following MS conditions were used for optimization of the TOF-MS parameters: ionization mode: electrospray ionization positive mode (ESI(+)) or negative mode (ESI(−)); source temperature: 120℃; desolvation temperature: 350℃; desolvation gas: 600 L/hr (N_2); cone gas: 50 L/hr (N_2); scan range: *m/z* 50–1,000; W mode (>10,000 full width at half maximum (FWHM), ESI(+) *m/z* 556.2771, ESI(−) *m/z* 554.2615); lock mass: leucine-enkephalin (0.2 μg/mL, methanol–water (1 : 1)).

4.3 MS conditions for optimization of LC-TOF-MS method

Optimization of the LC-TOF-MS method was performed using capillary and cone voltages of 3,000 V and 25 V, respectively. The aperture 1 voltage was set at 5, 20, and 40 V. Monitor ions for quantification are shown in Table 1.

5. Evaluation of matrix effects

Matrix-matched standards were prepared as follows: blank extract solutions of 100 μL were evaporated to dryness under a stream of nitrogen, and the residue was re-dissolved in 100 μL of standard mixture (0.01 or 0.1 μg/mL) in methanol. The matrix effect was evaluated by comparing the peak areas of matrix-matched standards to those of standards in methanol. Dimethomorph, ferimzone, tralkoxydim, and tridemorph were quantified by summation of the peak areas of the isomers.

Table 1. Elemental compositions, retention times, calculated exact masses, matrix effects, and LOQs of the studied pesticides

Compound	Elemental composition[a]	Retention time (min)	Type of ion	Calculated exact mass	Matrix effect[b]				LOQ (mg/kg)[d]	
					Soybean		Spinach		Soybean	Spinach
					0.1 (μg/mL)	0.01 (μg/mL)	0.1 (μg/mL)	0.01 (μg/mL)		
Acibenzolar-*S*-methyl	$C_8H_6N_2OS_2$	8.3	$[M+H]^+$	211.0000	1.11	—[c]	1.09	—[c]	0.07	0.02
Acifluorfen	$C_{14}H_7ClF_3NO_5$	8.0	$[M-H]^-$	359.9887	1.07	0.99	1.05	1.09	0.002	0.003
Aldicarb	$C_7H_{14}N_2O_2S$	5.9	$[M+NH_4]^+$	208.1120	0.99	1.00	0.90	0.96	0.006	0.008
Aldoxycarb	$C_7H_{14}N_2O_4S$	3.3	$[M+NH_4]^+$	240.1018	1.02	1.08	1.00	0.99	0.005	0.003
Anilofos	$C_{13}H_{19}ClNO_3PS_2$	9.4	$[M+H]^+$	368.0311	1.07	1.01	0.95	1.11	0.002	0.001
Aramite	$C_{15}H_{23}ClO_4S$	10.3	$[M+NH_4]^+$	352.1349	0.93	0.98	1.04	0.98	0.005	0.002
Avermectin B1a	$C_{48}H_{72}O_{14}$	11.1	$[M+NH_4]^+$	890.5266	0.89	—[c]	0.98	—[c]	0.02	0.02
Azafenidin	$C_{15}H_{13}Cl_2N_3O_2$	7.7	$[M+H]^+$	338.0463	1.00	0.95	0.92	1.02	0.003	0.003
Azamethiphos	$C_9H_{10}ClN_2O_5PS$	6.5	$[M+H]^+$	324.9815	0.82	0.53	0.83	0.94	0.004	0.002
Azimsulfuron	$C_{13}H_{16}N_{10}O_5S$	4.6	$[M+H]^+$	425.1104	0.98	0.94	0.97	0.94	0.002	0.003
Azinphos-methyl	$C_{10}H_{12}N_3O_3PS_2$	8.0	$[M+NH_4]^+$	335.0401	1.02	1.07	0.93	1.01	0.002	0.002
Azoxystrobin	$C_{22}H_{17}N_3O_5$	8.2	$[M+H]^+$	404.1246	1.03	0.98	0.97	1.03	0.001	0.001
Bendiocarb	$C_{11}H_{13}NO_4$	6.7	$[M+NH_4]^+$	241.1188	0.99	1.01	0.90	0.99	0.005	0.002
Bensulfuron-methyl	$C_{16}H_{18}N_4O_7S$	6.7	$[M+H]^+$	411.0974	1.00	0.95	0.89	0.95	0.002	0.001
Benzofenap	$C_{22}H_{20}Cl_2N_2O_3$	10.0	$[M+H]^+$	431.0929	0.99	0.87	0.97	1.06	0.003	0.002
Boscalid	$C_{18}H_{12}Cl_2N_2O$	8.4	$[M+H]^+$	343.0405	1.02	0.97	1.02	0.95	0.005	0.003
Bromoxynil	$C_7H_3Br_2NO$	4.9	$[M-H]^-$	275.8483	1.05	0.98	0.93	1.18	0.002	0.002
Butafenacil	$C_{20}H_{18}ClF_3N_2O_6$	8.8	$[M+NH_4]^+$	492.1149	1.02	0.98	0.98	0.84	0.002	0.001
Carbaryl	$C_{12}H_{11}NO_2$	7.1	$[M+NH_4]^+$	219.1134	1.01	1.04	0.92	0.95	0.003	0.002
Carbofuran	$C_{12}H_{15}NO_3$	6.7	$[M+H]^+$	222.1130	1.01	1.02	0.90	0.99	0.008	0.01
Carpropamid	$C_{15}H_{18}Cl_3NO$	9.4	$[M+H]^+$	334.0532	1.04	0.95	1.04	0.64	0.006	0.002
Chloridazon	$C_{10}H_8ClN_3O$	5.1	$[M+H]^+$	222.0434	0.98	1.01	0.98	0.97	0.001	0.001
Chlorimuron-ethyl	$C_{15}H_{15}ClN_4O_6S$	6.3	$[M+H]^+$	415.0479	1.00	0.97	0.90	0.98	0.002	0.001
4-Chlorophenoxyacetic acid	$C_8H_7ClO_3$	4.9	$[M-H]^-$	185.0005	1.05	—[c]	0.90	—[c]	0.02	0.02
Chloroxuron	$C_{15}H_{15}ClN_2O_2$	8.8	$[M+H]^+$	291.0900	1.02	0.99	1.00	0.91	0.001	0.001
Chlorsulfuron	$C_{12}H_{12}ClN_5O_4S$	4.9	$[M+H]^+$	358.0377	1.01	0.82	0.96	0.91	0.003	0.002
Chromafenozide	$C_{24}H_{30}N_2O_3$	8.8	$[M+H]^+$	395.2335	1.04	0.96	1.00	1.01	0.001	0.003
Cinosulfuron	$C_{15}H_{19}N_5O_7S$	4.7	$[M+H]^+$	414.1083	1.00	0.98	1.00	1.00	0.001	0.001
Clodinafop	$C_{14}H_{11}ClFNO_4$	7.2	$[M+H]^+$	312.0439	1.08	0.90	0.95	0.96	0.006	0.005
Clofentezine	$C_{14}H_8Cl_2N_4$	9.6	$[M+H]^+$	303.0204	1.01	0.93	1.05	0.65	0.007	0.003
Clomeprop	$C_{16}H_{15}Cl_2NO_2$	10.2	$[M+H]^+$	324.0558	0.98	0.97	0.83	0.98	0.004	0.002
Cloprop	$C_9H_9ClO_3$	5.5	$[M-H]^-$	199.0162	1.13	1.12	0.95	1.19	0.009	0.009
Cloquintocet-mexyl	$C_{18}H_{22}ClNO_3$	10.3	$[M+H]^+$	336.1366	1.08	1.05	0.98	0.96	0.001	0.001
Cloransulam-methyl	$C_{15}H_{13}ClFN_5O_5S$	6.5	$[M+H]^+$	430.0388	1.03	1.01	0.91	0.73	0.003	0.004
Clothianidin	$C_6H_8ClN_5O_2S$	4.6	$[M-H]^-$	248.0009	0.99	0.90	1.02	1.13	0.002	0.002
Cumyluron	$C_{17}H_{19}ClN_2O$	8.7	$[M+H]^+$	303.1264	1.03	0.92	0.97	1.00	0.001	0.001
Cyazofamid	$C_{13}H_{13}ClN_4O_2S$	9.0	$[M+H]^+$	325.0526	0.94	0.92	1.00	0.97	0.006	0.002
Cyclanilide	$C_{11}H_9Cl_2NO_3$	7.3	$[M-H]^-$	271.9881	1.10	0.96	1.03	1.19	0.001	0.001
Cycloate	$C_{11}H_{21}NOS$	9.9	$[M+H]^+$	216.1422	1.01	0.99	1.19	0.96	0.007	0.002
Cycloprothrin	$C_{26}H_{21}Cl_2NO_4$	10.7	$[M+NH_4]^+$	499.1191	0.89	0.83	1.03	0.84	0.004	0.003
Cyclosulfamuron	$C_{17}H_{19}N_5O_6S$	7.2	$[M+H]^+$	422.1134	1.03	0.98	0.93	1.00	0.001	0.001
Cyflufenamid	$C_{20}H_{17}F_5N_2O_2$	9.6	$[M+H]^+$	413.1288	1.02	0.89	1.02	1.01	0.001	0.001
Cyprodinil	$C_{14}H_{15}N_3$	9.4	$[M+H]^+$	226.1344	1.08	1.05	0.95	0.97	0.001	0.003
2,4-D	$C_8H_6Cl_2O_3$	6.2	$[M-H]^-$	218.9616	1.06	1.03	0.96	1.05	0.008	0.008
Daimuron	$C_{17}H_{20}N_2O$	8.6	$[M+H]^+$	269.1654	0.99	0.96	0.97	1.02	0.002	0.001
Di-allate	$C_{10}H_{17}Cl_2NOS$	10.0	$[M+H]^+$	270.0486	0.96	0.89	1.07	0.74	0.008	0.01
Dichlorprop	$C_9H_8Cl_2O_3$	6.9	$[M-H]^-$	232.9772	1.07	0.92	1.06	1.30	0.005	0.005
Diclomezine	$C_{11}H_8Cl_2N_2O$	9.2	$[M+H]^+$	255.0092	0.99	0.85	1.02	1.08	0.005	0.003
Diclosulam	$C_{13}H_{10}Cl_2FN_5O_3S$	6.7	$[M+H]^+$	405.9944	1.10	1.04	0.97	1.00	0.01	0.002
Diflubenzuron	$C_{14}H_9ClF_2N_2O_2$	9.1	$[M+H]^+$	311.0399	1.05	1.14	0.98	0.80	0.005	0.003
Dimethirimol	$C_{11}H_{19}N_3O$	7.4	$[M+H]^+$	210.1606	1.06	1.01	0.90	0.99	0.004	0.001
Dimethomorph	$C_{21}H_{22}ClNO_4$	8.3	$[M+H]^+$	388.1316	1.03	0.94	0.97	1.04	0.004	0.005
Diuron	$C_9H_{10}Cl_2N_2O$	7.8	$[M+H]^+$	233.0248	0.99	0.97	0.90	1.00	0.004	0.001
Epoxiconazole	$C_{17}H_{13}ClFN_3O$	9.0	$[M+H]^+$	330.0809	1.03	0.97	0.96	0.97	0.001	0.001
Ethametsulfuron-methyl	$C_{15}H_{18}N_6O_6S$	5.4	$[M+H]^+$	411.1087	0.97	0.97	0.91	0.99	0.001	0.001
Ethoxysulfuron	$C_{15}H_{18}N_4O_7S$	6.6	$[M+H]^+$	399.0974	1.01	0.99	0.90	0.98	0.001	0.001
Fenamidone	$C_{17}H_{17}N_3OS$	8.3	$[M+H]^+$	312.1171	1.01	0.95	0.96	0.98	0.001	0.001
Fenhexamid	$C_{14}H_{17}Cl_2NO_2$	8.8	$[M+H]^+$	302.0715	0.97	1.01	1.00	1.04	0.002	0.002

Table 1. Continued

Compound	Elemental composition[a]	Retention time (min)	Type of ion	Calculated exact mass	Matrix effect[b]				LOQ (mg/kg)[e]	
					Soybean		Spinach		Soybean	Spinach
					0.1 (μg/mL)	0.01 (μg/mL)	0.1 (μg/mL)	0.01 (μg/mL)		
Fenobucarb	$C_{12}H_{17}NO_2$	8.2	$[M+NH_4]^+$	225.1603	0.96	1.10	0.98	0.99	0.003	0.003
Fenoxaprop-ethyl	$C_{18}H_{16}ClNO_5$	10.1	$[M+H]^+$	362.0795	0.98	0.97	1.00	0.99	0.003	0.002
Fenoxycarb	$C_{17}H_{19}NO_4$	9.2	$[M+H]^+$	302.1392	1.03	0.99	0.99	1.01	0.003	0.003
(*E*)-Fenpyroximate	$C_{24}H_{27}N_3O_4$	10.7	$[M+H]^+$	422.2080	0.96	0.97	0.95	1.06	0.001	0.001
(*Z*)-Fenpyroximate	$C_{24}H_{27}N_3O_4$	10.3	$[M+H]^+$	422.2080	1.03	0.99	0.95	0.97	0.001	0.001
Ferimzone	$C_{15}H_{18}N_4$	8.3	$[M+H]^+$	255.1610	0.99	0.99	0.96	1.00	0.002	0.001
Flazasulfuron	$C_{13}H_{12}F_3N_5O_5S$	5.0	$[M+H]^+$	408.0590	0.96	0.90	0.92	0.89	0.001	0.001
Florasulam	$C_{12}H_8F_3N_5O_3S$	4.8	$[M+NH_4]^+$	377.0644	1.06	0.92	0.99	0.91	0.003	0.002
Fluazifop	$C_{15}H_{12}F_3NO_4$	7.2	$[M+H]^+$	328.0797	1.06	0.97	0.95	0.98	0.002	0.002
Flufenacet	$C_{14}H_{13}F_4N_3O_2S$	8.9	$[M+H]^+$	364.0743	1.02	1.01	0.98	0.95	0.001	0.001
Flufenoxuron	$C_{21}H_{11}ClF_6N_2O_3$	10.6	$[M-H]^-$	487.0284	1.07	1.04	—[d]	—[d]	0.002	—[d]
Flumetsulam	$C_{12}H_9F_2N_5O_2S$	3.7	$[M+H]^+$	326.0523	0.97	0.97	1.01	0.95	0.003	0.003
Fluridone	$C_{19}H_{14}F_3NO$	8.0	$[M+H]^+$	330.1106	1.02	1.03	0.91	0.99	0.001	0.001
Fluroxypyr	$C_7H_5Cl_2FN_2O_3$	4.2	$[M-H]^-$	252.9583	1.01	—[c]	0.85	—[c]	0.08	0.04
Fomesafen	$C_{15}H_{10}ClF_3N_2O_6S$	8.0	$[M+NH_4]^+$	456.0244	1.00	0.91	0.98	1.02	0.004	0.008
Foramsulfuron	$C_{17}H_{20}N_6O_7S$	4.7	$[M+H]^+$	453.1192	0.97	0.92	0.98	0.85	0.002	0.002
Forchlorfenuron	$C_{12}H_{10}ClN_3O$	7.7	$[M+H]^+$	248.0591	1.02	0.93	0.85	1.01	0.002	0.002
Furametpyr	$C_{17}H_{20}ClN_3O_2$	7.5	$[M+H]^+$	334.1322	1.05	1.01	0.90	0.98	0.001	0.001
Furathiocarb	$C_{18}H_{26}N_2O_5S$	10.1	$[M+H]^+$	383.1641	1.00	1.03	0.99	0.99	0.001	0.001
Gibberellic acid	$C_{19}H_{22}O_6$	4.3	$[M-H]^-$	345.1338	0.96	—[c]	0.82	—[c]	0.09	0.08
Halosulfuron-methyl	$C_{13}H_{15}ClN_6O_7S$	6.0	$[M+H]^+$	435.0490	0.99	0.95	0.88	0.94	0.001	0.001
Haloxyfop	$C_{15}H_{11}ClF_3NO_4$	8.2	$[M+H]^+$	362.0407	1.10	0.98	1.00	1.22	0.007	0.005
Hexaflumuron	$C_{16}H_8Cl_2F_6N_2O_3$	9.9	$[M-H]^-$	458.9738	1.10	0.92	1.01	1.10	0.001	0.001
Hexythiazox	$C_{17}H_{21}ClN_2O_2S$	10.5	$[M+H]^+$	353.1091	0.90	0.88	0.84	0.90	0.003	0.002
Imazalil	$C_{14}H_{14}Cl_2N_2O$	9.4	$[M+H]^+$	297.0561	1.07	1.01	0.99	0.99	0.001	0.001
Imazaquin	$C_{17}H_{17}N_3O_3$	4.8	$[M+H]^+$	312.1348	1.00	1.00	1.01	0.96	0.001	0.001
Imazosulfuron	$C_{14}H_{13}ClN_6O_5S$	5.1	$[M+H]^+$	413.0435	0.97	0.93	0.85	0.96	0.001	0.001
Imidacloprid	$C_9H_{10}ClN_5O_2$	4.6	$[M+H]^+$	256.0601	0.99	0.80	1.01	0.83	0.003	0.002
Indanofan	$C_{20}H_{17}ClO_3$	9.0	$[M+H]^+$	341.0944	0.97	—[c]	1.03	—[c]	0.06	0.04
Indoxacarb	$C_{22}H_{17}ClF_3N_3O_7$	9.8	$[M+H]^+$	528.0785	0.95	0.83	1.16	1.02	0.005	0.003
Iodosulfuron-methyl	$C_{14}H_{14}IN_5O_6S$	6.0	$[M+H]^+$	507.9788	0.94	0.93	0.86	0.96	0.002	0.003
Ioxynil	$C_7H_3I_2NO$	6.0	$[M-H]^-$	369.8226	1.02	0.96	0.98	1.18	0.001	0.001
Iprovalicarb	$C_{18}H_{28}N_2O_3$	8.8	$[M+H]^+$	321.2178	1.04	1.00	1.00	1.05	0.001	0.002
Isoxaflutole	$C_{15}H_{12}F_3NO_4S$	7.7	$[M-H]^-$	358.0361	1.12	0.82	1.10	0.93	0.004	0.004
Lactofen	$C_{19}H_{15}ClF_3NO_7$	10.2	$[M+NH_4]^+$	479.0833	0.98	0.92	1.03	1.04	0.002	0.002
Linuron	$C_9H_{10}Cl_2N_2O_2$	8.3	$[M+H]^+$	249.0198	1.15	0.93	1.04	1.06	0.005	0.005
Lufenuron	$C_{17}H_8Cl_2F_8N_2O_3$	10.3	$[M-H]^-$	508.9706	1.10	1.05	1.03	1.20	0.001	0.001
MCPA	$C_9H_9ClO_3$	6.2	$[M-H]^-$	199.0162	1.07	0.98	0.96	1.16	0.007	0.007
MCPB	$C_{11}H_{13}ClO_3$	7.5	$[M-H]^-$	227.0475	1.19	—[c]	1.12	—[c]	0.07	0.03
Mecoprop	$C_{10}H_{11}ClO_3$	6.8	$[M-H]^-$	213.0318	1.06	0.84	1.02	1.09	0.009	0.008
Mepanipyrim	$C_{14}H_{13}N_3$	8.8	$[M+H]^+$	224.1188	1.06	1.01	0.95	0.99	0.001	0.002
Mesosulfuron-methyl	$C_{17}H_{21}N_5O_9S_2$	5.0	$[M+H]^+$	504.0859	0.92	0.94	0.82	0.57	0.002	0.001
Methabenzthiazuron	$C_{10}H_{11}N_3OS$	7.5	$[M+H]^+$	222.0701	1.05	1.01	0.90	1.07	0.004	0.004
Methiocarb	$C_{11}H_{15}NO_2S$	8.4	$[M+NH_4]^+$	243.1167	1.01	0.97	0.95	1.05	0.005	0.005
Methomyl	$C_5H_{10}N_2O_2S$	3.7	$[M+H]^+$	163.0541	1.00	1.05	1.00	0.98	0.005	0.005
Methoxyfenozide	$C_{22}H_{28}N_2O_3$	8.6	$[M+H]^+$	369.2178	1.02	0.92	0.98	1.00	0.001	0.003
Metosulam	$C_{14}H_{13}Cl_2N_5O_4S$	6.2	$[M+H]^+$	418.0144	1.03	0.96	0.88	0.97	0.002	0.001
Metsulfuron-methyl	$C_{14}H_{15}N_5O_6S$	4.4	$[M+H]^+$	382.0821	1.00	0.81	0.96	0.71	0.001	0.002
Monolinuron	$C_9H_{11}ClN_2O_2$	7.2	$[M+H]^+$	215.0587	0.89	1.03	0.91	0.95	0.005	0.003
1-Naphthylacetic acid	$C_{12}H_{10}O_2$	5.4	$[M+NH_4]^+$	204.1025	0.92	0.73	0.89	0.70	0.006	0.006
Naproanilide	$C_{19}H_{17}NO_2$	9.1	$[M+H]^+$	292.1338	1.01	0.89	0.99	0.95	0.002	0.004
Naptalam	$C_{18}H_{13}NO_3$	5.9	$[M+H]^+$	292.0974	0.97	0.97	0.89	0.91	0.004	0.004
Novaluron	$C_{17}H_9ClF_8N_2O_4$	9.9	$[M-H]^-$	491.0045	1.06	0.92	1.08	1.16	0.001	0.001
Oryzalin	$C_{12}H_{18}N_4O_6S$	9.0	$[M-H]^-$	345.0869	1.14	1.00	1.04	1.13	0.007	0.001
Oxamyl	$C_7H_{13}N_3O_3S$	3.4	$[M+NH_4]^+$	237.1021	1.03	1.06	1.04	0.95	0.009	0.008
Oxaziclomefone	$C_{20}H_{19}Cl_2NO_2$	10.1	$[M+H]^+$	376.0871	1.02	1.00	1.00	0.96	0.001	0.001
Oxycarboxin	$C_{12}H_{13}NO_4S$	5.4	$[M+NH_4]^+$	285.0909	1.01	0.91	0.87	0.97	0.001	0.001
Pencycuron	$C_{19}H_{21}ClN_2O$	9.7	$[M+H]^+$	329.1421	1.05	0.94	0.96	1.01	0.002	0.001
Penoxsulam	$C_{16}H_{14}F_5N_5O_5S$	6.3	$[M+H]^+$	484.0714	1.02	1.00	0.95	0.96	0.001	0.001

Table 1. Continued

Compound	Elemental composition[a]	Retention time (min)	Type of ion	Calculated exact mass	Matrix effect[b]				LOQ (mg/kg)[e]	
					Soybean		Spinach		Soybean	Spinach
					0.1 (μg/mL)	0.01 (μg/mL)	0.1 (μg/mL)	0.01 (μg/mL)		
Pentoxazone	$C_{17}H_{17}ClFNO_4$	10.1	$[M+NH_4]^+$	371.1174	0.91	—[c]	0.92	—[c]	0.07	0.08
Phenmedipham	$C_{16}H_{16}N_2O_4$	8.0	$[M+NH_4]^+$	318.1454	1.09	1.07	0.97	1.00	0.002	0.002
Pirimicarb	$C_{11}H_{18}N_4O_2$	7.4	$[M+H]^+$	239.1508	1.05	0.91	0.90	0.98	0.003	0.001
Primisulfuron-methyl	$C_{15}H_{12}F_4N_4O_7S$	7.2	$[M-H]^-$	467.0285	1.18	1.05	1.05	1.29	0.001	0.001
Propaquizafop	$C_{22}H_{22}ClN_3O_5$	10.2	$[M+H]^+$	444.1326	1.02	1.02	0.98	0.96	0.001	0.001
Propoxycarbazone	$C_{15}H_{18}N_4O_7S$	5.3	$[M+NH_4]^+$	416.1240	1.03	0.83	0.81	0.48	0.001	0.001
Prosulfuron	$C_{15}H_{16}F_3N_5O_4S$	6.8	$[M+H]^+$	420.0953	1.00	0.96	0.87	0.96	0.001	0.001
Pyraclostrobin	$C_{19}H_{18}ClN_3O_4$	9.5	$[M+H]^+$	388.1064	1.08	1.07	0.98	0.99	0.001	0.001
Pyrazolynate	$C_{19}H_{16}Cl_2N_2O_4S$	9.7	$[M+H]^+$	439.0286	0.92	0.70	0.90	0.85	0.005	0.003
Pyrazosulfuron-ethyl	$C_{14}H_{18}N_6O_7S$	5.7	$[M+H]^+$	415.1036	0.94	0.86	0.87	1.00	0.002	0.001
Pyriftalid	$C_{15}H_{14}N_2O_4S$	8.0	$[M+H]^+$	319.0753	1.04	1.00	0.95	1.02	0.001	0.001
Quizalofop-ethyl	$C_{19}H_{17}ClN_2O_4$	10.1	$[M+H]^+$	373.0955	0.96	0.97	0.99	1.01	0.003	0.003
Silafluofen	$C_{25}H_{29}FO_2Si$	11.9	$[M+NH_4]^+$	426.2265	0.84	1.02	1.04	1.02	0.005	0.003
Simeconazole	$C_{14}H_{20}FN_3OSi$	8.9	$[M+H]^+$	294.1438	1.01	1.00	0.94	1.06	0.001	0.001
Spinosyn A	$C_{41}H_{65}NO_{10}$	11.2	$[M+H]^+$	732.4687	0.86	0.87	0.84	0.83	0.004	0.005
Spinosyn D	$C_{42}H_{67}NO_{10}$	11.4	$[M+H]^+$	746.4843	0.99	0.80	0.88	0.99	0.004	0.006
Sulfentrazone	$C_{11}H_{10}Cl_2F_2N_4O_3S$	6.6	$[M+NH_4]^+$	404.0162	1.03	1.04	0.92	1.28	0.002	0.002
Sulfosulfuron	$C_{16}H_{18}N_6O_7S_2$	5.1	$[M+H]^+$	471.0757	1.07	0.93	0.93	1.02	0.007	0.005
Tebufenozide	$C_{22}H_{28}N_2O_2$	9.2	$[M+H]^+$	353.2229	1.03	1.07	1.00	1.04	0.001	0.001
Tebuthiuron	$C_9H_{16}N_4OS$	6.9	$[M+H]^+$	229.1123	1.01	0.99	0.87	0.95	0.001	0.002
Teflubenzuron	$C_{14}H_6Cl_2F_4N_2O_2$	10.4	$[M-H]^-$	378.9664	1.07	0.83	1.10	1.11	0.002	0.001
Tetrachlorvinphos	$C_{10}H_9Cl_4O_4P$	9.2	$[M+NH_4]^+$	383.9307	1.04	1.03	0.93	1.01	0.002	0.002
Thiabendazole	$C_{10}H_7N_3S$	6.1	$[M+H]^+$	202.0439	1.00	1.00	0.88	1.02	0.001	0.001
Thiacloprid	$C_{10}H_9ClN_4S$	5.5	$[M+H]^+$	253.0315	1.00	0.99	0.92	0.96	0.003	0.002
Thiamethoxam	$C_8H_{10}ClN_5O_3S$	3.9	$[M+NH_4]^+$	309.0537	1.00	1.02	1.00	0.88	0.002	0.002
Thidiazuron	$C_9H_8N_4OS$	6.7	$[M-H]^-$	219.0341	1.01	0.90	1.02	1.33	0.001	0.001
Thifensulfuron-methyl	$C_{12}H_{13}N_5O_6S_2$	4.4	$[M+H]^+$	388.0386	1.01	0.92	0.97	0.95	0.001	0.001
Thiodicarb	$C_{10}H_{18}N_4O_4S_3$	7.2	$[M+H]^+$	355.0568	1.07	0.93	0.92	0.99	0.002	0.005
Tralkoxydim	$C_{20}H_{27}NO_3$	7.6	$[M-H]^-$	328.1913	1.10	0.89	1.05	0.97	0.007	0.007
Triasulfuron	$C_{14}H_{16}ClN_5O_5S$	5.2	$[M+H]^+$	402.0639	0.94	0.83	0.63	0.57	0.001	0.001
Tribenuron-methyl	$C_{15}H_{17}N_5O_6S$	5.0	$[M+H]^+$	396.0978	1.06	1.14	0.88	0.98	0.002	0.001
Triclopyr	$C_7H_4Cl_3NO_3$	6.6	$[M-H]^-$	253.9179	1.07	—[c]	1.05	—[c]	0.02	0.02
Tridemorph	$C_{19}H_{39}NO$	11.8	$[M+H]^+$	298.3110	0.98	1.02	1.01	0.98	0.004	0.004
Trifloxysulfuron	$C_{14}H_{14}F_3N_5O_6S$	5.3	$[M+H]^+$	438.0695	1.00	0.95	0.84	0.87	0.001	0.001
Triflumuron	$C_{15}H_{10}ClF_3N_2O_3$	9.6	$[M-H]^-$	357.0254	1.13	0.95	1.14	1.19	0.001	0.001
Triflusulfuron-methyl	$C_{17}H_{19}F_3N_6O_6S$	7.0	$[M+H]^+$	493.1117	1.03	1.00	0.90	0.92	0.001	0.001
Triticonazole	$C_{17}H_{20}ClN_3O$	8.9	$[M+H]^+$	318.1373	1.00	0.98	0.95	1.04	0.002	0.002

[a] Elemental composition of the neutral molecule.
[b] Peak area ratio of matrix-matched standard to that of standard in pure solvent.
[c] Not determined. Signal-to-noise ratio (S/N)<10.
[d] Found in blank sample.
[e] Limits of quantification (LOQs) were calculated as the analyte concentration that gave S/N=10 in a matrix-matched standard.

Results and Discussions

1. Optimization of TOF-MS parameters for quantifications

Since pesticides display a wide variety of structures and molecular weights, it can be speculated that the optimum TOF-MS parameters such as cone voltage and capillary voltage, differ for each pesticide. Because the full spectrum is recorded at all times, however, it is difficult to set these parameters for each pesticide separately, and that is why it is necessary to determine the optimum MS parameters covering a broad range of pesticides. Therefore, in this study, the three MS parameters of capillary voltage, cone voltage, and aperture 1 voltage, were optimized by flow injection analyses of 10 representative pesticides (5 pesticides for ESI(+) and 5 pesticides for ESI(−)) using 10 mmol/L ammonium formate–methanol (1 : 1) as a mobile phase (flow rate 0.05 mL/min, injection volume 3 μL). The selected pesticides ranged from relatively low molecular weight (MW) to high MW, *i.e.*, methomyl (MW=162), simeconazole (MW=293), hexythiazox (MW=353), azoxystrobin (MW=403), and avermectin B1a (MW=873) for ESI(+) mode, and MCPA (MW=201), 2,4-D (MW=221), acifluorfen (MW=362), propoxycarbazone (MW=398), and iodosulfuron methyl (MW=507) for ESI(−) mode. First, the effect

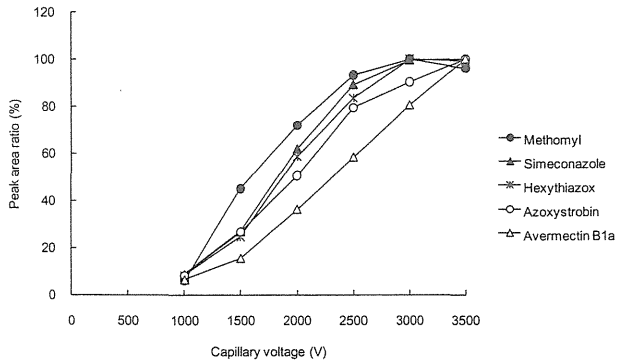

Fig. 1. Effect of capillary voltage on peak areas of methomyl, simeconazole, hexythiazox, azoxystrobin, and avermectin B1a

Cone voltage 25 V, aperture 1 voltage 5 V.
The maximum peak area obtained by varying the capillary voltage was taken as 100%.

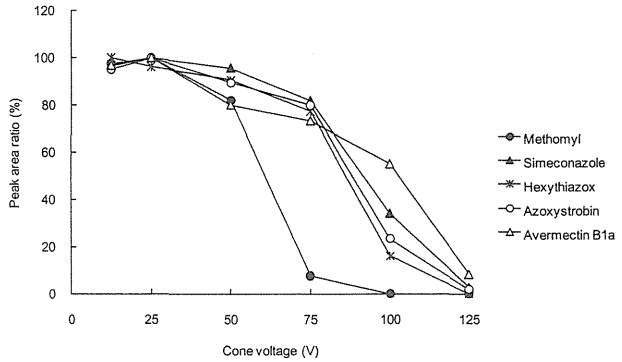

Fig. 2. Effect of cone voltage on peak areas of methomyl, simeconazole, hexythiazox, azoxystrobin, and avermectin B1a

Capillary voltage 3000 V, aperture 1 voltage 5 V.
The maximum peak area obtained by varying the cone voltage was taken as 100%.

of capillary voltage on peak areas was examined by varying the capillary voltage from 1,000 to 3,500 V. The cone voltages were set at 25 and 50 V, while aperture 1 voltages were 5 and 15 V. The results showed that an increase in capillary voltage from 1,000 to 3,000 V led to an increase in the relative peak areas of the tested pesticides, with a maximum at 3,000–3,500 V in both ESI(+) and ESI(−) modes (Fig. 1). Therefore, a capillary voltage of 3,000 V was applied in further experiments.

Subsequently, the effect of cone voltage on the peak areas was investigated in the range of 12.5 to 125 V, while the aperture 1 voltage was set at 5 and 15 V. The maximum peak area of the tested pesticides was obtained at a cone voltage between 12.5–25 V in ESI(+) mode (Fig. 2), and between 12.5–50 V in ESI(−) mode. A cone voltage of 25 V was therefore considered to be optimal.

Finally, the effect of aperture 1 voltage on the peak areas was investigated by varying the aperture 1 voltage from 5 to 60 V (the capillary and cone voltages were fixed at 3,000 and 25 V, respectively). In ESI(+) mode,

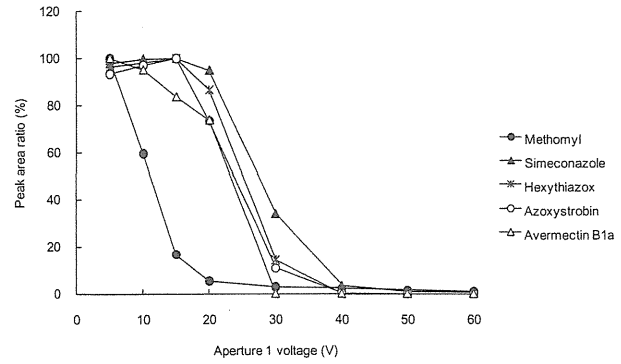

Fig. 3. Effect of aperture 1 voltage on peak areas of methomyl, simeconazole, hexythiazox, azoxystrobin, and avermectin B1a

Capillary voltage 3,000 V, cone voltage 25 V.
The maximum peak area obtained by varying the aperture 1 voltage was taken as 100%.

the results showed that the peak areas of the tested pesticides were highest at an aperture 1 voltage of 5–15 V, and decreased at higher voltages (Fig. 3). In the case of methomyl, the peak area significantly decreased above 10 V due to fragmentation. In ESI(−) mode, the peak areas were highest at an aperture 1 voltage of 5–10 V, and decreased above 30 V. Because a high aperture 1 voltage causes fragmentation leading to a decrease in peak areas, an aperture 1 voltage of 5 V was chosen for quantification for both ionization modes.

2. Optimization of TOF-MS parameters for monitoring fragment ions

LC-TOF-MS with high resolution can differentiate between co-eluting compounds based on the combination of accurate mass and retention time information. In complex food matrices, however, it is possible to find co-eluting compounds whose mass is similar to that of the target compounds for a given retention time. To establish a reliable LC-TOF-MS method, therefore, additional information is required to confirm the identity of the compound. Compounds containing chlorine, bromine, or sulfur can be differentiated by their isotopic profile, whereas for molecules that do not contain such isotopes, additional structural information can be obtained from in-source collision-induced dissociation (CID) fragmentation, which can be induced by setting a high aperture 1 voltage. The aperture 1 voltage was thus optimized for the monitoring of fragment ions using the same set of 10 pesticides as used for optimization of TOF-MS parameters for quantifications. In case of methomyl, the precursor ion $[M+H]^+$was observed at an aperture 1 voltage of 5 V, whereas two fragment ions, $[C_3H_8NOS]^+$ (calculated mass (calcd.) 106.0327) and $[C_3H_6NS]^+$ (calcd. 88.0221), were observed at 15 V, along with $[M+H]^+$ (Fig. 4). The peak areas of both fragment ions were highest at an aperture 1 voltage of 15–20 V (Fig. 5). In the case of acifluorfen, $[M-H]^-$ and its isotopic ion ($[C_{14}H_6{}^{37}ClF_3NO_5]^-$, calcd. 361.9857) were observed at an aperture 1 voltage of 5 V, while two fragment ions, $[C_{13}H_6ClF_3NO_3]^-$ (calcd.

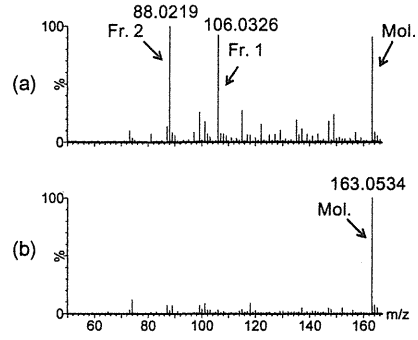

Fig. 4. Mass spectra of methomyl at (a) a high aperture 1 voltage of 15 V and (b) low aperture 1 voltage of 5 V

Mol.: $[M+H]^+$, Fr.1: $[C_3H_8NOS]^+$, Fr.2: $[C_3H_6NS]^+$.

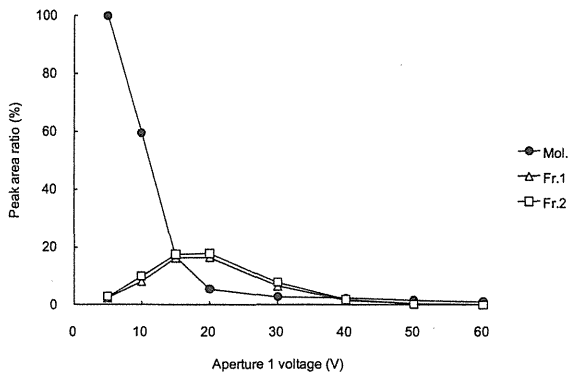

Fig. 5. Effect of the aperture 1 voltage on peak areas of methomyl and its fragment ions

Mol.: $[M+H]^+$, Fr.1: $[C_3H_8NOS]^+$, Fr.2: $[C_3H_6NS]^+$.
The peak area of Mol. at an aperture 1 voltage of 5 V was taken as 100%.

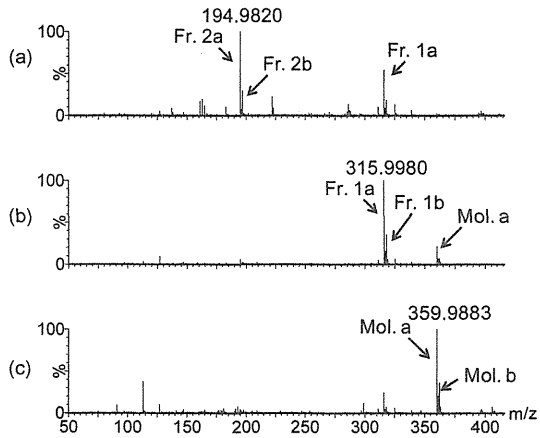

Fig. 6. Mass spectra of acifluorfen at aperture 1 voltages of (a) 50 V, (b) 20 V, and (c) 5 V

Mol.a: $[M-H]^-$, Mol.b: $[C_{14}H_6{}^{37}ClF_3NO_5]^-$, Fr.1a: $[C_{13}H_6ClF_3NO_3]^-$, Fr.1b: $[C_{13}H_6{}^{37}ClF_3NO_3]^-$, Fr.2a: $[C_7H_3ClF_3O]^-$, Fr.2b: $[C_7H_3{}^{37}ClF_3O]^-$.

315.9988) and $[C_7H_3ClF_3O]^-$ (calcd. 194.9825), and their isotopic ions were observed at voltages of 20 V and 50 V (Fig. 6). The maximum peak areas of the two fragment ions, $[C_{13}H_6ClF_3NO_3]^-$ and $[C_7H_3ClF_3O]^-$, were found at

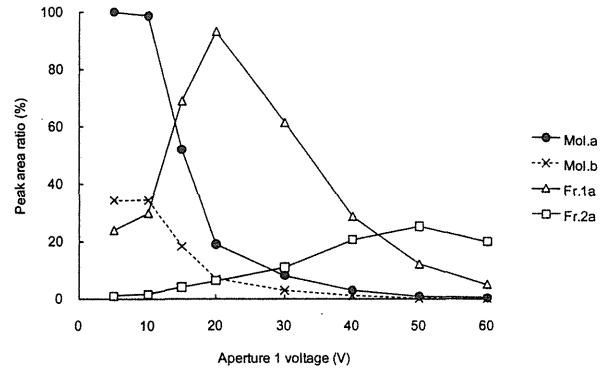

Fig. 7. Effect of the aperture 1 voltage on peak areas of acifluorfen, its isotopic ion, and fragment ions

Mol.a: $[M-H]^-$, Mol.b: $[C_{14}H_6{}^{37}ClF_3NO_5]^-$, Fr.1a: $[C_{13}H_6ClF_3NO_3]^-$, Fr.2a: $[C_7H_3ClF_3O]^-$.
The peak area of Mol.a at aperture 1 voltage of 5 V was taken as 100%.

20 and 50 V, respectively (Fig. 7). The maximum peak areas of the fragment ions of the other eight pesticides were also found at aperture 1 voltages from 20–50 V. Based on these results, 5 V was considered to be the optimal aperture 1 voltage for quantification, while 20 and 40 V were chosen for confirmation *via* CID fragmentation.

3. Optimization of LC-TOF-MS method

The LC-TOF-MS method was optimized by analyzing a standard mixture of 154 pesticides (0.1 μg/mL) (for a list of pesticides, see Table 1) in both ESI(+) and ESI(−) modes, using an ODS column with 10 mmol/L ammonium formate and methanol as a mobile phase. The peaks with highest signal-to-noise (*S/N*) ratios among the observed ions, (*i.e.*, $[M+H]^+$, $[M+NH_4]^+$, $[M+Na]^+$, and $[M-H]^-$), were selected for quantification. As a result, 128 pesticides out of 154 tested pesticides were quantified in ESI(+) mode, and 26 pesticides in ESI(−) mode. Mass accuracy was within ±5 ppm for all the tested pesticides in both ESI(+) and ESI(−) modes.

Matrix effects, *i.e.*, the ion suppression or enhancement caused by co-eluting matrices, frequently occur in LC-MS when using ESI mode. However, they can be reduced or eliminated by optimization of LC methods, sample cleanup, or by changing the type of ionization. Therefore, in this study, the LC conditions for chromatographic separation were optimized.

The maximum pressures at flow rates of 0.2, 0.3, and 0.4 mL/min were approximately 7,500, 10,700, and 13,600 psi, respectively (column temperature 40℃). Although the LC instrument used can be operated at a maximum of 15,000 psi, the flow rate in this study was set at 0.3 mL/min, since it is known that the pressure may increase in the presence of matrices.

The matrix effects with two gradient conditions, *i.e.*, gradient method (a) [0 min (A : B=95 : 5) → 10 min (A : B =5 : 95) → 15 min (A : B=5 : 95) → 15.1 min (A : B=0 : 100) → 25 min (A : B=0 : 100)], and gradient method (b)

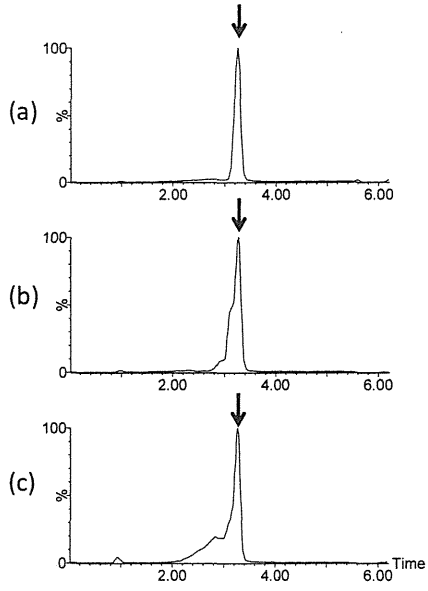

Fig. 8. Comparison of peak shapes of aldoxycarb with injection volume (a) 3 μL, (b) 5 μL, and (c) 7 μL

Concentration 0.1 μg/mL, *m/z* 240.1018, mass window 20 mDa.

[0 min (A : B=95 : 5) → 5 min (A : B=5 : 95) → 10 min (A : B=5 : 95) → 10.1 min (A : B=0 : 100) → 20 min (A : B =0 : 100)], were evaluated by comparing the peak areas of a matrix-matched standard of soybean (0.1 μg/mL) with the peak areas of the standards in pure solvent (0.1 μg/mL). Using gradient method (a), the peak area ratios of all of the tested pesticides were in the range of 0.82 to 1.15, and no significant matrix effect was observed. On the other hand, using the shorter gradient method (b), the peak area ratios of 3 pesticides (azamethiphos, pyrazolynate, and pyrazosulfuron ethyl) were lower than 0.80 while that for acibenzolar-*S*-methyl was above 1.20, suggesting that gradient method (b) was more likely to cause matrix effects than gradient method (a). Therefore, gradient method (a) was chosen for the separation.

To optimize LC-TOF-MS conditions, attention was also paid to the injection solvent and its volume. Taking into account the solubility of hydrophobic target pesticides or co-extracts, we chose methanol as the injection solvent. Since methanol is less polar solvent than the initial mobile phase, it may cause peak broadening of polar pesticides at high injection volumes. A comparison of the peak shapes of aldoxycarb at 3, 5, and 7 μL injection volumes showed that a good peak shape was observed only at 3 μL injection volume (Fig. 8). Based on this result, 3 μL was used as the injection volume.

4. Setting mass windows

Although setting of a narrow mass window for extracting chromatograms leads to a reduction of the chemical noise, and thus increases selectivity, it may also remove target compounds from the chromatogram, and this is especially relevant for low intensity ions. To select the optimal mass window, the standard mixture of 154 pesticides (0.1 μg/mL) was analyzed 5 times and the relative standard deviations (RSD) of the peak areas were compared for mass windows of 5, 10, and 20 mDa. The use of mass windows of 10 or 20 mDa led to RSDs below 9% for the peak areas of all of the tested pesticides, whereas the peak area RSD of 6 pesticides was over 10% using a 5 mDa mass window. A mass window of 20 mDa was, therefore, used for quantification.

5. Matrix effects, LOQ, and selectivity

To evaluate the matrix effect in the optimized LC-TOF-MS method, matrix-matched standards were prepared from the soybean and spinach extracts, and the peak areas of the matrix-matched standards were compared with those of the standards in pure solvent. In addition, based on an *S/N* ratio of 10, the limits of quantification (LOQs) were estimated by LC-TOF-MS analysis of the matrix-matched standards. Since *S/N* ratios depend on the mass window used for extracting chromatograms, the LOQs were calculated by setting the mass window of 20 mDa, which was also used for quantification.

At a concentration level of 0.1 mg/kg, the ratios of the peak areas of matrix-matched standards to that of standards in pure solvent were within the range of 0.80 to 1.20 for spinach and soybean, except for triasulfuron and flufenoxuron in spinach. No significant matrix effect was thus observed for most of the tested pesticides (Table 1). In the case of triasulfuron in the spinach extract, the peak area ratio was 0.63, indicating ion suppression due to co-eluting matrices in the sample solution.

The chromatogram of the blank spinach extract showed a peak at the same retention time as flufenoxuron. The mass spectrum at an aperture 1 voltage of 5 V showed two signals at *m/z* 487.0282 and *m/z* 489.0249, whose accurate mass matched well with the precursor ion of flufenoxuron (calcd. 487.0284) as well as with its isotopic ion $[C_{21}H_{10}{}^{37}ClF_6N_2O_3]^-$ (calcd. 489.0255) (mass accuracy was −0.4 and −1.2 ppm, respectively). In addition, the mass spectrum at an aperture 1 voltage of 40 V showed a signal at *m/z* 156.0257, corresponding to the characteristic fragment ion of flufenoxuron $[C_7H_4F_2NO]^-$ (calcd. 156.0261) (mass accuracy −2.6 ppm), which lends further support to the presence of flufenoxuron in the blank spinach extract. Its concentration in spinach was 0.6 mg/kg, which is well below the Japanese MRL (10 mg/kg). These results demonstrate the utility of obtaining fragmentation information by in-source CID.

At a concentration level of 0.01 mg/kg, out of the 154 pesticides analyzed, 145 showed peak areas of *S/N*>10 in either ESI(+) or ESI(−) mode (Table 1). In addition, the ratios of the peak areas of matrix-matched standards to those of the standards in pure solvent were within the range of 0.80 to 1.20 for most of the tested pesticides (soybean extract: 142 pesticides, spinach extract: 130 pesticides). Furthermore, no significant interfering peak was observed in the chromatogram of the blank extracts, indicating high selectivity of the method. Together, the results indicate that, although further

studies are warranted, the LC-TOF-MS multi-residue method can be successfully applied to the determination of pesticides in foods at concentration levels as low as 0.01 mg/kg, which is the uniform limit for agricultural chemicals in Japan.

Conclusion

In this study, LC-TOF-MS conditions were carefully optimized and applied to the LC-TOF-MS determination of pesticide residues in agricultural products. For most of the tested pesticides, no significant matrix effect was observed at a concentration level of 0.01 mg/kg, while LOQs were mostly less than 0.01 mg/kg. In contrast to LC-MS/MS, LC-TOF-MS enables monitoring a large number of compounds within one run, and can be used for identifying both non-target and unknown compounds based on accurate mass. Thus, the improved sensitivity and resolution of TOF-MS instruments make the LC-TOF-MS method a potentially efficient tool for multi-residue analysis of pesticides in foods.

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