Determination of 44 Pesticides in Foodstuffs by LC / MS / MS

Application

Food Safety

Authors

Masahiko Takino
Agilent Technologies, Inc.
Hachioji, Tokyo
Japan

Toshitsugu Tanaka
Kobe Institute of Health
Department of Food Chemistry
Chuo-ku, Kobe
Japan

Abstract

A sensitive and selective analytical method for the determination of 44 pesticide residues in several foodstuffs using the Agilent G6410AA Triple Quadrupole Mass Spectrometer (QQQ) was developed. This method use two different sample preparation methods followed by LC / MS / MS (liquid chromatography / tandem mass spectrometry). The limits of detection for all pesticides were less than 10 ng / mL in foodstuff. The sensitivity of QQQ easily met the maximum residue limits (MRLs) of all investigated pesticides in Japan Food Hygiene Law.

Introduction

Pesticides are widely used in agricultural practices. The main application can be classified in production and post-harvest treatment of agricultural commodities for transport purposes. In this sense, production agriculture comprises the main category of use of pesticides subject to control requirements and, therefore, maximum residue levels (MRLs) have been fixed to assess food safety. In recent years, the established regulations regarding MRLs in commodities have been more and more stringent. In Japan, the positive list system was introduced this year, and MRLs have been set for over 500 pesticides in all foodstuffs. This new system sets different MRLs for each pesticide within each food group. Typically, the MRLs range from 0.01 to 3 µg/g depending on the commodities and pesticides. The low MRLs fostered the development of more sensitive analytical methods to meet the requirements of complex samples. In this sense, liquid chromatography/tandem mass spectrometry (LC/MS/MS) with QQQ in multiple reaction monitoring (MRM) mode has become so far the most widely used techniques for the quantitation of polar pesticides in food. MRM mode provides for more specific detection in a complex matrix such as food. In this work, 44 pesticides (Tables 1 and 2) are analyzed in two separate runs with sample analytical conditions. The sensitivity requirements set by the positive lost system for these pesticides are easily met.

Experimental

Chemicals

The acetonitrile was of LC/MS grade from Wako Pure Chemical Ind (Japan). Toluene, acetone, n-hexane, formic acid, sodium chloride, and anhydrous sodium sulfate were of analytical grade from Wako Pure Chemical. All SPE cartridges were purchased from Spelco Japan (Japan). Pesticide standards were obtained from Hayashi Pure Chemical (Japan).

Sample Preparation

Extraction

Vegetable and fruit samples were obtained from the local markets. A sample of 10 to 500 g was chopped in a food processor to obtain thoroughly mixed homogenates. A 20-g portion of sample homogenate was weighed in a 200-mL PTFE centrifuge tube. Then 50 mL of acetonitrile was added and blended in a Polytoron. The extract was then filtered by applying vacuum. The filtrate was collected and the residue was re-extracted with 20 mL of acetonitrile. The filtrates were combined in a 100-mL volumetric flask and made up to volume with acetonitrile. A 20-mL portion of the extract was transferred into a PTFE centrifuge tube, and 10 g of NaCl and 20 mL of 0.5 M phosphate buffer (pH 7.0) were added to the extract followed by shaking for 5 min. Five grams of anhydrous Na_2SO_4 were added to the acetonitrile layer obtained after salting out. After removing anhydrous Na_2SO_4, the extract was evaporated to dryness by rotary evaporator (water bath temperature did not exceed 40 °C). The residue was dissolved in 2 mL of acetonitrile-toluene (3:1).

Cleanup

Group 1 - The extract was loaded into a GCB/amino propyl SPE cartridge (500 ng/500 mg) preconditioned with 10 mL of acetonitrile-toluene (3:1). The 20 mL of acetonitrile-toluene (3:1) was further added to the SPE cartridge. All eluate was collected and evaporated by rotary evaporator. The residue was dissolved in 4 mL of methanol.

Group 2 - The extract was loaded into a silica gel SPE cartridge (500 mg) preconditioned with 10 mL each of methanol, acetone, and n-hexane (10 mL of methanol, 10 mL of acetone, and 10 mL of n-hexane, total volume is 30 mL). The 10 mL of acetone-triethylamine-n-hexane (20:0.5:80) was further added to the SPE cartridge. All eluate was discarded. The 20 mL of acetone-methanol (1:1) was applied and the eluate was collected and evaporated by rotary evaporator. The residue was dissolved in 4 mL of methanol.

Standard Preparation

Stock solutions of individual pesticides were prepared in methanol at 1 µg/mL. Serial dilutions using methanol produced a range of standard mixture solutions at 0.001 µg/mL to 1 µg/mL.

The blank matrix residues were fortified with a mixture of pesticides studied at 10 ng/g.

LC / MS / MS Instrument

The LC/MS/MS system used in this work consists of an Agilent 1100-series vacuum degasser, binary pump, well-plate autosampler, thermostatted column compartment, and the Agilent G6410 Triple Quadrupole Mass Spectrometer with an electrospray ionization source (ESI). The objective of the method development was to obtain a fast and sensitive analysis for quantifying pesticides in fruits and vegetables. For chromatographic resolution and sensitivity, different solvents and columns were optimized. It was found that a simple solvent system using water, acetonitrile, formic acid, formic acetate, and a 1.8-µm particle size C18 column would work very well.

LC Conditions

Instrument:	Agilent 1100 HPLC
Column:	ZORBAX Extend C18, 100 mm × 2.1 mm, 1.8 µm (p/n 728700-902)
Column temp:	40 °C
Mobile phase:	A = 0.1% formic acid +5 mM ammonium formate in water B= Acetonitrile
Gradient:	10% B at 0 min, 80% B at 30 min
Flow rate:	0.2 mL/min
Injection vol:	5 µL

MS Conditions

Instrument:	Agilent 6410 QQQ
Source:	Positive ESI
Drying gas flow:	10 L/min
Nebulizer:	50 psig
Drying gas temp:	350 °C
V_{cap}:	4000 V
Scan:	*m/z* 100 to 550
Fragmentor:	Variable 100 V
MRM ions:	Shown in Tables 1 and 2
Collision energy:	Shown in Tables 1 and 2

LC / MS / MS Method

Quantitative analysis was carried out using MRM mode with time program. The parameters of MRM transition are shown in Tables 1 and 2.

Table 1. Data Acquisition Parameters of MRM Transitions of Each Pesticide in Group 1

No	Pesticides	RT (min)	Molecular weight	Precursor ion (*m/z*)	Product ion (*m/z*)	Collision energy(V)
1	Thiabendazole	5.018	201	202	175	20
2	Thiamethoxam	6.16	291	292	211	5
3	Clothianidin	7.83	249	250	169	10
4	Chloridazon	8.19	221	222	104	10
5	Imidacloprid	8.39	255	256	209	20
6	Dimethirimol	8.8	209	210	171	20
7	Oxycarboxine	11.02	267	268	175	10
8	Thiacloprid	11.03	252	253	126	20
9	Azamethiophos	12.87	324	325	183	10
10	Ferimzone(E)	13.21	254	255	124	20
11	Ferimzone(Z)	13.7	254	255	132	20
12	Phenmedipham	17.77	317	318	136	20
13	Azinphos-methyl	17.9	318	132	77	15
14	Simeconazole	18.5	293	294	70	15
15	Isoxaflutol	18.7	359	360	251	15
16	Pyriftalid	18.7	318	319	139	20
17	Tridemorph	19.21	297	298	130	15
18	Methoxyfenozide	20.06	312	313	149	20
19	Chromafenozide	20.57	394	175	141	20
20	Fenoxycarb	20.63	301	302	88	15
21	Naproanilide	21.27	291	292	171	10
22	Butafenacil	21.55	491	492	331	20
23	Cyazofamide	21.7	324	325	108	10
24	Anilofos	22.5	367	368	199	10
25	Pyrazolate	23.5	438	439	173	15
26	Benzofenap	24	430	431	105	20
27	Cyflufenamid	24.3	412	413	241	20
28	Indoxacarb	24.37	527	528	150	15
29	Clomeprop	24.78	372	373	299	5
30	Cloquincet-mexyl	24.8	335	336	238	15
31	Furathiocarb	25.7	365	383	195	15
32	Lactofen	26.3	478	479	344	15
33	Tralkoxydim	26.7	329	330	284	10

Table 2. Data Acquisition Parameter of MRM Transitions of Each Pesticide in Group 2

No	Pesticides	RT (min)	Molecular weight	Precursor ion (*m/z*)	Product ion (*m/z*)	Collision energy (V)
1	Flumetsulam	9.96	325	326	129	20
2	Thidiazuron	11.95	220	221	102	10
3	Imazaquin	12.25	311	312	267	20
4	Thifensulfuron-methyl	12.89	387	388	167	10
5	Florasulam	13.75	359	360	129	20
6	Forchlorfenuron-methyl	14.63	247	248	129	10
7	Clorasulam-methyl	16.41	429	430	398	10
8	Diclosulam	16.83	405	406	161	20
9	Fomesafen	18.27	438	456	344	10
10	Triflusulfuron-methyl	19.29	492	493	264	15
11	Haloxyfop	19.67	361	362	316	15

Results and Discussion

Optimization of MRM Transitions

Determination of the optimal MRM transitions for each pesticide was carried out using full scan mode followed by product ion scan mode using two pesticide standard mixtures at 1 µg/mL. TICs of these standard mixtures in full scan mode and product ion scan mode are shown in Figures 1 and 2. The mass spectrum of each pesticide by full scan mode exhibited protonated molecular ions; $[M+H]^+$ as the base peak ion except azinphos-methyl, furathiocarb, and fomesafen, which exhibited fragment ion and ammonium adduct ion $[M+NH_4]^+$. These ions were selected as precursor ions for MRM mode. It was possible to generate individual product ion MS/MS spectrum of each pesticide by using multiple acquisition and time programming mode. As shown in Tables 1 and 2, 10 time segments for 33 pesticides in group 1 and 7 time segments for 11 pesticides in group 2 were used for MRM mode.

Total ion chromatograms of pesticide standard mixture corresponding to the minimum MRL value for pesticides (10 ng/mL) are shown in Figure 3. These show excellent signal-to-noise (S/N) ratios for all pesticides. The limit of detection (LOD) for each pesticide was determined using an S/N ratio of 3 with an MRM chromatogram of each pesticide at 1 ng/mL (see Table 3). To evaluate the linearity of the calibration curves, various concentrations of pesticide standard solutions ranging from 0.001 ng/mL to 1 ng/mL were analyzed. As shown in Table 3, the linearity was very good for all pesticides with correlation coefficients (r^2) greater than 0.998

The matrix effect of this method was investigated by using orange, apple, potato, and cabbage extracts spiked with pesticide standards at 10 ng/mL. Typical MRM chromatograms of orange extract are shown in Figures 4 and 5. The other chromatograms of apple, potato, and cabbage extract are shown in Figure 6. There was not additional peak from sample matrix in all food when compared with the pesticide standard mixture. These results indicate that MRM mode has very high selectivity.

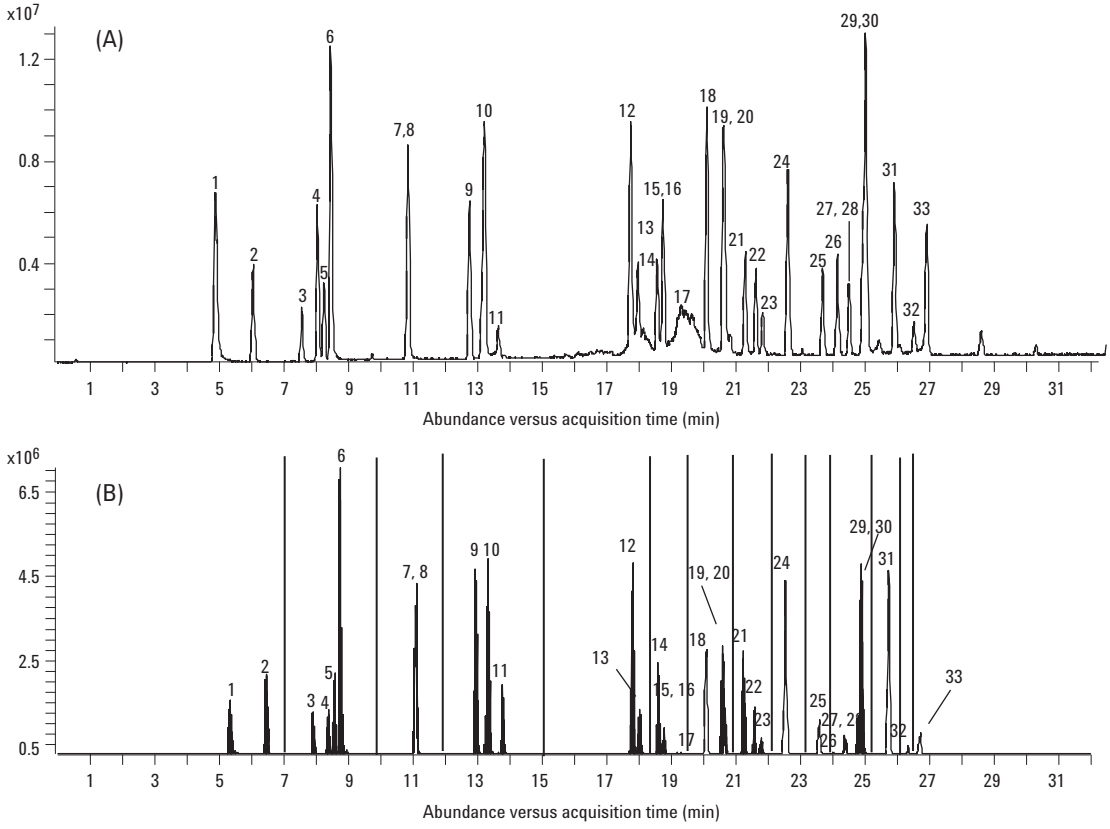

Figure 1. TIC of 33 pesticides standard in full scan mode (A) and product ion scan mode (B) at 1 µg / mL.

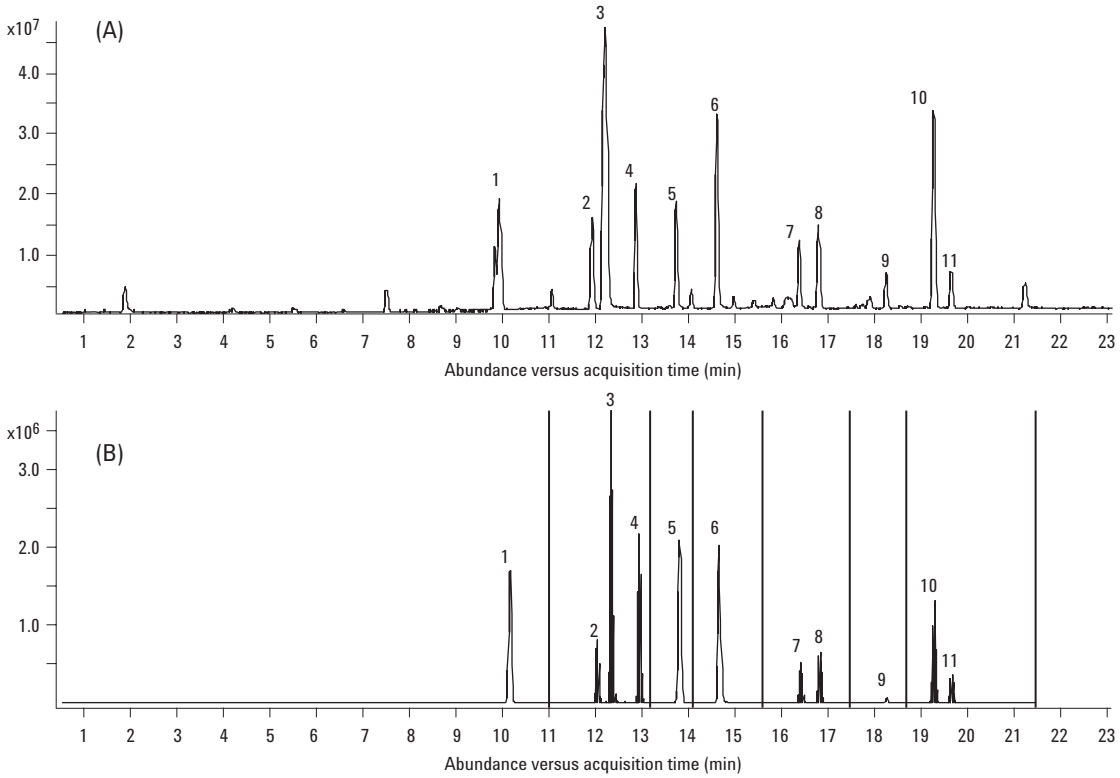

Figure 2. TIC of 11 pesticides standard in full scan mode (A) and product ion scan mode (B) at 1 µg / mL.

Furthermore, the change on the peak intensity of each pesticide by sample matrix was calculated by comparing with the peak intensity of pesticide standards. As these results show in Table 4, the relative intensity of each pesticide ranged from 91 to 116%. Thus, matrix effect such as ion suppression may be insignificant and it was possible to use external standards instead of matrix matched standards. The repeatability of each pesticide in orange extract is also shown in Table 4, and the RSD of each pesticide was in the range from 1.7 to 5.9%.

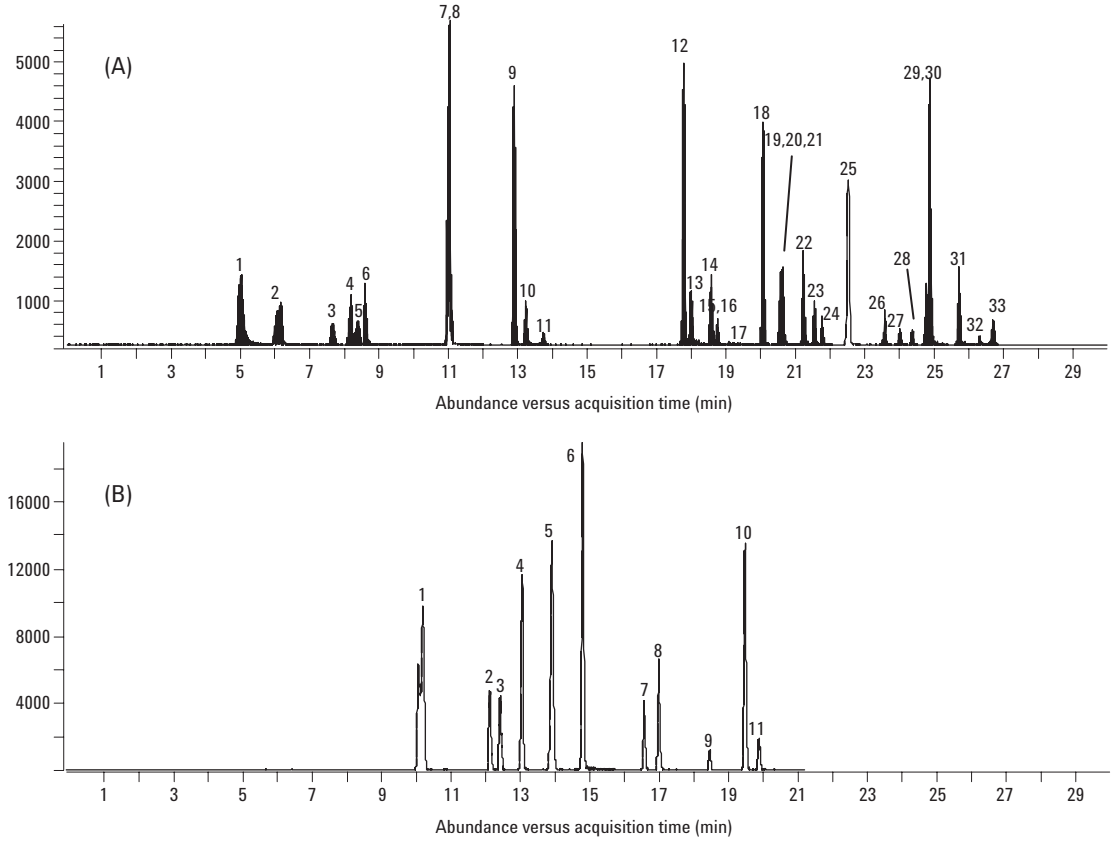

Figure 3. TIC of 33 pesticide standards (A) and 11 pesticides standard (B) at 10 ng / mL in MRM mode.

Table 3. Linearity and LOD of 44 Pesticide Standard Solutions

No	Pesticides	r^2	LOD (ng / mL)	No	Pesticides	r^2	LOD (ng / mL)
Group 1							
1	Thiabendazole	0.9999	<0.1	18	Methoxyfenozide	0.9993	0.55
2	Thiamethoxam	0.9992	<0.1	19	Chromafenozide	0.9992	0.49
3	Clothianidin	0.9999	<0.1	20	Fenoxycarb	0.9988	<0.1
4	Chloridazon	0.9993	<0.1	21	Naproanilide	0.9993	<0.1
5	Imidacloprid	0.9995	<0.1	22	Butafenacil	0.9994	<0.1
6	Dimethirimol	0.9989	<0.1	23	Cyazofamide	0.9987	0.43
7	Oxycarboxine	0.9993	<0.1	24	Anilofos	0.9991	<0.1
8	Thiacloprid	0.9991	<0.1	25	Pyrazolate	0.9990	0.51
9	Azamethiophos	0.9988	<0.1	26	Benzofenap	0.9982	0.49
10	Ferimzone(E)	0.9993	0.34	27	Cyflufenamid	0.9993	0.43
11	Ferimzone(Z)	0.9995	0.53	28	Clomeprop	0.9993	0.61
12	Phenmedipham	0.9993	<0.1	29	Indoxacarb	0.9991	1.04
13	Azinphos-methyl	0.9997	<0.1	30	Quinclorac-methyl	0.9988	0.63
14	Simeconazole	0.9992	<0.1	31	Furathiocarb	0.9987	<0.1
15	Isoxaflutol	0.9991	<0.1	32	Lactofen	0.9987	1.10
16	Pyriftalid	0.9988	<0.1	33	Tralkoxydim	0.9992	0.52
17	Tridemorph	0.9991	1.21				
Group 2							
1	Flumetsulam	0.9996	<0.1	7	Clorasulam-methyl	0.9987	<0.1
2	Thidiazuron	0.9994	<0.1	8	Diclosulam	0.9989	<0.1
3	Imazaquin	0.9992	<0.1	9	Fomesafen	0.9989	0.32
4	Thifensulfuron-methyl	0.9989	<0.1	10	Triflusulfuron-methyl	0.9992	<0.1
5	Florasulam	0.9969	<0.1	11	Haloxyfop	0.9995	0.19
6	Forchlorfenuron-methtyl	0.9977	<0.1				

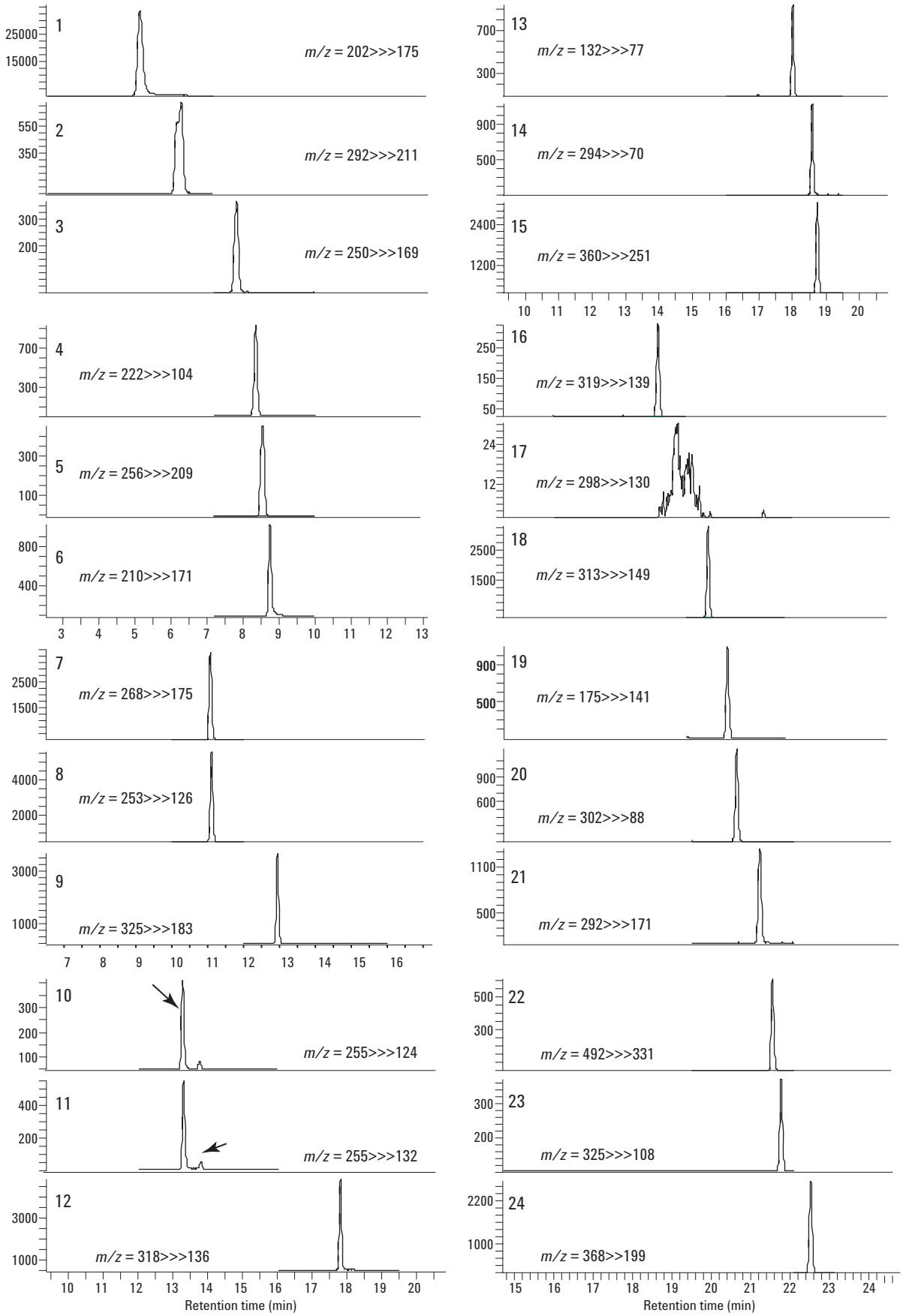

Figure 4. MRM of 33 pesticides in orange extract spiked at 10 ng / mL. (Continued)

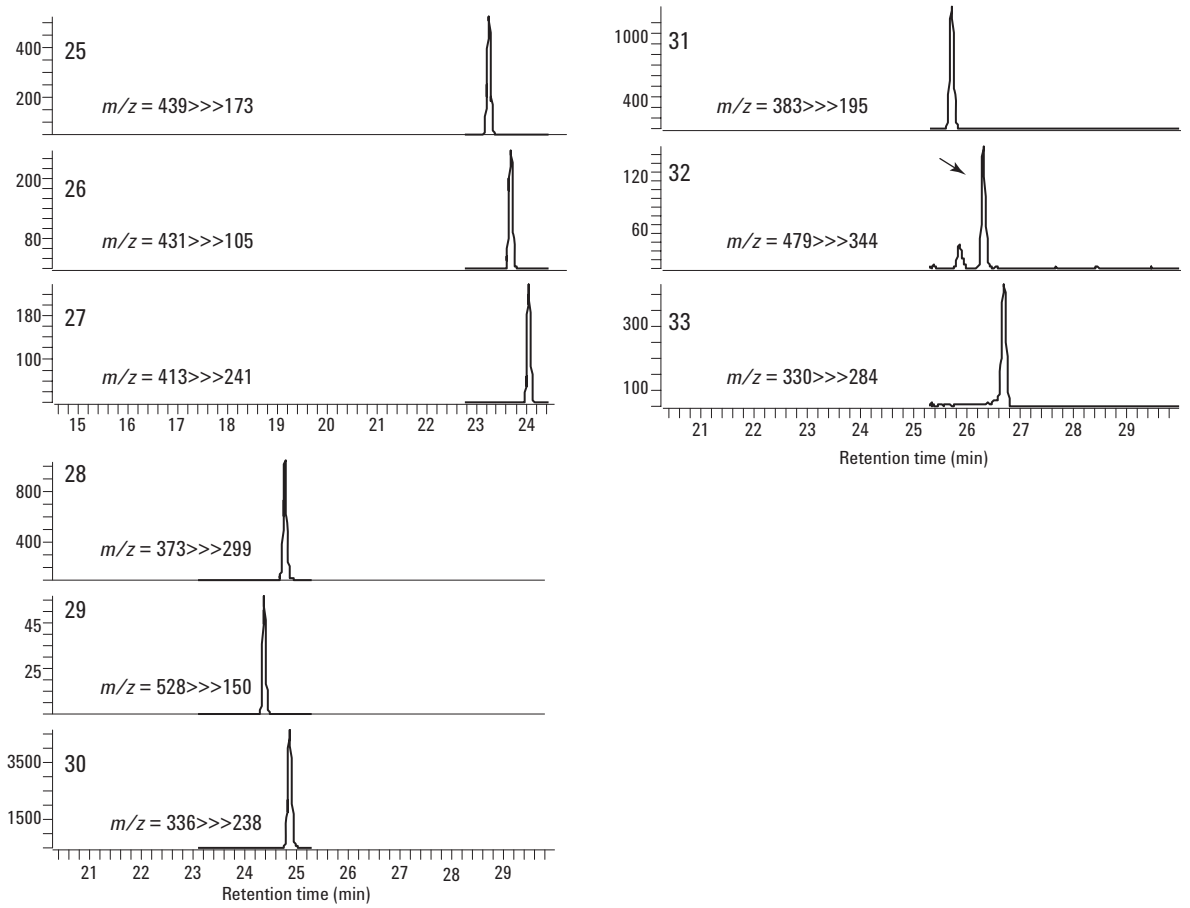

Figure 4. MRM of 33 pesticides in orange extract spiked at 10 ng / mL.

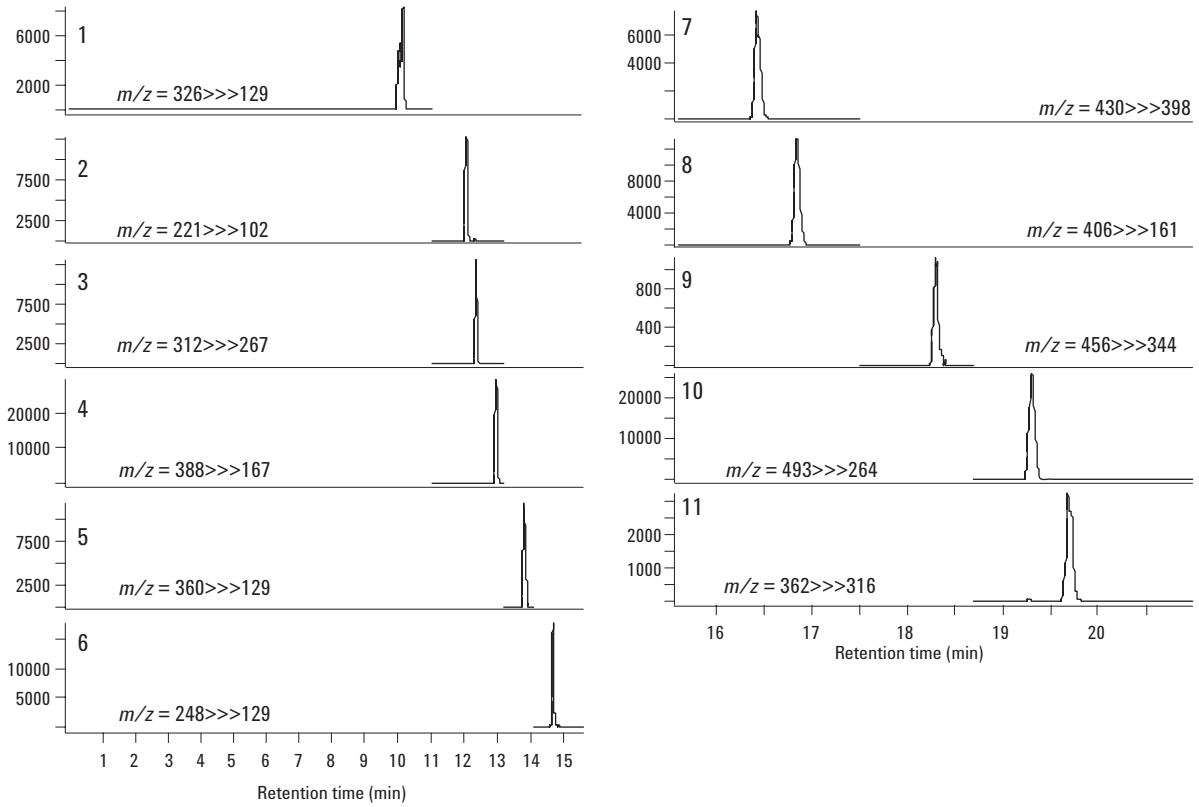

Figure 5. MRM of 11 pesticides in orange extract spiked at 10 ng / mL.

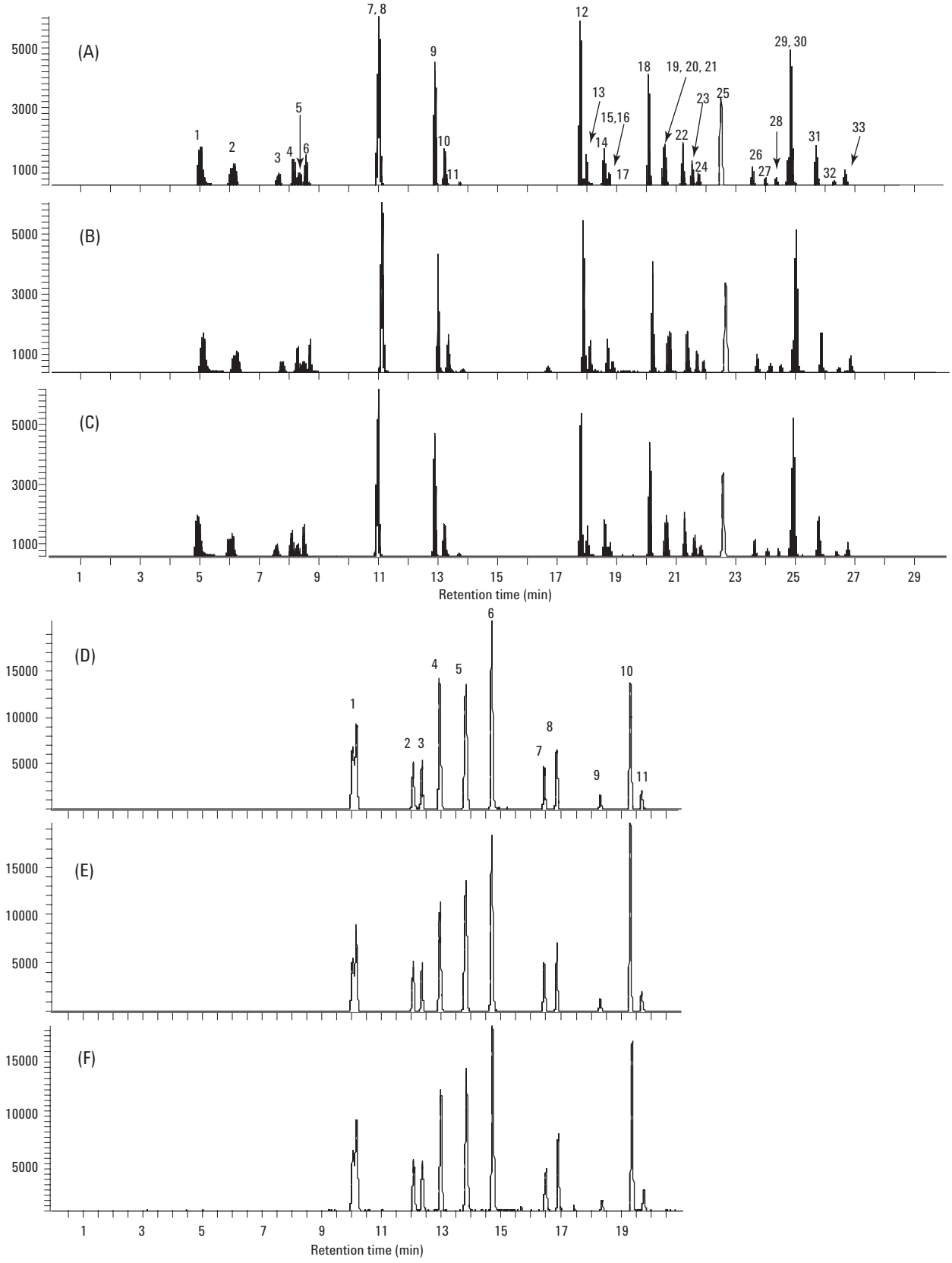

Figure 6. TIC of spiked at 10 ng / mL.

Table 4. Relative Intensity of Each Pesticide in Sample Extracts

No	Pesticides	Relative intensity(%)			
Group 1		**Orange***	**Cabbage**	**Apple**	**Potato**
1	Thiabendazole	105 (3.2)	101	116	107
2	Thiamethoxam	103 (2.1)	98	104	105
3	Clothianidin	106 (2.9)	101	109	112
4	Chloridazon	105 (3.3)	106	101	109
5	Imidacloprid	102 (1.7)	97	102	104
6	Dimethirimol	103 (4.6)	107	103	108
7	Oxycarboxine	106 (3.7)	102	104	106
8	Thiacloprid	104 (3.1)	104	106	108
9	Azamethiophos	93 (4.6)	90	94	84
10, 11	Ferimzone(E,Z)	116 (4.1)	109	102	112
12	Phenmedipham	96 (5.3)	99	100	104
13	Azinphos-methyl	90 (2.1)	103	104	110
14	Simeconazole	104 (4.4)	102	106	110
15	Isoxaflutol	102 (2.7)	104	108	103
16	Pyriftalid	97 (4.1)	103	104	93
17	Methoxyfenozide	92 (3.1)	99	104	97
18	Chromafenozide	96 (2.8)	102	103	101
19	Tridemorph	97 (3.4)	96	100	111
20	Fenoxycarb	99 (2.1)	105	102	101
21	Naproanilide	91 (4.3)	97	98	103
22	Butafenacil	102 (2.6)	114	104	114
23	Cyazofamide	93 (3.5)	92	87	95
24	Anilofos	102 (2.7)	105	103	107
25	Pyrazolate	103 (4.7)	101	103	97
26	Benzofenap	108 (5.2)	111	98	108
27	Cyflufenamid	108 (3.4)	110	105	101
29	Indoxacarb	109 (2.6)	105	100	111
28	Clomeprop	105 (4.2)	107	106	104
30	Cuinclorac-methyl	105 (4.1)	104	104	105
31	Furathiocarb	102 (1.8)	104	105	101
32	Lactofen	100 (3.7)	109	105	112
33	Tralkoxydim	101 (3.3)	111	102	117
Group 2					
1	Flumetsulam	97 (2.6)	110	156	104
2	Thidiazuron	104 (4.8)	101	102	113
3	Imazaquin	105 (3.1)	100	100	101
4	Thifensulfuron-methyl	106 (2.9)	112	116	113
5	Florasulam	99 (3.1)	106	103	109
6	Forchlorfenuron-methyl	101 (4.4)	103	100	108
7	Clorasulam-methyl	94 (3.9)	104	97	142
8	Diclosulam	95 (3.3)	102	96	107
9	Fomesafen	99 (5.9)	101	95	109
10	Triflusulfuron-methyl	97 (4.1)	111	104	108
11	Haloxyfop	108 (4.8)	114	110	124

*( ): RSD,% calculated based on five replicates within one day

Conclusions

The multiresidue method by LC/MS/MS described here was suitable for the determination of 44 pesticides in a variety of food samples due to its high sensitivity and high selectivity. Another advantage of this method is that ion suppression was not observed for all food samples studied. Thus, it may eliminate the need for matrix-matched standards, which make analysis more tedious for samples from different origins.

For more details concerning this application, please contact masahiko_takino@agilent.com

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