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Simultaneous determination of pesticide residues in vegetable extract by liquid chromatograph tandem mass spectrometry for high recovery rate

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Simultaneous determination of pesticide residues in vegetable extract by liquid chromatograph tandem mass spectrometry for high recovery rate

Overview

Development of simultaneous determination of pesticide residues in food by LC-MS/MS.

Introduction

In order to protect the food safety, it is important to establish detection criteria of pesticide residues and methods to improve the accuracy for measuring the concentration of the targets. In Japan, the detection criteria of pesticide residues are established as the Positive List System by the Ministry of Health, Labor and Welfare, as well as the standard test methods.
For monitoring of pesticide by LC-MS/MS, recovery rate should be the critical issue in addition to sensitivity. Generally, standard addition method as well as matrix-matched calibration curve are useful techniques rather than absolute calibration method in order to reduce matrix effects; however, these techniques are not always appropriate methods since each sample requires its independent calibration curve, respectively, for a wide variety of samples.
In this report, we introduce the results of investigation for basic parameters around the ESI ionization to avoid matrix effects and to maximize recovery rate of target pesticides using absolute calibration methods.

Methods and Materials

The test blank matrix solution (vegetable extract such as carrot) was prepared by a solid-phase extraction technique with QuEChERS (STQ method, performed by the Institute of Public Health in Sagamihara city, Kanagawa, Japan). Pesticides mixture solutions PL-7-2, PL-14-2 and PL-15-1 (FUJIFILM Wako Pure Chemical) were used as reference standards. Range of calibration curve for standard concentrations were set from 0.1 to 50 ng/mL by diluting with acetonitrile. The pesticides determination was performed using a triple quadrupole mass spectrometer LCMS-8050 equipped with Nexera™ X2 UHPLC (Shimadzu).

Chromatographic separation was performed by YMC-Triart C18 reversed phase column with mobile phase as 5 mmol/L ammonium acetate and methanol containing 5 mmol/L ammonium acetate. The measurement was performed by MRM, positive and negative ion quantities simultaneously with electrospray ionization method, and the dwell time was set to 5 to 200 msec for each compounds. Analytes were loaded into the mass spectrometer at 2 to 21 minutes using a flow switching valve.

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UHPLC conditions (Nexera™ X2 system)	
Column	: Shim-pack Scepter C18-120 (100 mm x 2.0 mm, 1.9 µm)
Mobile phase A	: 5 mmol/L Ammonium acetate-water
Mobile phase B	: 5 mmol/L Ammonium acetate-Methanol
Flow rate	: 0.2 mL/min (0-21 min, 27.01-32min), 0.6 mL/min (21.01-27 min) or 0.4 mL/min (0-21 min, 27.01-32min), 0.6 mL/min (21.01-27 min)
Time program	: B conc. 3% (0 min) → 10% (2 min) → 55% (6 min) → 100% (21-26 min) → 3% (26.01-32 min)
Column temp.	: 40 °C
Injection vol.	: 5 µL
Rince R0	: Water
Rince R1	: Methanol / Acetone / IPA = 1 / 1 / 1 including 0.1% Formic acid
Rince R2	: 5 mmol/L Oxalic Acid Dihydrate-Methanol
Needle rinse program	: inside) R1 → R2 → R0, 1000 µL each, outside) R1

MS conditions (LCMS-8050)	
Ionization	: ESI, Positive/Negative simultaneous MRM mode
DL temp.	: 150 °C
Interface temp.	: 200 °C
Heat block temp.	: 500 °C
Nebulizer gas	: 2.0 L/min
Heating gas	: 10 L/min
Drying gas	: 10 L/min
Probe position	: 2 mm or 3 mm

High Speed Mass Spectrometer
Ultra Fast Polarity Switching -5 msec
Ultra Fast MRM -Max.555 transition/sec

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Table 1. MRM transitions of pesticides.

No.	Name	Retention Time (min)	Polarity	Transition
1	Abamectin B1a	17.675	+	890.30→305.30
2	Acibenzolar-S-methyl	10.270	+	210.90→136.05
3	Aldicarb	6.637	+	208.20→115.85
4	Aldicarb-sulfone (Aldoxycarb)	4.284	+	240.10→ 86.20
5	Anilofos	12.568	+	368.00→125.00
6	Azamethiphos	7.163	+	325.00→182.90
7	Azinphos-methyl	9.566	+	318.00→132.05
8	Azoxystrobin	10.056	+	404.00→371.95
9	Bendiocarb	7.376	+	224.20→109.10
10	Benzofenap	14.541	+	431.15→105.25
11	Boscalid	10.259	+	343.00→306.95
12	Butafenacil	11.240	+	492.10→330.85
13	Carbaryl (NAC)	7.820	+	202.10→145.10
14	Carbofuran	7.393	+	222.10→123.15
15	Carpropamid	12.651	+	334.10→139.10
16	Chloridazon	6.085	+	222.10→104.10
17	Chloroxuron	10.986	+	291.10→ 72.15
18	Chromafenozide	11.379	+	395.20→175.15
19	Clofentezine	13.785	+	303.00→138.15
20	Cloquintocet-mexyl	15.210	+	336.10→237.90
21	Clothianidin	5.642	+	250.00→132.05
22	Cumyluron	10.875	+	303.20→185.10
23	Cyazofamid	11.655	+	325.00→108.10
24	Cycloate	13.541	+	216.10→154.00
25	Cycloprothrin	16.851	+	499.00→181.10
26	Cyflufenamid	13.496	+	413.10→295.05
27	Cyprodinil	12.797	+	226.10→108.00
28	Daimuron (Dymron)	10.654	+	269.25→151.15
29	Diflubenzuron	11.924	+	311.00→158.10
30	Dimethirimol	8.199	+	210.20→ 71.00
31	Dimethomorph (E, Z)	10.113	+	388.10→301.00
32		10.546		
33	Diuron (DCMU)	8.894	+	233.00→ 72.10
34	Epoxiconazole	11.544	+	330.00→121.10
35	Fenamidone	10.103	+	312.10→236.00
36	Fenoxaprop-ethyl	14.620	+	362.10→287.90
37	Fenoxycarb	12.169	+	302.10→ 88.00
38	Fenpyroximate (E, Z)	15.640	+	422.30→366.20
39		16.876		
40	Ferimzone (E, Z)	10.243	+	255.20→ 91.05
41		10.405		
42	Flufenacet	11.269	+	364.10→152.05

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Table 1. MRM transitions of pesticides.

No.	Name	Retention Time (min)	Polarity	Transition
43	Flufenoxuron	16.413	+	489.00→158.10
44	Fluridone	9.828	+	330.10→309.00
45	Furametpyr	8.531	+	334.10→157.10
46	Furathiocarb	14.816	+	383.20→195.00
47	Hexaflumuron	14.647	-	458.80→439.00
48	Hexythiazox	15.683	+	353.10→228.00
49	Imazalil	12.430	+	297.10→159.05
50	Imidacloprid	5.608	+	256.10→174.95
51	Indanofan	11.640	+	341.10→175.15
52	Indoxacarb	14.161	+	528.10→203.00
53	Iprovalicarb	11.131	+	321.20→119.15
54	Isoxaflutole	8.748	+	360.10→251.00
55	Linuron	9.924	+	248.80→182.05
56	Lufenuron	15.943	-	508.90→339.00
57	Mepanipyrim	11.498	+	224.10→ 77.00
58	Methabenzthiazuron	8.639	+	222.10→150.10
59	Methiocarb	9.990	+	226.10→121.10
60	Methomyl	4.804	+	163.00→ 87.90
61	Methoxyfenozide	10.828	+	369.20→149.15
62	Monolinuron	8.060	+	215.10→ 99.10
63	Naproanilide	12.093	+	292.25→171.25
64	Novaluron	14.782	+	493.00→158.00
65	Oryzalin	11.329	+	347.10→288.00
66	Oxamyl	4.519	+	237.10→ 72.10
67	Oxaziclomefone	14.670	+	376.20→190.15
68	Oxycarboxin	6.226	+	268.10→175.00
69	Pencycuron	13.575	+	329.10→125.00
70	Pentoxazone	14.788	+	371.10→286.00
71	Pirimicarb	8.351	+	239.20→ 72.00
72	Propaquizafop	15.067	+	444.10→100.15
73	Pyrazolynate	13.657	+	439.10→ 91.15
74	Pyriftalid	9.746	+	319.10→139.10
75	Quizalofop-ethyl	14.641	+	373.10→298.90
76	Silafluofen	19.913	+	426.30→287.15
77	Simeconazole	11.078	+	294.10→ 69.95
78	Spinosyn A	18.045	+	732.60→142.20
79	Spinosyn D	18.633	+	746.60→142.10
80	Tebufenozide	12.083	+	353.20→133.10
81	Tebuthiuron	7.575	+	229.10→172.00
82	Teflubenzuron	15.282	-	378.80→339.00
83	Tetrachlorvinphos (CVMP)	12.101	+	366.90→127.15
84	Thiabendazole	7.196	+	202.00→175.00

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Table 1. MRM transitions of pesticides.

No.	Name	Retention Time (min)	Polarity	Transition
85	Thiacloprid	6.424	+	253.00→126.05
86	Thiamethoxam	4.936	+	292.00→211.10
87	Thiodicarb	8.387	+	355.00→ 88.00
88	Triflumuron	13.318	+	359.00→156.05
89	Triticonazole	11.153	+	318.10→ 70.15

Result

Confirmation of quantitative range

It was confirmed that 89 compounds can obtain linearity at 0.1 to 20 ng/mL by the LCMS-8050 with the basic condition. In addition, linearity was obtained at 0.1 to 50 ng/mL with 78 compounds. The MRM chromatogram of the standard at 1 ng/mL is shown in figure 1.

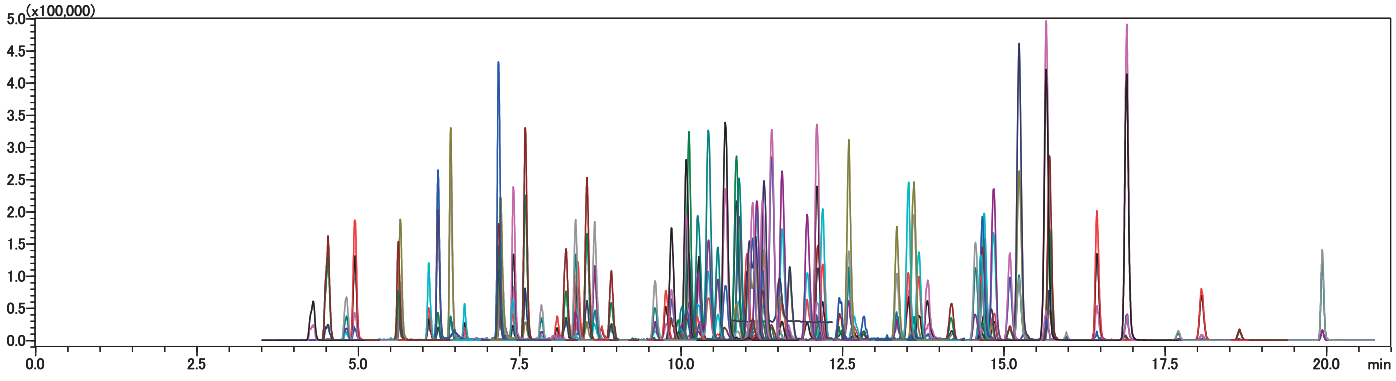

Figure 1. MRM chromatogram of reference standard (1 ng/mL) of pesticides.

Optimization for Recovery rate and reproducibility by flow rate and/or ESI probe position

In comparison between matrix-matched calibration curve and standard addition method, absolute calibration method is strongly prone to be affected by matrix effects such as ion suppression, thus the recovery rate would be less. Yet, according to the evaluation for several measurement parameters, we obtained the result that the flow rate and ESI probe position contributed to improve the recovery ratio and reproducibility.

For the analyses of the carrot extract spiked with 1 ng/mL as final concentration of the target pesticides, at 0.4 mL/min flow rate with 3 mm probe position, recovery rate and reproducibility of pesticides were generally increased. Detailed results were summarized in figure 2 and figure 3.

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Table 2. Content of conditions.

Condition	T.Flow (mL/min)	ESI probe position (mm)
a)	0.2	2
b)	0.2	3
c)	0.4	2
d)	0.4	3

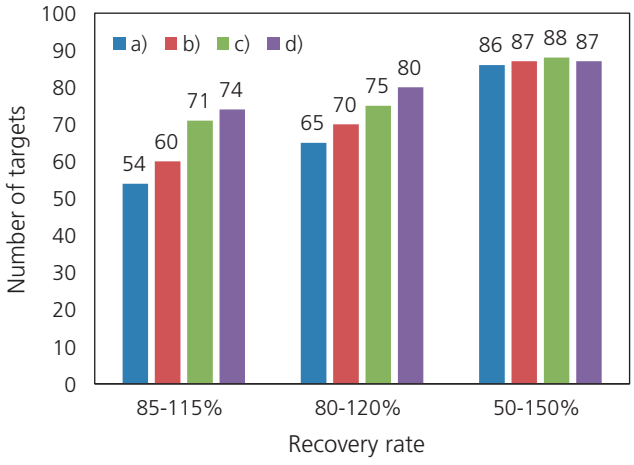

Figure 2. The recovery rate of carrot extract spiked with target pesticides.

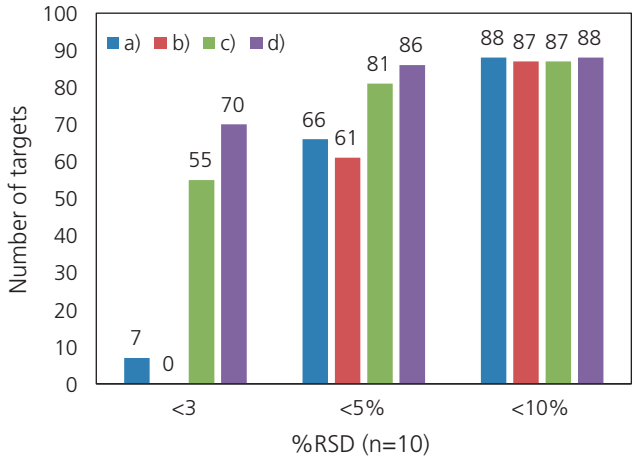

Figure 3. The reproducibility of carrot extract spiked with target pesticides.

Variance of peak area in response to changes of flow rate and/or ESI probe position

The number of targets in each ratio of the area value changed by changing the flow rate from 0.2 mL/min to 0.4 mL/min is shown in figure 4. From the figure 4, when the flow rate was increased, the area values of both the standard sample and the matrix-added sample became smaller. In addition, the number of targets in each ratio of the area value fluctuated depending on the probe position from 2 mm to 3 mm is shown in figure 5. According to the figure 5, the area value increased for both the standard sample and the matrix-added sample at the long distance of the probe position.

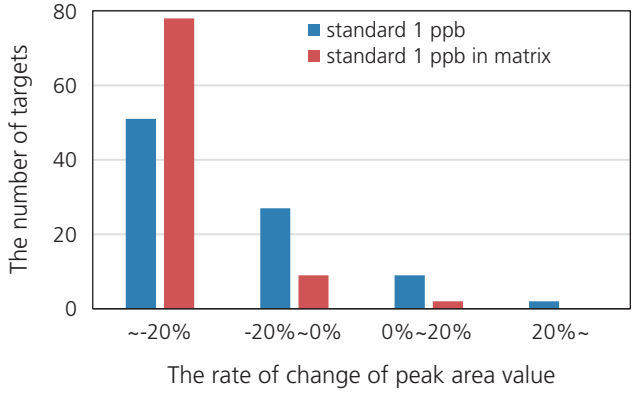

Figure 4. The number of targets of peak area changed by increasing the flow rate.

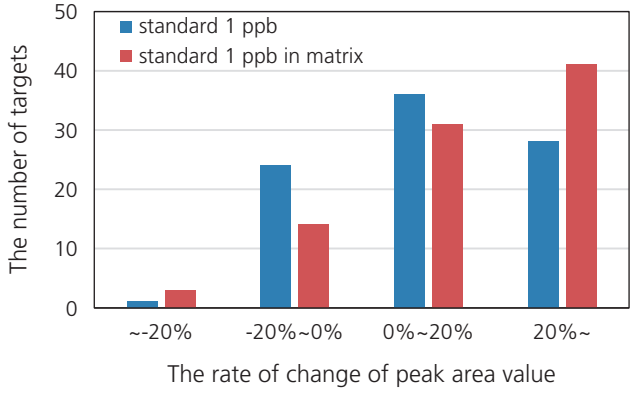

Figure 5. The number of targets of peak area changed depending on the position of the probe from 2 mm to 3 mm.

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MRM chromatogram of pesticides

MRM chromatogram of negative detect compounds and low sensitivity compounds of reference standard in 1 ng/mL and carrot extract spiked with 1 ng/mL as final concentration are shown in figure 6.

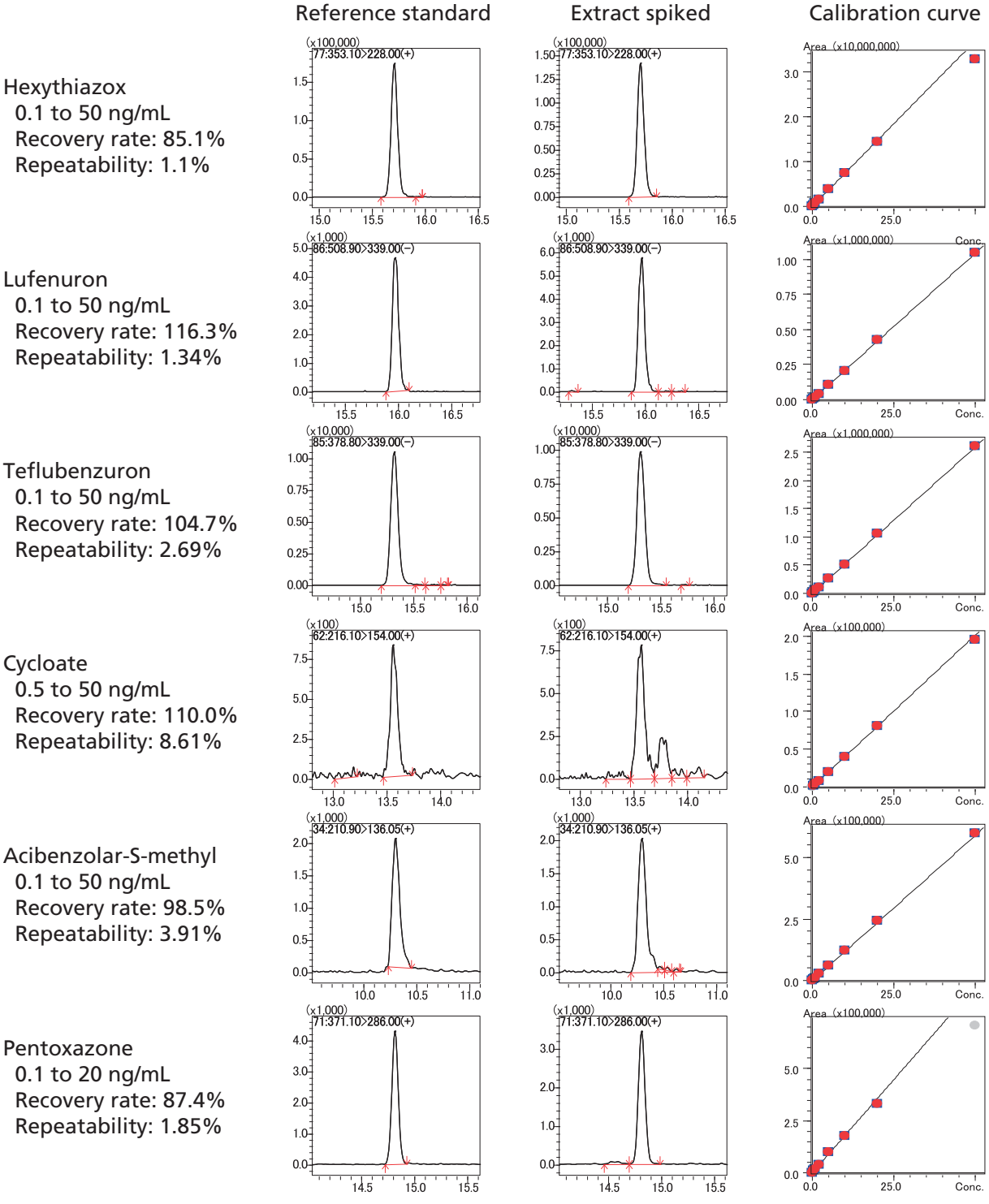

Figure 6. MRM chromatograms of reference standard 1 ng/mL, extract spiked with 1 ng/mL as final concentration with calibration curve.

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Conclusions

- We established a method to obtain high recovery conditions and repeatability with the absolute calibration curve.
- 89 compounds of pesticides were detected within 32 minutes including equilibration time.
- All compounds can be quantified in the range of 0.5 to 20 ng/mL. 87 compounds can be quantified in the range of 0.1 to 20 ng/mL and 78 compounds can be quantified in the range of 0.5 to 50 ng/mL.
- For further optimization, it was found that the recovery rate was significantly improved by the optimized flow rate and probe position.
- By rising the flow rate up to 0.4 mL/min and/or the probe position at up to 3 mm, recovery rate and repeatability were most improved.

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