SHIMADZU
Excellence in Science

Application News

No.C136

Liquid Chromatography Mass Spectrometry

Expanding Capabilities in Multi-Residue Pesticide Analysis Using The LCMS-8060

David R. Baker[1], Laëtitia Fages[2], Eric Capodanno[2], Neil Loftus[1]
[1]Shimadzu Corporation, UK; [2]Phytocontrol, France

Abstract

With an increasing global population, food security is increasingly under threat and there is a growing challenge for agriculture to produce more food, safely and more sustainably. The use of herbicides, insecticides, and fungicides reduce crop losses both before and after harvest, and increase crop yields. However, pesticide residues resulting from the use of plant protection products on crops may pose a risk to human health and require a legislative framework to monitor pesticide residues in food.

National programs for pesticide monitoring in the US, Europe and Japan have set Maximum Residue Levels (MRL's) or tolerance information (EPA) for pesticides in food products. A default value of 0.01 mg/kg is applied for MRL enforcement, which therefore requires highly sensitive and specific analytical technologies to monitor an increasing number of pesticides.

This application note describes the expanded capability of the LCMS-8060 to help accelerate method development workflows and support increased pesticide monitoring programs. Using the Shimadzu Pesticide MRM Library (the Library includes information on 766 certified reference materials) a single multi-residue LC/MS/MS method was developed for 646 pesticides (3 MRM transitions for over 99 % targeted pesticides resulting in 1,919 transitions in total, with a polarity switching time of 5 msec).

Keywords: Pesticides; food safety; LCMS-8060; Pesticide MRM Library, 776 compound library

Introduction

There are more than 1,000 pesticides used globally on soil and crops. With the ever increasing international trade of the food industry, regulatory bodies around the world have increased the number of regulated pesticides and the maximum residue levels (MRLs) allowed in food commodities. In the EU, regulation 396/2005/EC and its annexes set MRLs for over 500 pesticides in 370 food products.[1)] In the US, tolerances for more than 450 pesticides and other ingredients are established by the US EPA[2)] and Japan's positive list system for agricultural chemical residues in foods contains MRLs for over 400 pesticides in various commodities.[3)]

National pesticide monitoring programs create new challenges for food safety laboratories as the number of pesticides required for analysis is increasing together with an expanded range of food products.

In this application paper we present the development of a LC-MS/MS method for screening and quantifying over 646 pesticides in a single method. The method was quickly and efficiently set up using the Shimadzu Pesticide MRM Library. For each target pesticide analysis, up to 3 MRMs (Multiple Reaction Monitoring) transitions were imported from the library. 3 MRMs transitions provided additional data confidence in reporting results in comparison to the conventional 2 transitions used in most methods. As the LCMS-8060 has a high data acquisition speed 1,919 transitions were acquired using a polarity switching speed of 5 msec over a 10.5 minutes gradient elution.

To evaluate the method QuEChERS extracts of mint, tomato and apple were provided by a commercial laboratory as raw acetonitrile extracts and spiked with 646 pesticides (data is presented on the mint extract as it is the more complex sample matrix). The method was evaluated in matrix to ensure that the reporting limits were in agreement with recognised MRL's.

Experiment

Food extracts of mint, tomato and apple were supplied by Phytocontrol, France, following established QuEChERS protocols. Final extracts were prepared in acetonitrile without any dilution. Certified reference materials for the Shimadzu Pesticide MRM Library were obtained from ACSD, France as stock solutions. All solvents were of LCMS quality purchased from Sigma-Aldrich.

A six point calibration curve from 0.002 - 0.1 mg/kg (2 - 100 pg/µL) were generated using internal standard method. Two internal standards (Atrazine-d5 and Diuron-d6) were spiked in during the auto-sampler sequence for quantitation.

The robustness of the LCMS-8060 was assessed by peak area response for 646 pesticides spiked into mint, tomato and apple matrix extracts at 0.05 mg/kg.

LC/MS/MS method development

The Shimadzu Pesticide MRM Library has 766 pesticides in its database (Application News No. C135). For each pesticide several MRM's are included in the database and in this analysis the default value used was 3 MRM's. For this method, 1,919 transitions were selected in both positive and negative ionisation mode using a switching time of 5 msec (1,819 MRM transitions were in positive mode and 100 MRM transitions in negative mode).

To optimize ion source conditions (for example, DL temperature, interface temperature, heating block temperature, heating gas flow, drying gas flow and nebulizer gas flow) the interface setting software was used. This tool provides an optimized response for all compounds.

Table 1 LC and MS/MS Acquisition Parameters

Liquid chromatography	
UHPLC	Nexera LC system
Analytical column	Restek Raptor Biphenyl (2.1 mm I.D. × 100 mm L., 2.7 µm)
Column temperature	35 ˚C
Flow rate	0.4 mL/min
Solvent A	2 mmol/L ammonium formate + 0.002 % formic acid - Water
Solvent B	2 mmol/L ammonium formate + 0.002 % formic acid - Methanol
Binary Gradient B.Conc.	3 % (0 min) - 10 % (1.00 min) - 55 % (3.00 min) - 100 % (10.50 - 12.00 min) - 3 % (12.01 - 15.00 min)
Injection volume	2 µL sample (plus 40 µL water)

Mass spectrometry	
LC/MS/MS	LCMS-8060
Ionisation mode	Heated electrospray
Polarity switching time	5 msec
Pause time	1 msec
Total MRM transitions	1,919 (1,819 positive; 100 negative)
MRM Dwell	4 msec (target ion); 1 msec (reference ion)
Interface temperature	350 ˚C
Heating block	300 ˚C
Desolvation line	150 ˚C
Heating gas	10 L/min
Drying gas	10 L/min
Nebulizer gas	3 L/min

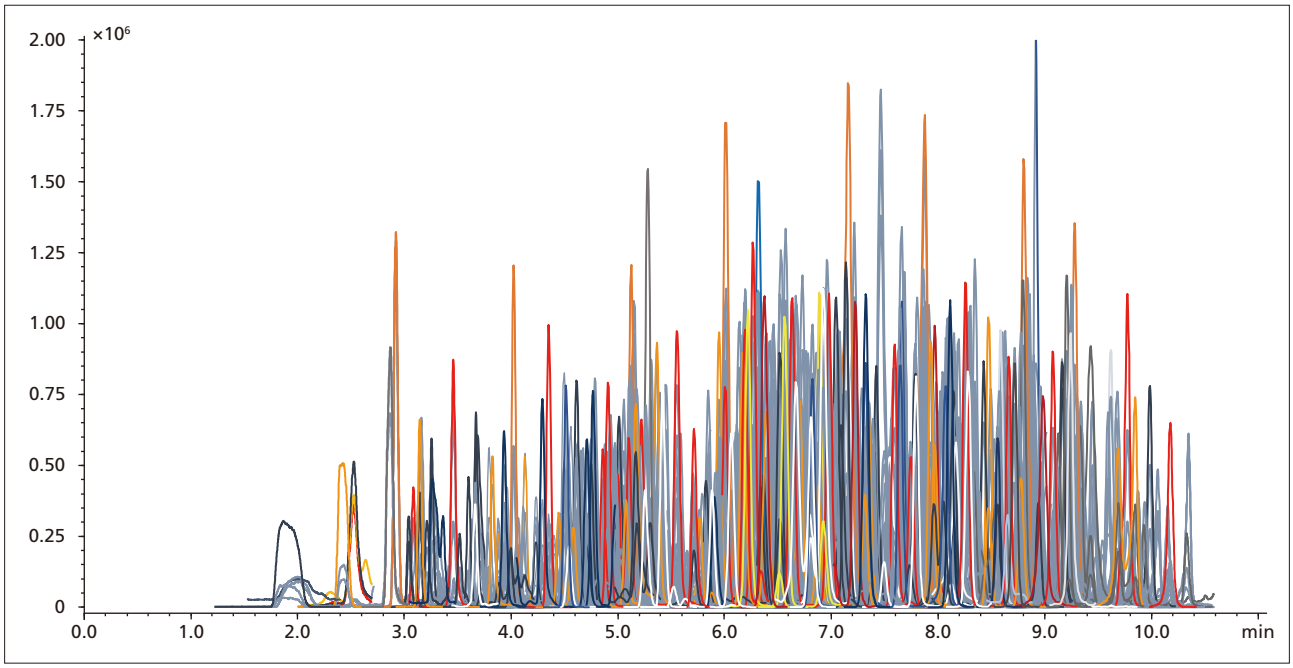

Fig. 1 MRM chromatograms of 646 pesticides spiked into a mint extract at 0.01 mg/kg (Up to 3 MRMs per compound and 5 msec polarity switching time).

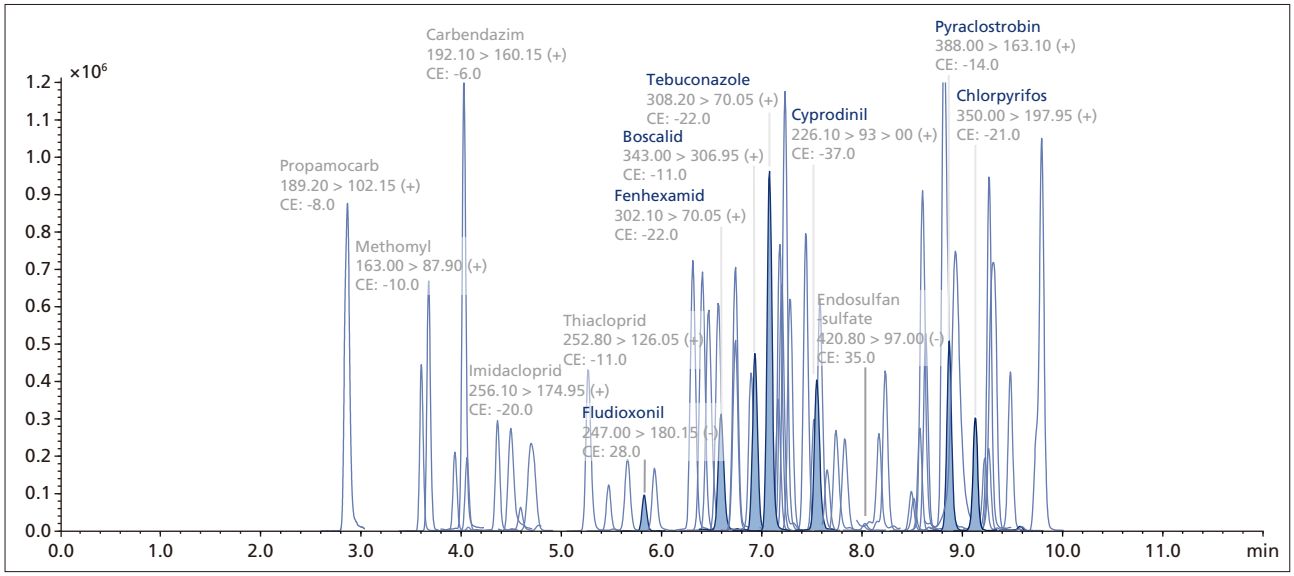

Fig. 2 MRM chromatograms for pesticides most commonly detected in plant products listed in the 2015 European Food Safety Journal. In this report, residues exceeding the legal limits were related to 58 different pesticides. Compounds such as boscalid, chlorpyriphos, cyprodinil, fenhexamid, fludioxonil, pyraclostrobin and tebuconazole (highlighted in the MRM chromatogram) are some of the most frequently detected compounds present in more than 4 % of the samples analyzed.

The MRM chromatograms show the response to each pesticide spiked into a food matrix at the default MRL of 0.01 mg/kg.

Results and Discussion

Shimadzu Pesticide MRM Library

(Application News No. C135)

A flexible tool for expanding capabilities in pesticide monitoring programs

The Pesticide MRM Library has been created using 766 certified reference materials and is designed to help accelerate method development and compound management.

The library contains an average of 8 optimized MRM transitions for each compound (including positive and negative ion modes). In total, more than 6,000 MRM transitions are held within the 766 compound library. The library itself documents CAS#, formula, activity, mono-isotopic mass and adduct masses, rank of MRM transitions, synonyms, InChI, InChIKey, compound names translation (Japanese and Chinese) and links to websites offering further information (for example; alanwood.net, PAN pesticide database, Chemical Book, ChemSpider).

The library also serves as a powerful data repository for reporting and checking pesticide data sources.

Creating flexible pesticide monitoring methods

Building a new LC/MS/MS method

To create new pesticide LC/MS/MS methods the user simply needs to select the target compounds from the library, identify the required number of MRMs for each compound and confirm the analytical column for the analysis. (The new method can be used to expand current capabilities or to create focused methods with a limited number of pesticides). The new method is simply imported into LabSolutions.

As the LCMS-8060 has a high data acquisition speed of 30,000 u/sec, high sensitivity and a polarity switching speed of 5 msec, the capabilities of the library can be expanded to meet the future needs of any laboratory.

Expanded capability of the LCMS-8060

The LCMS-8060 has a data acquisition speed of 30,000 u/sec which creates new opportunities for expanding compound lists.

As one example, between 6.45 and 6.60 minutes 25 pesticide compounds elute (Fig. 3). Even with high data density acquisitions the average variation in peak area response was less than 3 %RSD (varying between 1.1 - 5.9 %RSD).

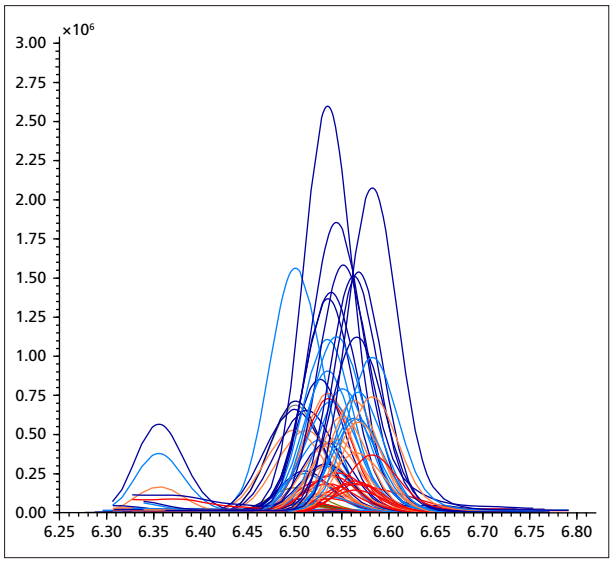

Fig. 3 The LCMS-8060 can acquire MRM data at a high speeds and enables precise quantitation even with high data density. Between 6.45 and 6.60 minutes 25 compounds were monitored (Table 2).

Table 2 Peak area variation (%RSD; n=6) for 25 pesticides eluting over a nine-second time window (6.45 - 6.60 minutes) spiked into a mint matrix extract at the reporting limit of 0.01 mg/kg.

Compound Name	CAS number	Formula	M	Polarity	MRM Quantitation Ion	RT	Average Peak Area	%RSD (n=6)
Trinexapac-ethyl	95266-40-3	C13H16O5	252.0998	+	252.90 > 69.05	6.45	1,780,015	3.1
Iprovalicarb	140923-17-7	C18H28N2O3	320.2100	+	321.20 > 119.15	6.46	1,442,486	2.8
Dodemorph	1593-77-7	C18H35NO	281.2719	+	282.30 > 116.15	6.47	658,920	4.2
Fluopyram	658066-35-4	C16H11ClF6N2O	396.0464	+	397.00 > 145.00	6.47	2,439,146	1.9
Flutolanil	66332-96-5	C17H16F3NO2	323.1133	+	324.10 > 242.00	6.48	3,372,285	2.7
Trifloxysulfuron	145099-21-4	C14H14F3N5O6S	437.0617	+	438.00 > 182.15	6.48	1,822,340	2.5
Azaconazole	60207-31-0	C12H11Cl2N3O2	299.0228	+	300.00 > 159.00	6.50	1,580,445	2.0
Terbutryn	886-50-0	C10H19N5S	241.1361	+	242.10 > 157.95	6.50	755,446	3.4
Prometryn	7287-19-6	C10H19N5S	241.1361	+	242.10 > 158.00	6.50	1,300,193	2.6
Azimsulfuron	120162-55-2	C13H16N10O5S	424.1026	+	425.10 > 182.10	6.50	2,498,050	1.8
Metominostrobin	133408-50-1	C16H16N2O3	284.1161	+	285.10 > 193.95	6.51	2,929,500	1.7
Thifluzamide	130000-40-7	C13H6Br2F6N2O2S	525.8421	+	528.60 > 148.05	6.51	193,982	5.9
Nicarbazin	330-95-0	C13H10N4O5	302.0651	-	301.10 > 137.15	6.52	973,101	2.6
Bromobutide	74712-19-9	C15H22BrNO	311.0885	+	312.10 > 194.10	6.53	1,829,781	2.1
Saflufenacil	372137-35-4	C17H17ClF4N4O5S	500.0544	+	501.00 > 198.00	6.53	465,224	2.3
Cyproconazole	94361-06-5	C15H18ClN3O	291.1138	+	292.10 > 70.05	6.54	1,174,967	1.7
Clomazone	81777-89-1	C12H14ClNO2	239.0713	+	239.90 > 125.00	6.54	3,409,656	1.7
Fensulfothion	115-90-2	C11H17O4PS2	308.0306	+	309.00 > 281.00	6.54	4,267,514	1.4
Oxasulfuron	144651-06-9	C17H18N4O6S	406.0947	+	407.10 > 150.15	6.54	2,911,533	1.1
Rimsulfuron	122931-48-0	C14H17N5O7S2	431.0569	+	432.00 > 182.00	6.55	4,722,065	1.8
Fenthion-oxon	6552-12-1	C10H15O4PS	262.0429	+	263.10 > 231.00	6.55	3,075,195	1.4
Nitrothal-isopropyl	10552-74-6	C14H16NO6Na	317.0875	+	295.10 > 230.95	6.56	2,199,581	3.0
Chlorantraniliprole	500008-45-7	C18H14BrCl2N5O2	480.9708	+	483.90 > 452.90	6.57	2,407,025	2.7
Fipronil-sulfone	120068-36-2	C12H4Cl2F6N4O2S	451.9336	-	451.00 > 414.90	6.57	2,843,708	2.0
Valifenalate	283159-90-0	C19H27ClN2O5	398.1608	+	399.20 > 155.00	6.59	3,845,335	1.9

Final method performance for 646 pesticides

In order to test the performance of the developed method, linearity, repeatability and longer term robustness were assessed for all 646 pesticides.

Linearity

Linearity was assessed over a six point calibration curve from 0.002 - 0.1 mg/kg (2 - 100 pg/µL). All 646 pesticides achieved excellent R^2 values greater than 0.99 in both tomato and mint spiked extracts with typical values greater than 0.996. Calibration curves were generated using a linear curve fit type and 1/C weighting. Typical calibration curve data is presented below in Fig. 4.

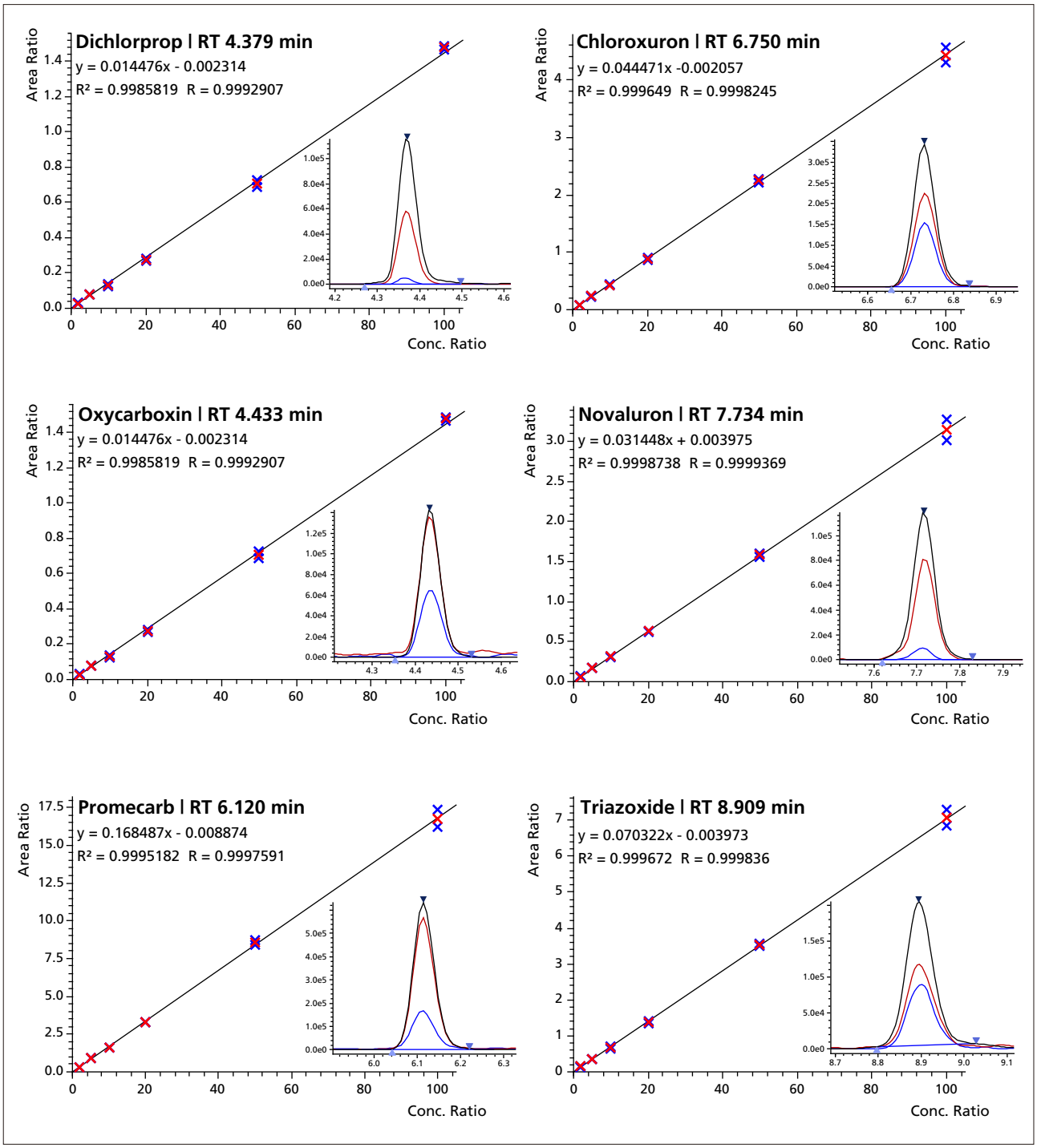

Fig. 4 Calibration curves for selected pesticides spiked into a mint matrix extract in the range 0.002 - 0.1 mg/kg. The quantitation MRM chromatogram is shown in black (qualifier ion MRM chromatograms are shown in red and blue).

Repeatability

To assess the robustness of the system and the developed method during routine analysis, repeat injections of a mint matrix sample spiked with 646 pesticides at 0.05 mg/kg, were analyzed over a 24 hour period.

The results for selected compounds are displayed below in Fig. 5.

Compounds were selected throughout the run at equi-distant points (closest elution points to 3, 4, 5, 6, 7, 8, 9 and 10 minutes), including positive and negative ion detection, (Table 3).

The peak area variance was less than 5.7 % for all pesticides measured.

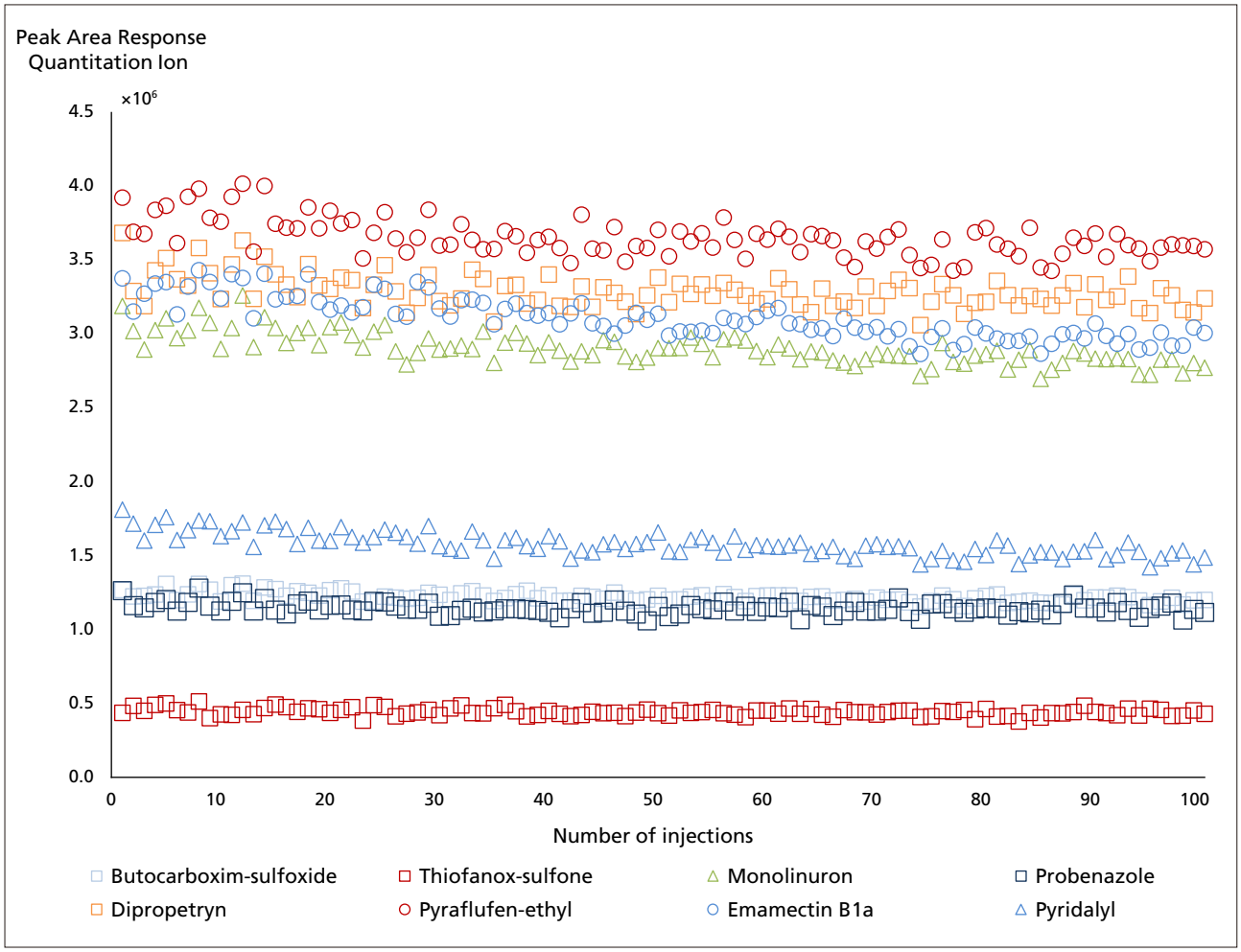

Fig. 5 Peak area response for several pesticides following 100 repeat injections of a 0.05 mg/kg spiked into mint matrix extract.

Table 3 Peak area variance for selected following the repeated injection of a 0.05 mg/kg spiked into mint matrix extract (number of sample replicates was 100; the analysis sequence was 24 hours).

Compound Name	CAS Number	Formula	M	Polarity	MRM Quantitation Ion	RT (mins)	Average Peak Area	%RSD (n=100)
Butocarboxim-sulfoxide	34681-24-8	C7H14N2O3S	206.0725	+	207.10 > 75.10	3.042	1,220,391	2.6
Thiofanox-sulfone	39184-59-3	C9H18N2O4S	250.0987	+	268.10 > 57.00	4.001	442,724	5.7
Monolinuron	1746-81-2	C9H11ClN2O2	214.0509	+	215.10 > 99.10	4.985	2,904,116	3.7
Probenazole	27605-76-1	C10H9NO3S	223.0303	+	224.00 > 41.05	5.995	1,145,189	3.5
Dipropetryn	4147-51-7	C11H21N5S	255.1518	+	256.20 > 144.05	6.999	3,289,597	3.4
Pyraflufen-ethyl	129630-19-9	C15H13Cl2F3N2O4	412.0204	+	413.00 > 339.00	8.004	3,653,333	3.5
Emamectin B1a	138511-97-4	C56H81NO15	1007.5606	+	886.40 > 158.20	9.008	3,109,562	4.5
Pyridalyl	179101-81-6	C18H14Cl4F3NO3	488.9680	-	491.90 > 109.05	10.171	1,579,422	5.0

Response to differing matrices

One of the major challenges in the quantitative LC/MS/MS analysis for pesticides in food is that compound and matrix-dependent response suppression or enhancement may occur. Although matrix effects can affect the peak area response between different food types following a QuEChERS extraction protocol, the peak area variance should be minimized within a single matrix.

Food extracts of apple, mint and tomato following QuEChERS extraction were spiked with 646 pesticides at 0.05 mg/kg and were repeatedly injected on the LCMS-8060 (n=100 repeat injections for each matrix; 300 injections in the same batch sequence). Fig. 6 shows the response for 3 selected pesticides analyzed in a single batch sequence corresponding to a 72 hour analysis sequence. Within a matrix, variance was less than 5.9 %RSD for all compounds.

Although the absolute peak area changes with different food matrices, the response between injection 1 and injection 100 for 2 pesticides (probenazole and dipropetryn) within a single matrix has a variance less than 5.7 %RSD.

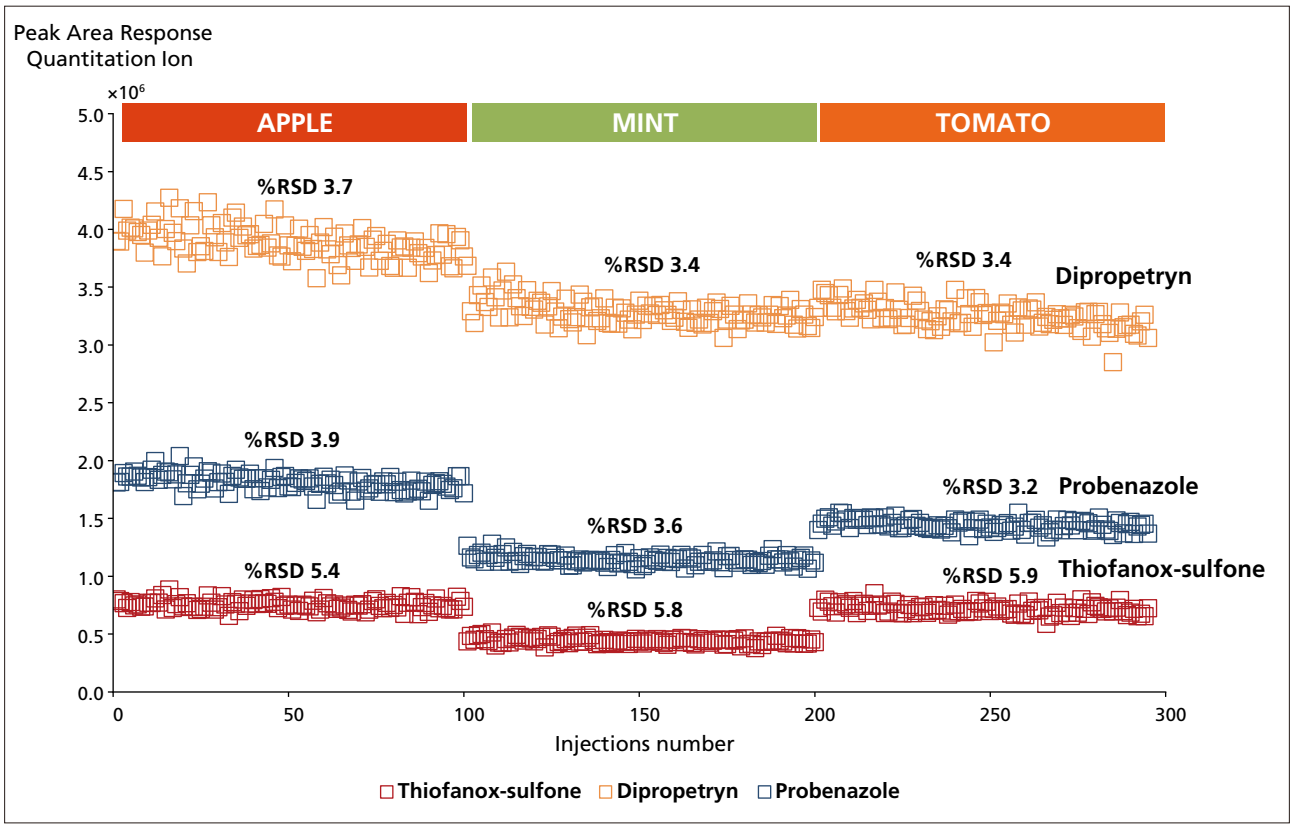

Fig. 6 Peak area response for three pesticides spiked into apple, mint and tomato matrix extracts at 0.05 mg/kg over 72 hours. As in Fig. 5, compounds were selected to reflect peak area response throughout the chromatographic run (Table 3).

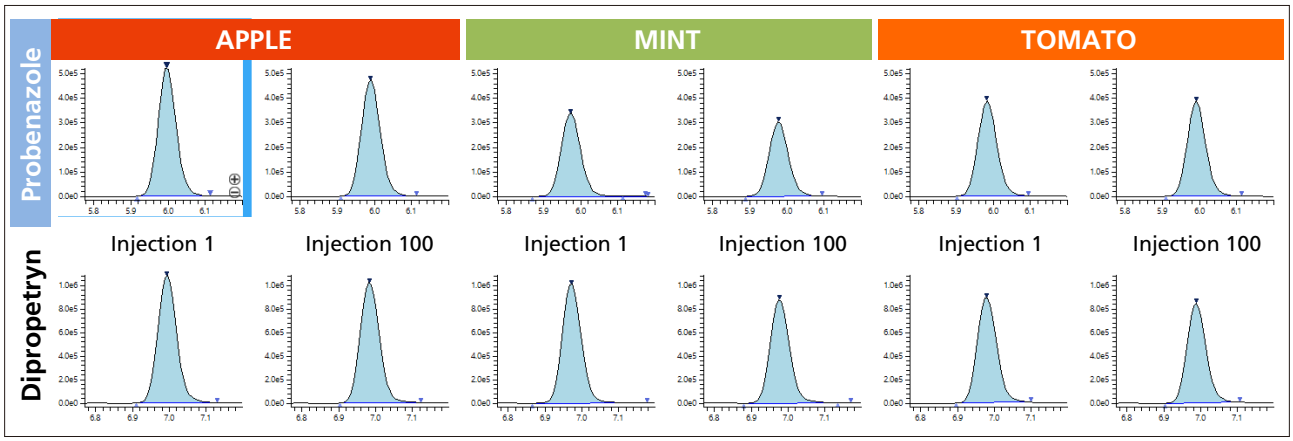

Fig. 7 MRM chromatograms for probenazole (RT 5.995 minutes) and dipropetryn (RT 6.999 minutes) for injection 1 and injection 100 spiked into apple, mint and tomato matrix extracts. The extracts were spiked at 0.05 mg/kg and analyzed over 72 hours.

Reducing matrix effects by extensively diluting the sample

The need to test for more pesticides in a wider range of samples at high sensitivity is very challenging as matrix effects from the sample extraction will influence both ion suppression and enhancement. Ion suppression can lead to errors in the detection capability, accuracy and precision of the method.

To reduce the effect of interfering compounds in the quantitation of complex samples extensive sample dilution is now widely used in routine analysis. It is an approach which is simple to build into multi-residue extraction methods and is cost effective.

This approach leads to greater robustness as a consequence of a reduced sample injection in the LC/MS/MS, higher data quality and increased instrument uptime.

Fig. 8 shows the results of diluting a matrix sample spiked at 0.005 mg/kg with dilution factors of 1:5, 1:10, 1:20, 1:50 and 1:100.

As matrix effects can be both significant and variable for different compounds Table 4 shows recovery data for a series of pesticides diluted from 0 to a dilution factor of 1:100.

Matrix suppression was reduced for most compounds when the sample was diluted 1:10 with recoveries in the range of 70 - 120 % with an associated repeatability RSDr ≤ 20 %. Relative standard deviations in relation to the mean values were typically less than 10 %.

Diluting the sample by a factor of 20 or 50 resulted in acceptable signal suppression from the matrix.

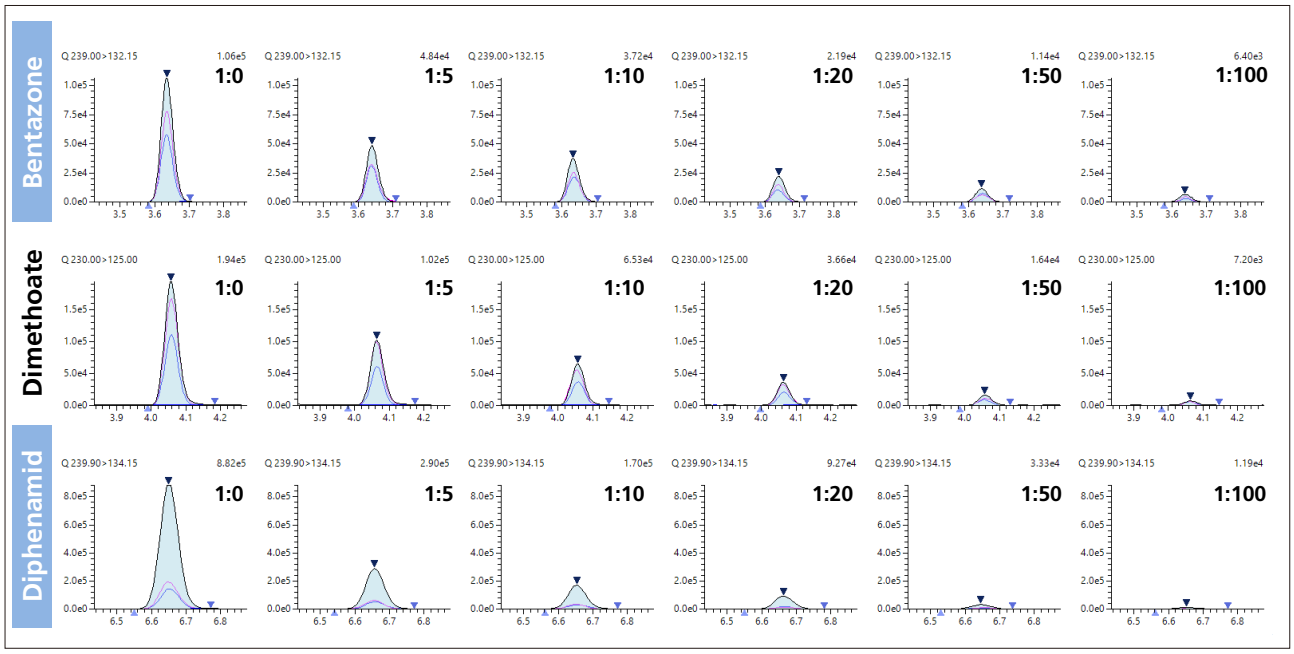

Fig. 8 MRM chromatograms for 3 selected compounds spiked into a mint extract at 0.005 mg/kg and diluted 1:5, 1:10, 1:20, 1:50 and 1:100 with water.

Table 4 Diluting a sample matrix extract spiked with 0.005 mg/kg with water reduced matrix ion suppression.

				Dilution series					
Compound	CAS	Formula	M	0	1:5	1:10	1:20	1:50	1:100
				Recovery					
Bentazone	25057-89-0	C10H12N2O3S	240.0569	32.1	44.6	65.5	72.7	91.7	98.1
Demeton-S-methyl-sulfone	17040-19-6	C6H15O5PS2	262.0099	51.1	78.5	89.6	91.1	114.2	116.8
Dimethoate	60-51-5	C5H12NO3PS2	228.9996	36.2	65.3	88.5	92.2	92.4	94.2
Isocarbamid	30979-48-7	C8H15N3O2	185.1164	28.8	57.1	81.8	98.7	102.5	96.4
Vamidothion	2275-23-2	C8H18NO4PS2	287.0415	53.6	76.3	98.2	98.5	101.5	114.1
Thiazafluron	25366-23-8	C6H7F3N4OS	240.0293	32.8	62.9	80.5	84.2	87.1	97.4
Demeton-S-methyl	919-86-8	C6H15O3PS2	230.0200	57.8	82.1	93.1	87.6	108.5	102.4
Sebuthylazine	7286-69-3	C9H16ClN5	229.1094	28.7	53.3	69.8	79.8	88.5	95.8
Flutriafol	76674-21-0	C16H13F2N3O	301.1027	27.3	46.1	71.4	76.1	81.8	87.3
Furametpyr	123572-88-3	C17H20ClN3O2	333.1244	48.3	69.8	86.9	86.2	97.6	101.9
Fenobucarb	3766-81-2	C12H17NO2	207.1259	60.9	79.2	100.7	96.1	102.8	103.9
Benodanil	15310-01-7	C13H10INO	322.9807	50.9	69.8	86.3	96.5	102.4	94.8
Terbuthylazine	5915-41-3	C9H16ClN5	229.1094	50.4	66.6	83.2	87.2	89.8	91.0
Dimethachlor	50563-36-5	C13H18ClNO2	255.1026	75.1	86.1	106.0	107.1	106.2	108.0
Dimethenamid	87674-68-8	C12H18ClNO2S	275.0747	72.6	84.9	102.9	100.0	103.6	97.3
Furalaxyl	57646-30-7	C17H19NO4	301.1314	82.2	89.1	106.6	108.6	106.2	102.4
Bixafen	581809-46-3	C18H12Cl2F3N3O	413.0310	66.8	79.3	99.0	95.6	103.7	97.1
Triflumuron	64628-44-0	C15H10ClF3N2O3	358.0332	54.2	71.8	95.5	84.9	95.3	101.7
Epoxiconazole	133855-98-8	C17H13ClFN3O	329.0731	61.6	77.2	98.8	95.3	90.0	101.2
Teflubenzuron	83121-18-0	C14H6Cl2F4N2O2	379.9742	41.8	50.9	80.1	86.8	100.0	97.7

Conclusion

A fast, selective and highly sensitive method has been developed for the quantitation of 646 pesticides using a single method with 1,919 transitions (corresponding to up to 3 MRM transitions per compound) and a LC gradient time of only 10.5 minutes.

As the LCMS-8060 has a rapid polarity switching time of 5 msec, the single multi-residue LC/MS/MS method supported the analysis of 34 pesticides in negative ion mode and 612 compounds in positive ion mode.

The enhanced performance and higher sensitivity of the LCMS-8060 has created new opportunities in sample dilution to reduce ion signal suppression and matrix effects. For most compounds a dilution factor of 1:20 or 1:50 was sufficient to provide recoveries in the range 70 - 120 %.

References

1. Commission Regulation (EC). 2005. No 396/2005 of the European Parliament and of the Council, maximum residue levels of pesticides in or on food and feed of plant and animal origin. Official Journal of the European Union, L 70: 1-16

2. US Environmental Protection Agency, Electronic code of federal regulation: Title 40: Part 180 - tolerances and exemptions for pesticide chemical residues in food. http://www.ecfr.gov/cgi-bin/text-idx?c=ecfr&tpl=/ecfrbrowse/Title40/40cfr180_main_02.tpl

3. Japanese Ministry of Health, Labour and Welfare, Department of Food Safety. 2006. Director Notice about Analytical Methods for Residual Compositional Substances of Agricultural Chemicals, Feed Additives, and Veterinary Drugs in Food (Syoku-An No. 0124001 January 24, 2005; amendments May 26, 2006).

First Edition: Jun. 2016

Shimadzu Corporation
www.shimadzu.com/an/