

Quantitative analysis of 646 pesticides (1,919 MRMs) by LC-MS/MS with a fast 10.5 minute cycle time

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Introduction

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 paper 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 (typically 3 MRM transitions for each targeted pesticide resulting in 1,919 transitions in total, with a polarity switching time of 5 msec).

Materials and Methods

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 an internal standard method. Two internal standards (Atrazine-d5 and Diuron-d6) were spiked in during the auto-sampler sequence for quantitation.

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Table 1. LC and MS/MS acquisition parameters used to create the generic LC-MS/MS method.

Liquid chromatography																	
UHPLC	: Nexera LC system																
Analytical column	: Restek Raptor biphenyl (100 x 2.1 mm. 2.7 µm)																
Column temperature	: 35°C																
Flow rate	: 0.4mL/minute																
Solvent A	: 2 mmol/L ammonium formate in 0.002% formic acid solution																
Solvent B	: 2 mmol/L ammonium formate in 0.002% formic acid solution																
Binary Gradient	: <table border="1" style="margin-left: 20px;"> <thead> <tr> <th>Time (mins)</th> <th>%B</th> </tr> </thead> <tbody> <tr> <td>0</td> <td>3</td> </tr> <tr> <td>1</td> <td>10</td> </tr> <tr> <td>3</td> <td>55</td> </tr> <tr> <td>10.5</td> <td>100</td> </tr> <tr> <td>12</td> <td>100</td> </tr> <tr> <td>12.01</td> <td>3</td> </tr> <tr> <td>15</td> <td>Stop</td> </tr> </tbody> </table>	Time (mins)	%B	0	3	1	10	3	55	10.5	100	12	100	12.01	3	15	Stop
Time (mins)	%B																
0	3																
1	10																
3	55																
10.5	100																
12	100																
12.01	3																
15	Stop																
Injection volume	: 2µL sample (plus 40µL water)																
Mass spectrometry																	
LC/MS/MS	: LCMS-8060																
Ionisation mode	: Heated electrospray																
Polarity switching time	: 5 msec																
Pause time	: 1 msec																
Total MRM transitions	: 1919 (1819 positive; 100 negative)																
MRM Dwell	: 4msec (target ion); 1msec (reference ion)																
Interface temperature	: 350°C																
Heating block	: 300°C																
Desolvation line	: 150°C																
Heating gas	: 10 L/min																
Drying gas	: 10 L/min																
Nebulising gas	: 3 L/min																

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Results

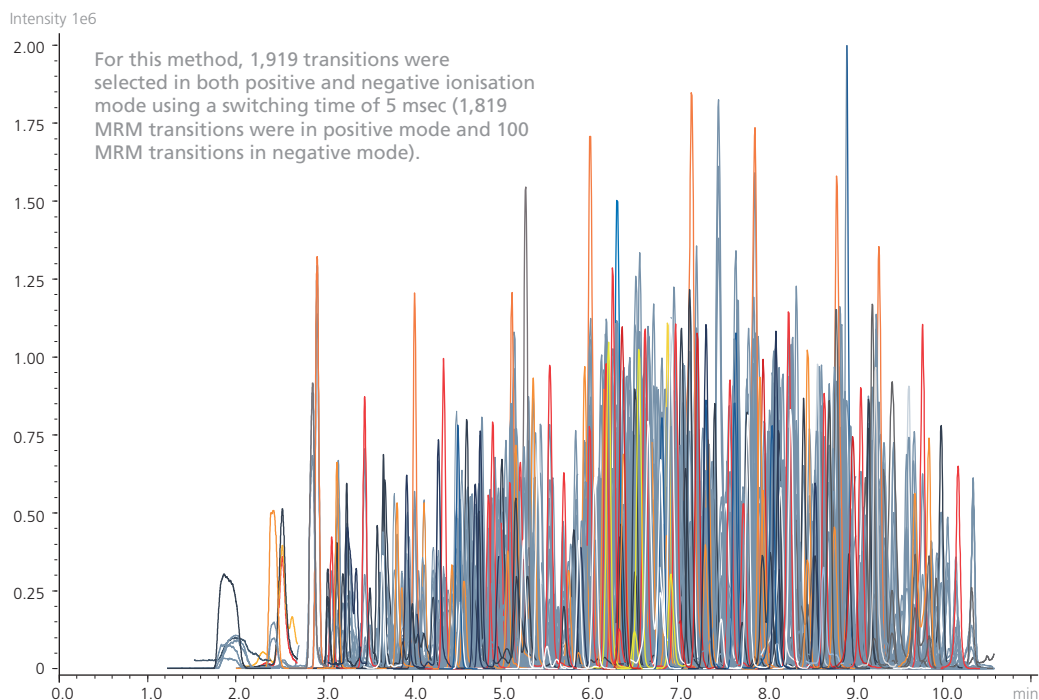


Figure 1. Chromatogram of 646 pesticides spiked into a mint extract at 0.010 mg/kg. (Up to 3 MRMs per compound and 5 msec polarity switching time).

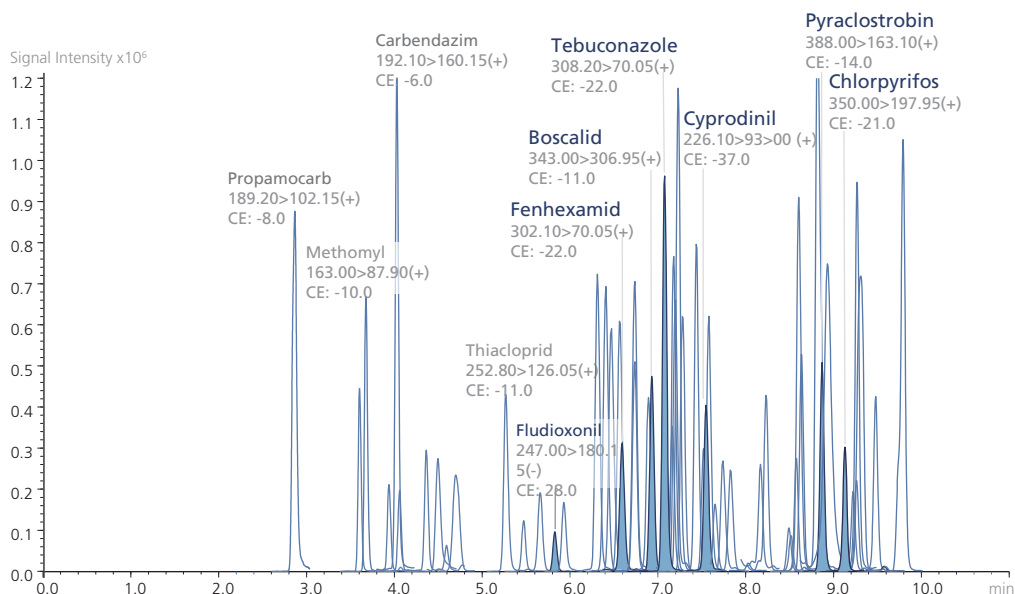


Figure 2. MRM chromatograms for pesticides most commonly detected in plant products listed in the 2015 European Food Safety Journal. In this report, residues exceeding the legal limits were related to 58 different pesticides. Compounds such as boscalid, chlorpyrifos, cyprodinil, fenhexamid, fludioxonil, 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.

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High data density acquisitions

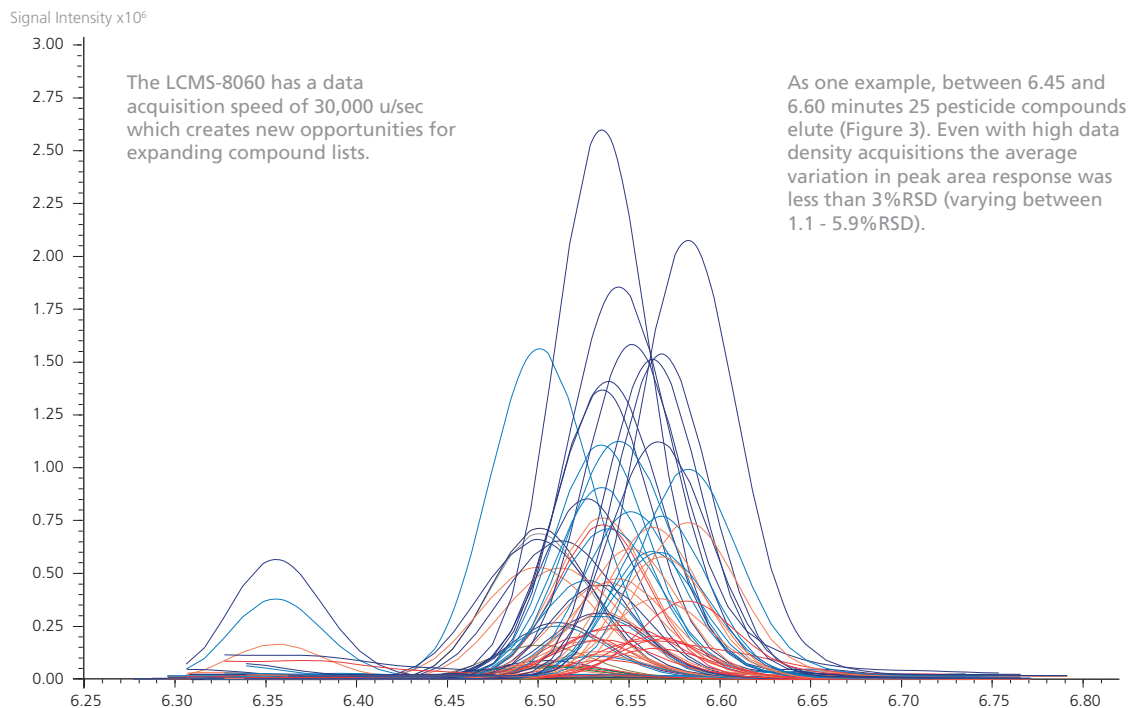


Figure 3. The LCMS-8060 can acquire MRM data at a high speeds and enables precise quantitation even with high data density. Between 6.45 and 6.60 minutes 25 compounds were monitored (Table 2).

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Table 2. 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 with a peak area variation less than 6% (%RSD; n=6). (The sample is a mint matrix extract at the reporting limit of 0.01 mg/kg).

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

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Robustness

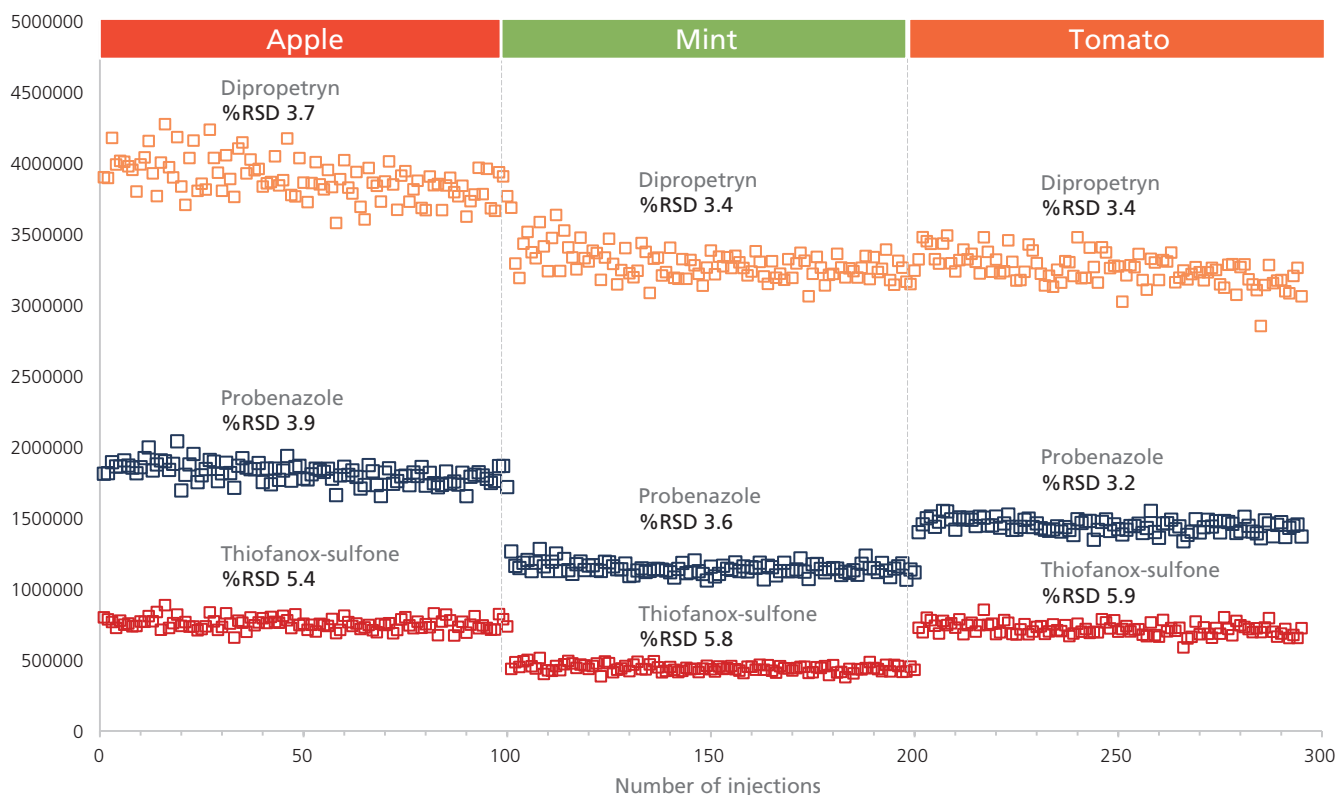


Figure 4. Peak area response for three pesticides spiked into apple, mint and tomato matrix extracts at 0.05 mg/kg over 72 hours. As in Figure 3, compounds were selected to reflect peak area response throughout the chromatographic run (Table 2).

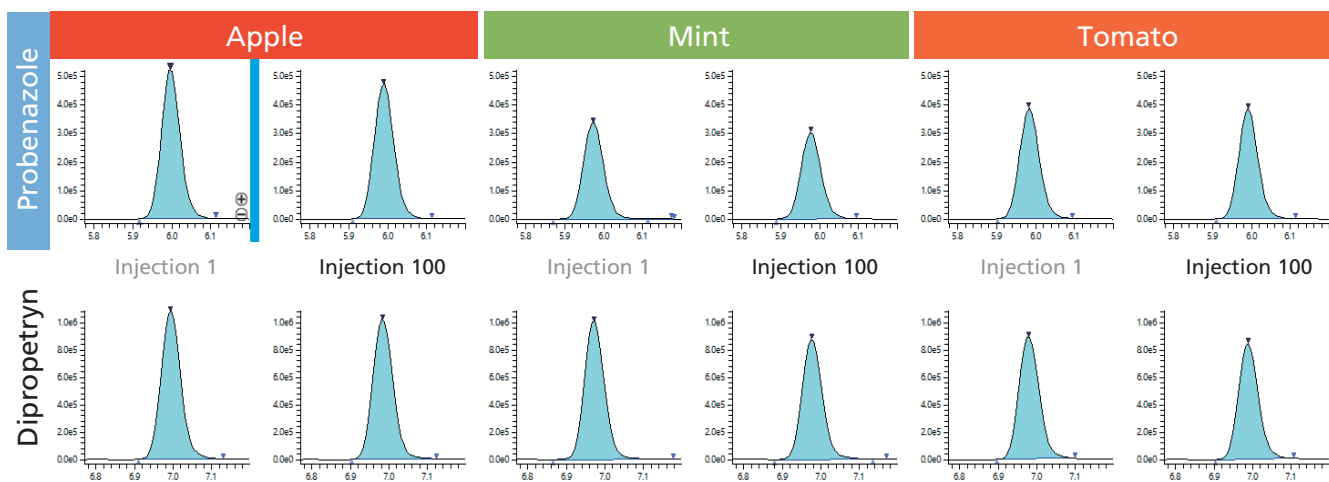


Figure 5. Peak area response for probenazole (RT 5.995 min) and dipropetryn (RT 6.999 min) for injection 1 and injection 100 spiked into apple, mint and tomato matrix extracts. The extracts were spiked at 0.05 mg/kg and analyzed over 72 hours.

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Conclusions

- 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%.

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