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Applying 'MRM Spectrum Mode' and Library Searching for Enhanced Reporting Confidence in Routine Pesticide Residue Analysis

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Introduction

To help reduce the incidence of false positive and false negative reporting in pesticide residue monitoring routine multiple-reaction monitoring (MRM) methods have been enhanced to monitor a higher number of fragment ion transitions to increase specificity and reporting confidence. In this workflow, typically 6-10 fragment ion transitions were monitored for each target pesticide as opposed to a conventional approach using 2-3 fragment ions. By acquiring a high number of fragment ion transitions, each target pesticide had a corresponding fragmentation spectra which could be used in routine library searching and compound verification using reference library match scores. This 'MRM Spectrum mode' was applied to quantify and identify 193 pesticides using 1,291 MRM transitions without compromising limits of detection, linearity or repeatability using a high speed data acquisition triple quadrupole MS/MS.

Materials and Methods

Pesticide spiked samples, extracted using established QuEChERS based methods, were provided by Concept Life Sciences, UK. Matrices included turmeric, plum, peppermint, parsnip, cherry, lime, pumpkin, tomato and potato. Final extracts were prepared in acetonitrile without any dilution and directly injected into the LC-MS/MS. A water co-injection method, performed automatically in the auto-sampler, was used to improve early eluting peak shapes in addition to a sub 2 micron particle size column to improve peak capacity (Table 1) .

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

Liquid chromatography	
UHPLC	: Nexera LC system
Analytical column	: HSS T3 (100 x 2.1, 1.7µm)
Column temperature	: 40°C
Flow rate	: 0.4mL/minute
Solvent A	: 5 mmol/L ammonium formate and 0.004% formic acid
Solvent B	: 5 mmol/L ammonium formate and 0.004% formic acid in methanol
Binary Gradient	:

Time (mins)	%B
1.50	35
11.50	100
13.00	100
13.01	3
15.00	Stop

Injection volume	: 0.1 µl (plus 30µl water)

LC-MS/MS Mass spectrometry	MRM Spectrum Mode : generating library searchable spectra
Target number of compounds	: 193
Total number of MRM transitions	: 1,291 transitions (1,229 in ESI+ and 62 in ESI-)
Pause time/dwell time	: 1 msec/3 msec
Ionisation mode	: ESI +/-
Polarity switching time	: 5 msec
Source temperatures (interface; heat block; DL)	: 350°C; 300°C; 150°C
Gas flows (nebulising; heating; drying)	: 3L/min; 10 L/min; 10L/min

LC-MS/MS Mass spectrometry	2 MRM method
Target number of compounds	: 193
Total number of MRM transitions	: 386 (374 in ESI+ and 12 in ESI-)
Pause time/dwell time	: 1 msec/3 msec
Ionisation mode	: ESI +/-
Polarity switching time	: 5 msec
Source temperatures (interface; heat block; DL)	: 350°C; 300°C; 150°C
Gas flows (nebulising; heating; drying)	: 3L/min; 10 L/min; 10L/min

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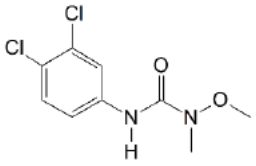
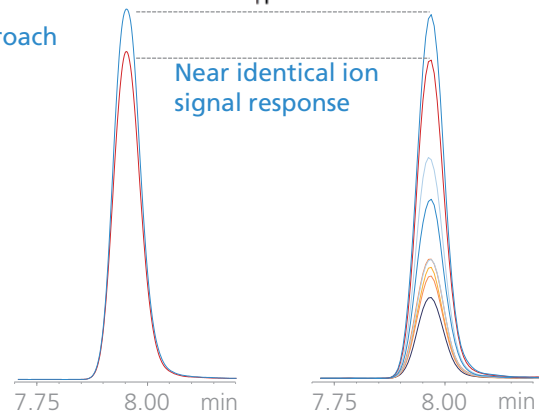
Results

Compound Name Linuron
Formula C9H10Cl2N2O2
CAS 330-55-2



Higher specificity
Higher reporting confidence
Library searchable fragment data

Conventional approach
2 MRM's
1:248.80>160.00
2:248.80>182.10



MRM Spectrum Mode
9 MRM's
1:248.80>160.00
2:248.80>182.10
3:250.80>162.00
4:248.80>133.10
5:250.80>135.00
6:248.80>161.00
7:250.80>184.10
8:248.80>125.00
9:248.80>153.00

Figure 1. Using a high speed triple quadrupole mass analyser a higher number of fragment ions were acquired in MRM increasing the specificity of detection and reducing false negative and false positive reporting. In the case of linuron, 9 precursor-fragment ion transitions were used to increase confidence in assay specificity. There is no compromise in data quality between methods despite a higher number of fragment ions monitored. Signal intensity, linearity, reproducibility are in good agreement between both methods.

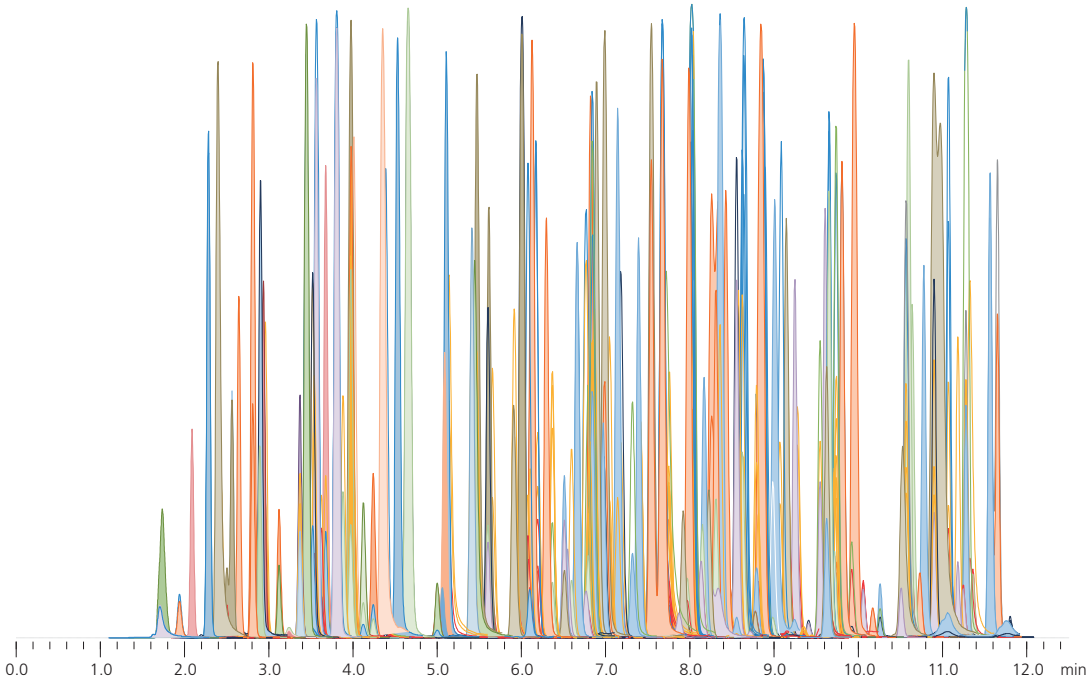


Figure 2. MRM chromatogram for all 193 pesticides spiked at 0.010 mg/kg measured with MRM Spectrum mode. Using this mode 1,291 MRM transitions were measured for 193 pesticides. Despite the high data density acquired with MRM Spectrum mode (for example, 151 MRM transitions were registered in the same time window during the analysis, see Figure 2) sensitivity was not affected by the high data acquisition rate.

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On average 7 MRM transitions were applied to each compound, with more than 10 MRM transitions applied to 34 compounds. All MRM transitions were acquired throughout the MRM window without the need for triggering thresholds. The method includes a total of 1,291 MRM transitions for 193 pesticides in a run time of only 15 minutes. A dwell time of 3 msecs was applied to every MRM transition. In order to evaluate the data quality from the MRM Spectrum Mode method, the same method was set up with 2 MRMs applied to each compound (386 MRMs in total) using the same acquisition method (Table 1).

MRM Spectrum based identification

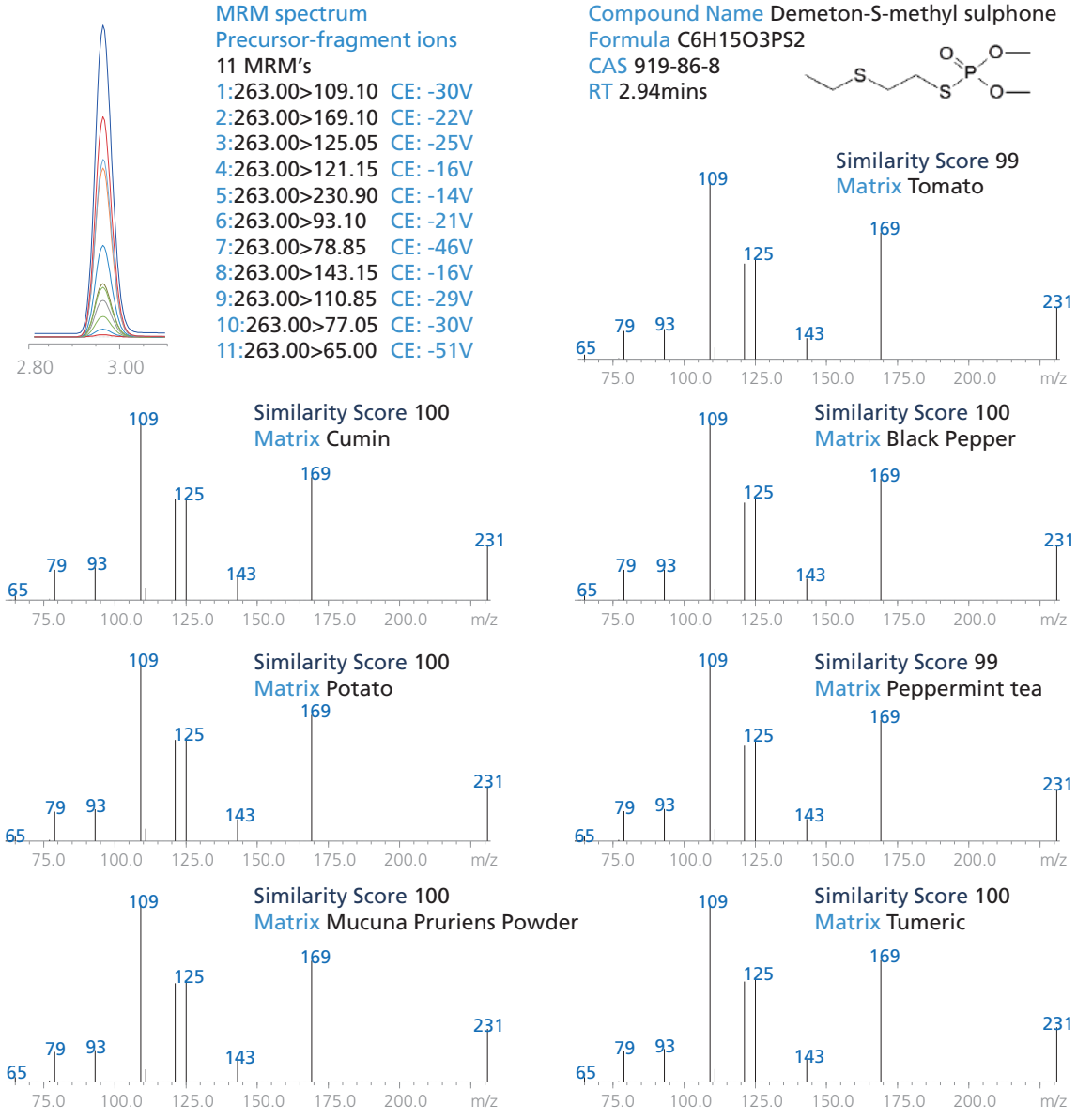

Figure 3. MRM spectrum identification in different matrices for demeton-S-methyl sulphone. In this study, the number of qualifier fragment ion transitions was increased for each pesticide and the combined transitions were used to create a MRM product ion spectrum. The product ion spectrum was used in conventional library matching routines comparing against a reference spectrum to generate a similarity score.

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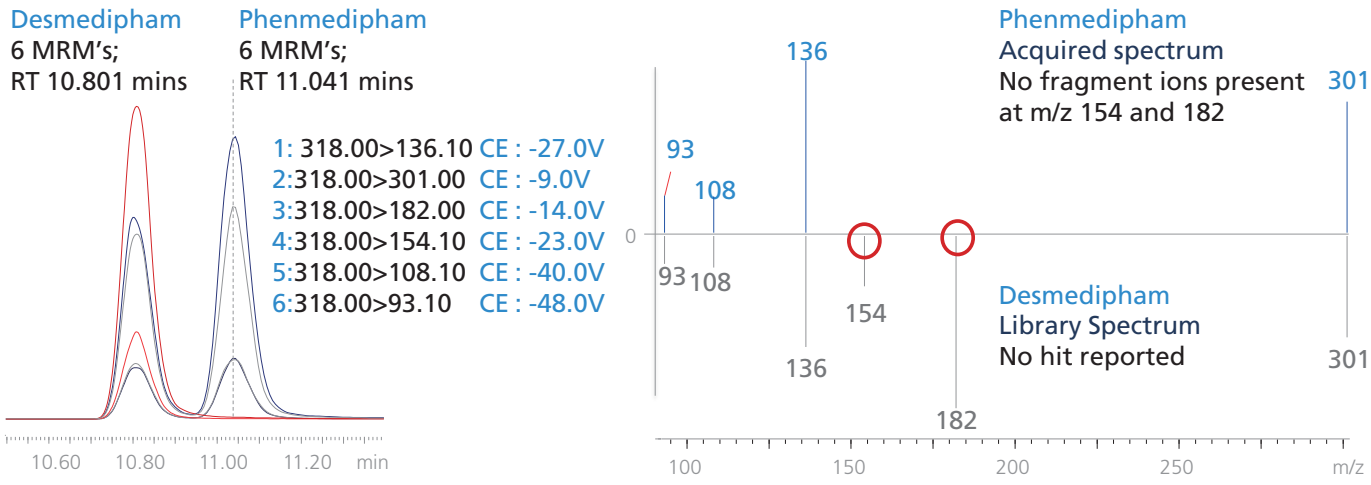

Figure 4. MRM chromatogram for desmedipham and phenmedipham spiked into a cumin extract at 0.1 mg/kg. As phenmedipham shares common transitions and elutes at a similar retention time as desmedipham the MRM spectrum can be used to distinguish between both pesticides to avoid false positive reporting.

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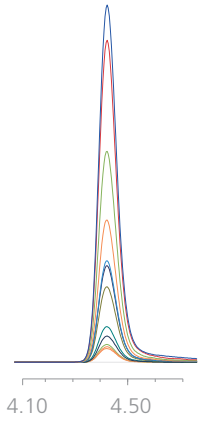
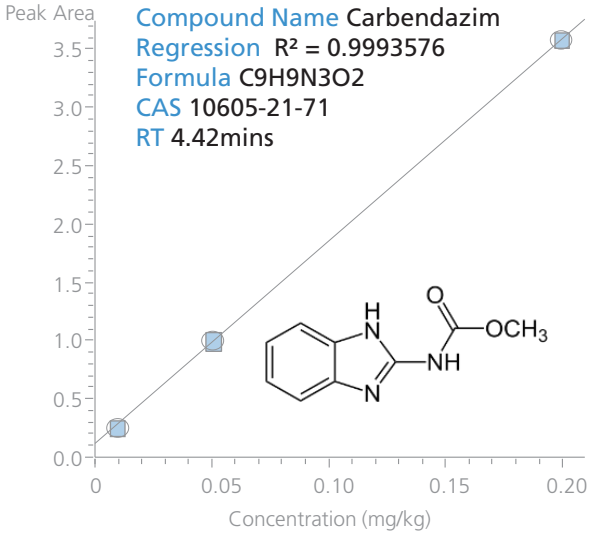
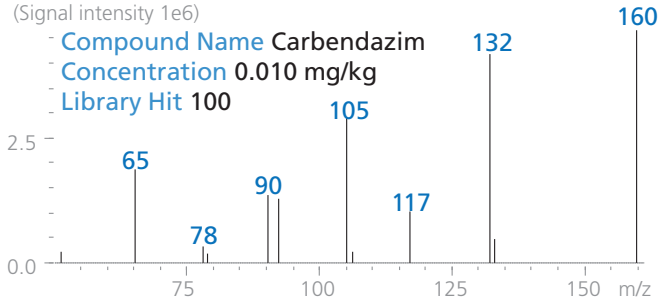
MRM Spectrum Quantitation and Library Searching

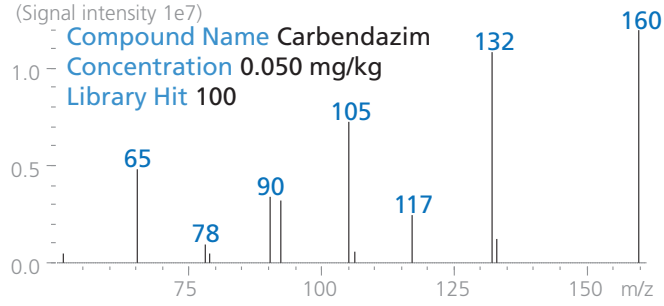


MRM spectrum Mode
Precursor-fragment ions
12 MRM's

1:	192.10>159.95	CE: -34V
2:	192.10>132.10	CE: -32V
3:	192.10>105.15	CE: -41V
4:	192.10>65.10	CE: -48V
5:	192.10>90.15	CE : -42V
6:	192.10>92.15	CE: -36V
7:	192.10>117.15	CE : -33V
8:	192.10>78.15	CE : -55V
9:	192.10>133.10	CE : -32V
10:	192.10>51.10	CE : -60V
11:	192.10>106.20	CE : -42V
12:	192.10>78.90	CE : -50V







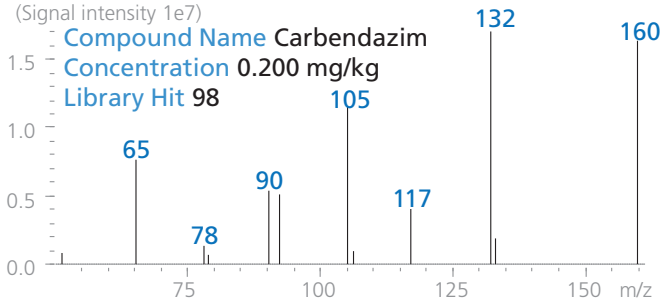


Figure 5. The limit on the number of MRM transitions used to generate a product ion spectrum is dependent on the chemical structure of the pesticide molecule. In the case of carbendazim, several bonds could be broken using collision energies between 10-60V resulting in a product ion spectrum of 12 fragment ions. The product ion spectrum can then be used for library search and analyte confirmation as shown above. For each calibration level ranging from 0.010-0.200mg/kg the library similarity score was greater than 99 confidently confirming the target analyte. The advantage of this technique is that library searchable product ion spectrum data is used in target compound identification without compromising sensitivity, accuracy and robustness in quantitative data reporting.

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Conclusions

- False positive results are a major issue for all pesticide residue monitoring laboratories. EU regulations require that retention time and the ion ratio between 2 MRM transitions are within a set threshold. However, even applying this criteria false positives may occur for certain pesticide/commodity combinations.
- We have applied MRM Spectrum mode to identify and quantify 193 target pesticides in a number of different sample matrices. In this workflow the library score is used as an additional identification criterion in order to improve confidence when reporting results.
- Acquisition of the MRM Spectrum method (1,291 MRM transitions) did not compromise data quality when compared to a conventional 2 MRM per compound method (386 MRM transitions) with consistent signal response and repeatability in both methods. The MRM product ion spectrums were consistent across the linear range and between different matrices. The method acquired data in both positive and negative ion modes with a polarity switching time of 5 msec enabling fast cycle times and a high data collection rate.

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