

Analysis of Pesticides in Wine by LCMS



What's in Your Wine?

People like to think of wine as “just grapes.”

But there is a lot more in your wine glass than fermented grapes.

For example:

- yeast are added to aid fermentation.
- Salts, sugars and acids may be added to control and direct the fermentation process.

When we compare the differences between :

- organically-certified wine,
- wine made with organic grapes,
- conventionally-made wine

we need to look at how many chemicals are added and where they come from.

lexique

Case 1:

Conventionally-grown wine grapes **can be treated** with synthetic pesticides, fungicides and insecticides.

Case 2:

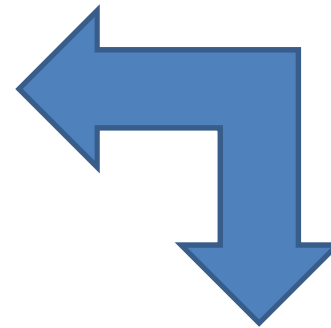
Organically-grown grapes **cannot be treated** with any synthetic pesticides, fungicides, insecticides, or fertilizers.

NB:

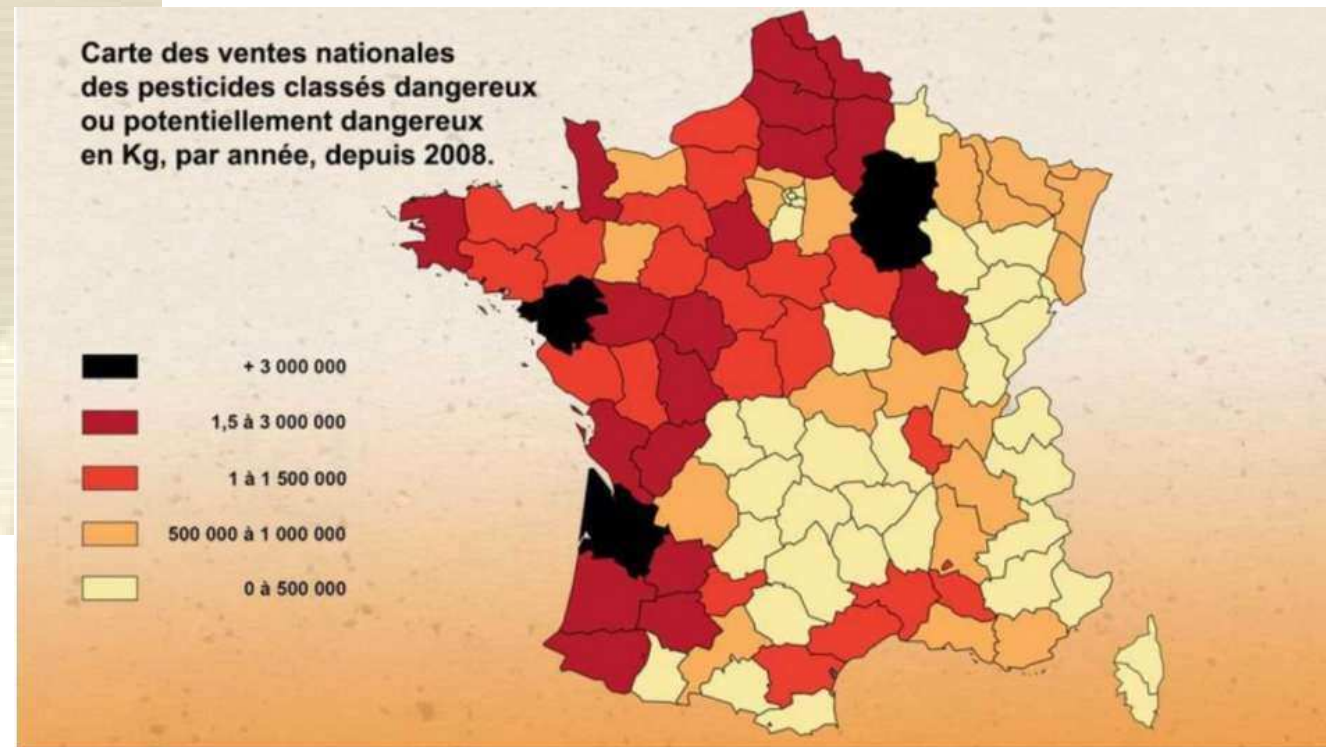
In US, the **Pesticide Action Network** (PAN) classifies about a million pounds of those chemicals dispersed on wine grapes as “bad actors,” meaning that **they are known or probable causes of cancer, are neurotoxins, or groundwater contaminants**.

Roundup, a herbicide, is widely used on wine grapes in conventional farming. A recent study has linked Roundup with health dangers, including Parkinson’s, infertility, and cancers.

Pesticides & Wines ...



Bordeaux area
Champagne area
Loire area



Pesticides: a major concern

[Bordeaux Town Moves to Restrict Vineyard Pesticide Spraying to Protect Sites Where Local Children Live and Play](#)

Vineyard owners in the Gironde district of Bordeaux face new restrictions on pesticide spraying, due to local protestors' concerns about children's health risks in the region.

While the prefecture voted in 2014 to protect schools from spraying at certain times, this week the region voted to expand the list of protected sites to include other places where children play or live, including day care, nurseries, playgrounds and health facilities, according to La France Agricole and the [French TV](#) as well as other French news sources.

Gironde growers will be encouraged to install protective vegetation, such as hedges, and employ anti-drift measures, during spraying. The government-recommended steps include maintaining a distance of 50 meters from sites, unless using specialized spray equipment, in which case the distance can be reduced to 20 or 5 meters from the site).

The government took these steps after public hearings were held, [which were attended by parents and local growers and winemakers.](#)

In the past year, the region has become a lightning rod for anti-pesticide activists who have become alarmed over children's health risks from vineyard pesticides, which was sparked in part by a 2014 incident at the school in Villeneuve-de-Blaye when [vineyards sprayed fungicides during a windy day](#), sending 23 children to the hospital. One of those spraying was the town's mayor.



Parents protesting in Feb. in the Gironde

Pesticides in wine

Study by the magazine « QUE CHOISIR »:

French Wines Sampled for Pesticide Residue, October 2013

Data from Que Choisir

Region	Number of Wines Sampled	Wines with more than 5 detected pesticides			Wines with total pesticide residue >50 ppb concentration		
		6-10 (Number/%)	>11 (Number/%)	6 or more (Number/%)	50 - 100 ppb (Number/%)	>100 ppb (Number/%)	Total >50 ppb (Number/%)
Bordeaux	20	7/35%	1/5%	8/40%	4/20%	10/50%	14/70%
Bourgogne	7	5/71%	0/0%	5/71%	2/28%	3/43%	5/71%
Champagne	4	4/100%	0/0%	4/100%	0/0%	4/100%	4/100%
Côtes du Rhône	21	7/33%	1/5%	8/38%	3/14%	3/14%	6/28%
Languedoc-Roussillon	22	9/41%	0/0%	9/41%	6/27%	7/32%	13/59%
Loire	6	3/50%	1/17%	4/67%	1/17%	3/50%	4/67%
Provence	4	0/0%	0/0%	0/0%	0/0%	0/0%	0/0%
Vins du Sud-Ouest	3	2/67%	1/33%	3/100%	0/0%	3/100%	3/100%
Vins de France	5	2/40%	2/40%	4/80%	2/40%	3/60%	5/100%
Total Sampled	92	39/42%	6/6%	45/49%	18/20%	36/39%	54/59%

Regulation ...?

There is no regulation for wine currently.

SANTE/11945/2015 regulation is for fruits & Vegetables so only grappes are concernend with MRL (Maximum Residue Level).

Maximum residue level. In Regulation 396/2005 list MRLs for pesticide/commodity combinations, an asterisk indicates that the MRL* is set at or about the LOQ, with the LOQ being here a consensus figure rather than a measured value.



EUROPEAN COMMISSION
DIRECTORATE-GENERAL FOR HEALTH AND FOOD SAFETY
Safety of the Food Chain
Pesticides and biocides

SANTE/11945/2015
30 November -1 December 2015 rev. 0

Guidance document on analytical quality control and method validation procedures
for pesticides residues analysis in food and feed.

SANTE/11945/2015

Supersedes

SANCO/12571/2013

Implemented by 01/01/2016

SHIMADZU's offer



8060



8050



8045

For LabSolutions Version 5.82 and Later

LC/MS/MS Method Package for Residual Pesticides Ver. 2

- World's largest compound panel (646 pesticides in a single method)
- Ultra-high-speed method, with detection in 10.5 minutes
- Ultra-high-speed detection delivers robust and reproducible data quality for extended pesticide programs
- Pretreatment program to improve the shape of peaks for polar pesticides
- Excellent data stability in combination with the LCMS-8000 series



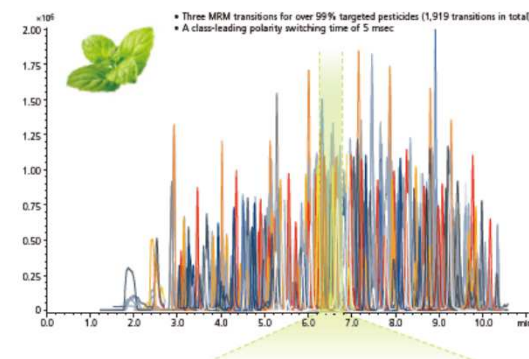
LCMS-8060

Pesticide Screening Analysis for 646 Pesticides in 10.5 Minutes

Using this method package in combination with the LCMS-8050/8060 enables performing a pesticide analysis of 646 pesticides in 10.5 minutes.

Since the LCMS-8050/8060 have a rapid polarity switching time of 5 msec, a single multi-residue LC/MS/MS method supports the analysis of 612 pesticides in positive ion mode and 34 compounds in negative ion mode (Three MRM transitions for over 99% targeted pesticides, resulting in 1,919 transitions in total).

MRM Chromatograms for 646 Pesticides Spiked into a Mint Extract at 0.01 mg/kg



SHIMADZU's strategy

Table 4. Identification requirements for different MS techniques²

MS detector / characteristics	Typical systems (examples)	Acquisition	Requirements for identification	
			minimum number of ions	other
Unit mass resolution	quadrupole, ion trap, TOF	full scan, limited m/z range, SIM	3 ions	$S/N \geq 3\sigma$ Analyte peaks in the extracted ion chromatograms must fully overlap. Ion ratio within ±30% (relative) of average of calibration standards from same sequence
MS/MS	triple quadrupole, ion trap, Q-trap, Q-TOF, Q-Orbitrap	selected or multiple reaction monitoring (SRM, MRM), mass resolution for precursor-ion isolation equal to or better than unit mass resolution	2 product ions	
Accurate mass measurement	High resolution MS: (Q-)TOF (Q-)Orbitrap FT-ICR-MS sector MS	full scan, limited m/z range, SIM, fragmentation with or without precursor-ion selection, or combinations thereof	2 ions with mass accuracy ≤ 5 ppm ^{a,b,c}	
		combined single stage MS and MS/MS with mass resolution for precursor-ion isolation equal to or better than unit mass resolution	<u>2 ions:</u> 1 molecular ion, (de)protonated molecule or adduct ion with mass acc. ≤ 5 ppm ^{a,c} <u>plus</u> 1 MS/MS product ion ^d	

More than
3 MRM

Multi MRM

2 MRM method vs “MRM Spectrum mode”

Comparison of a conventional 2 MRM method (404 MRMs)

Against the MRM Spectrum mode method (1335 transitions)

Higher specificity, higher reporting confidence, no significant change in response

Conventional approach

2 MRM's

ESI+

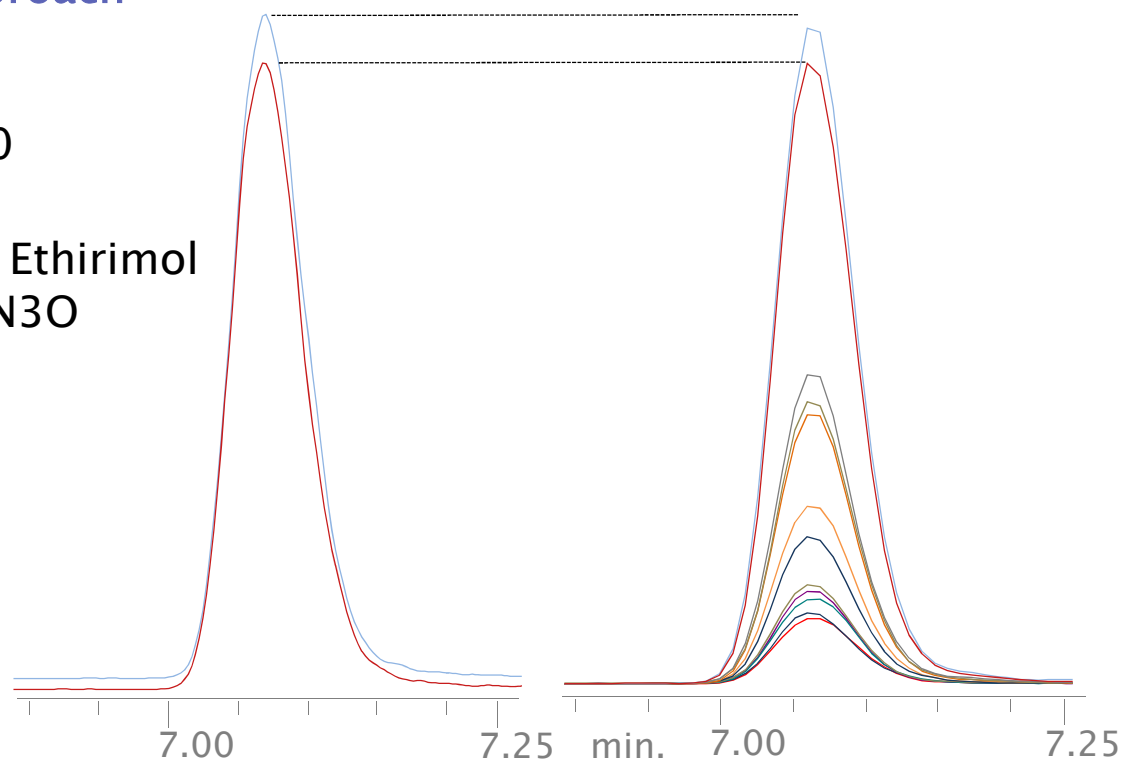
1:210.20>140.20

2:210.20>98.20

Compound Name Ethirimol

Formula C₁₁H₁₉N₃O

CAS 23947-60-6



MRM Spectrum mode

12 MRM's

ESI+

1:210.20>140.20

2:210.20>98.20

3:210.20>182.20

4:210.20>193.05

5:210.20>70.20

6:210.20>165.20

7:210.20>71.20

8:210.20>138.10

9:210.20>150.20

10:210.20>95.15

11:210.20>107.25

12:210.20>167.20

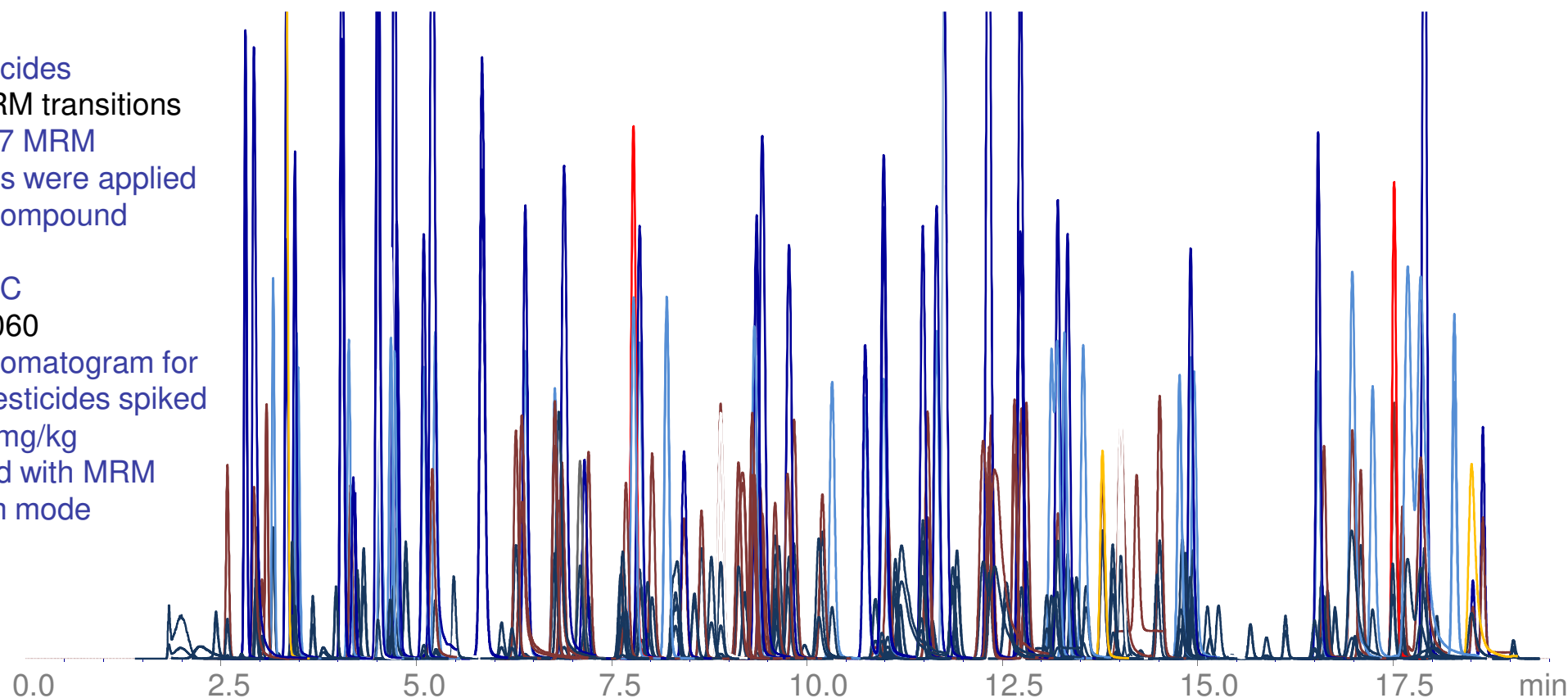
Pesticide analysis | MRM Spectrum Mode

MRM Spectrum mode and Library Searching

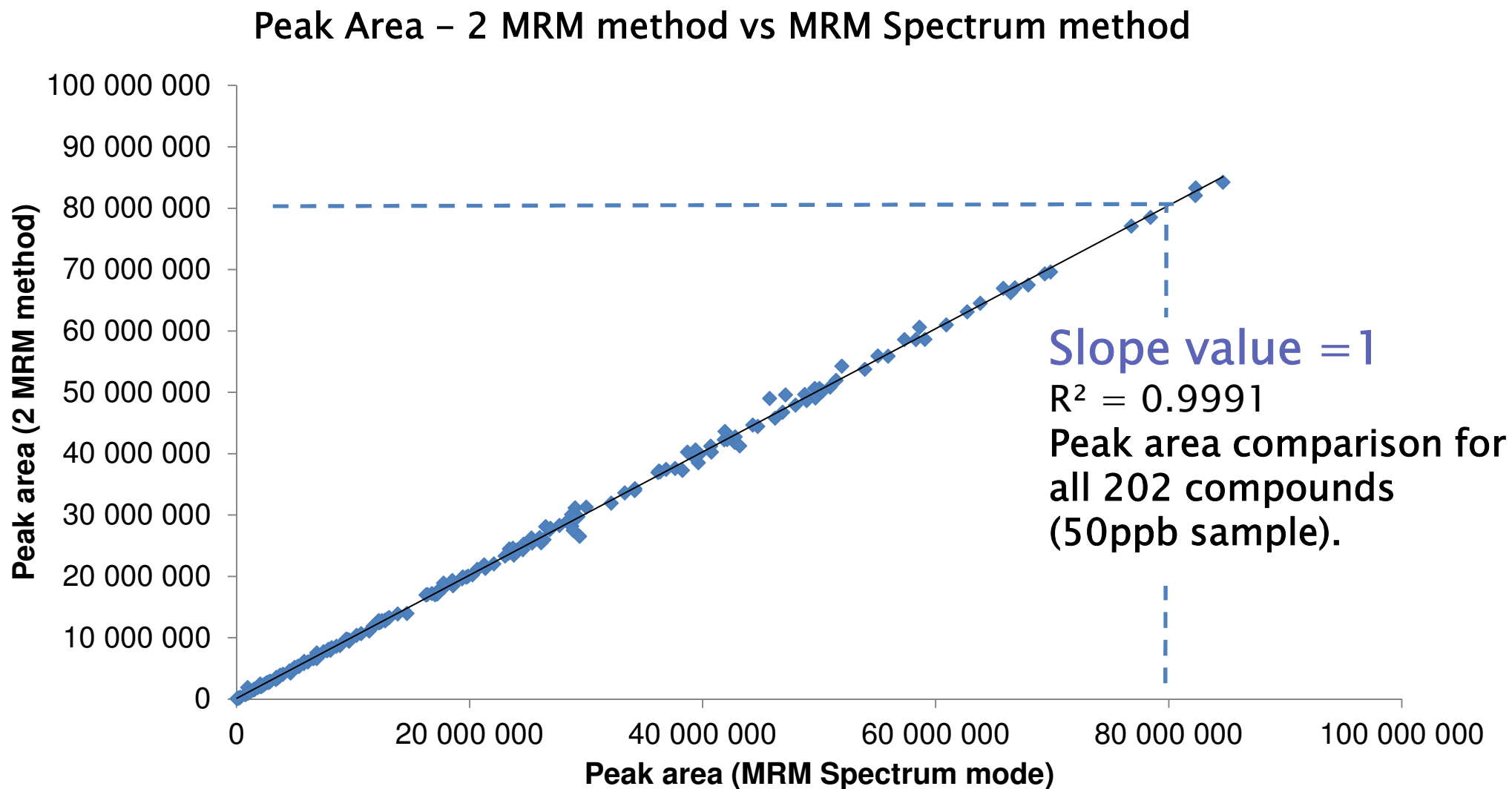
In this workflow, typically 6-10 fragment ion transitions were monitored for each target pesticide as opposed to a conventional approach using 2-3 fragment ions. By acquiring a high number of fragment ion transitions, each target pesticide had a corresponding fragmentation spectra which could be used in routine library searching and compound verification using reference library match scores.

193 pesticides
1,291 MRM transitions
Average 7 MRM
transitions were applied
to each compound

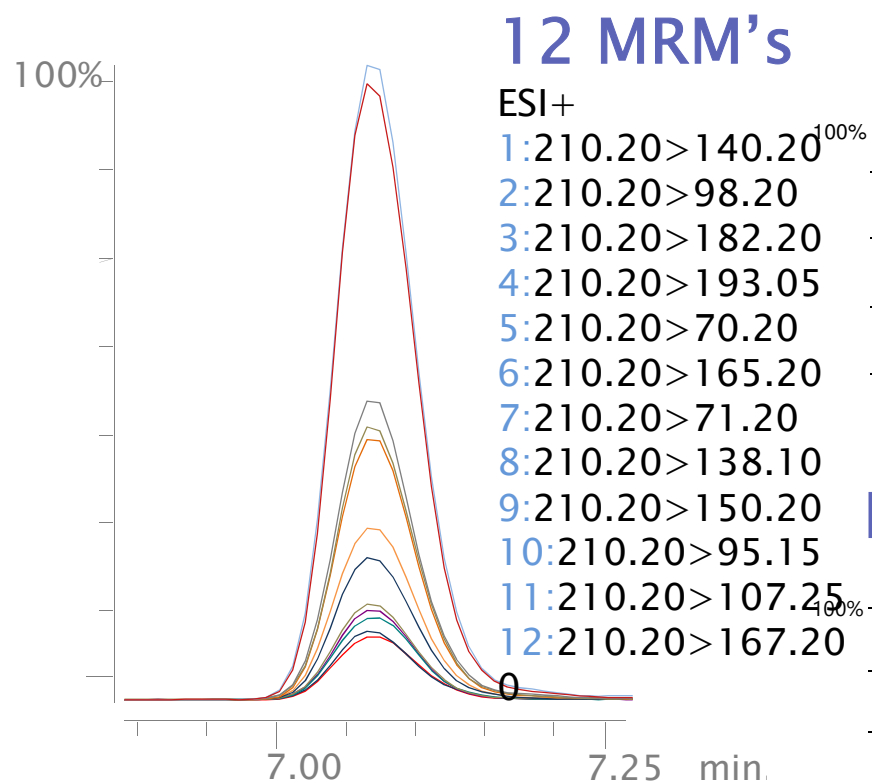
Nexera LC
LCMS-8060
MRM chromatogram for
all 193 pesticides spiked
at 0.010 mg/kg
measured with MRM
Spectrum mode



2 MRM method vs MRM Spectrum mode



MRM Spectrum



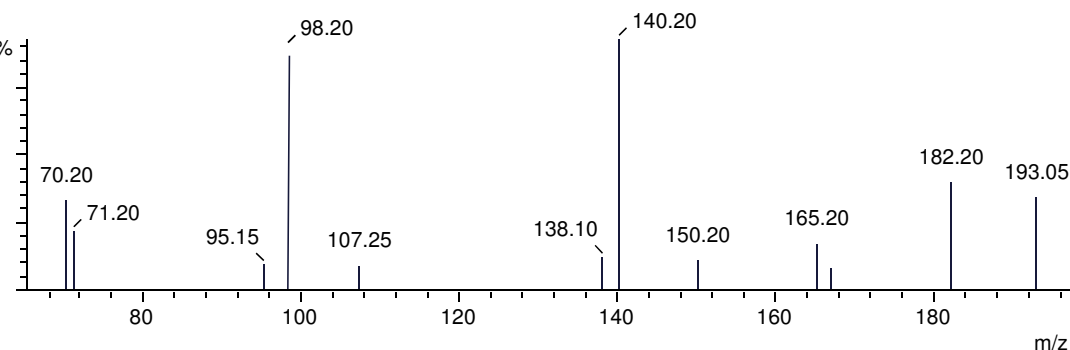
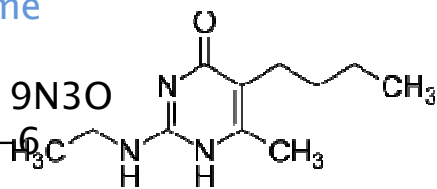
Compound Name

Ethirimol

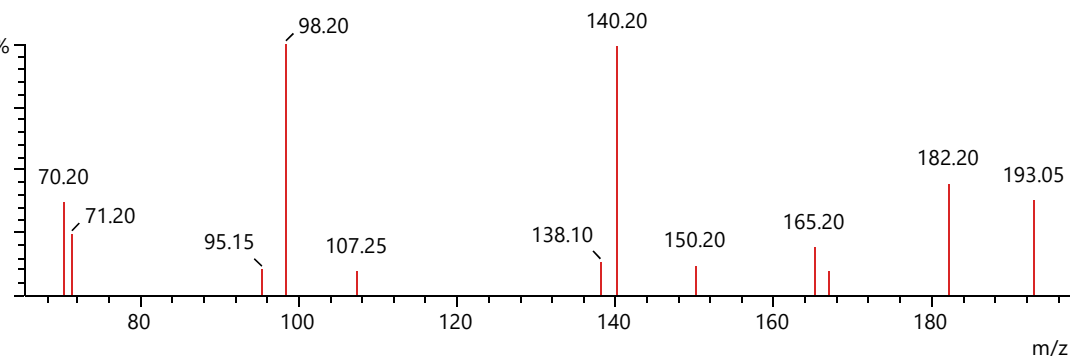
Formula C₁₁H₁₉N₃O

CAS 23947-60-4

RT 7.02mins



Library spectrum: Score 99%



The unknown MRM spectrum is searched against a reference spectrum to provide a similarity score

Consistent MRM spectrum

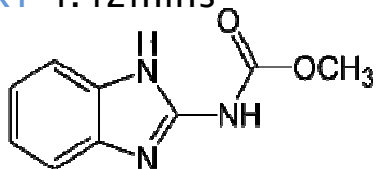
Compound Name

Carbendazim

Formula C₉H₉N₃O₂

CAS 10605-21-71

RT 4.42mins



MRM spectrum Mode

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

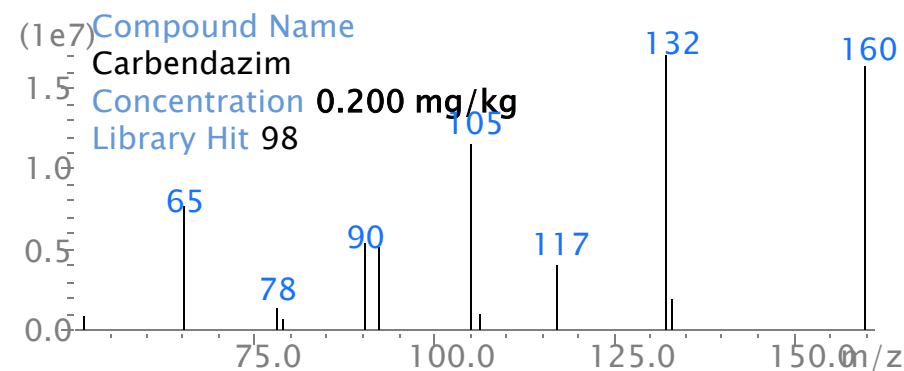
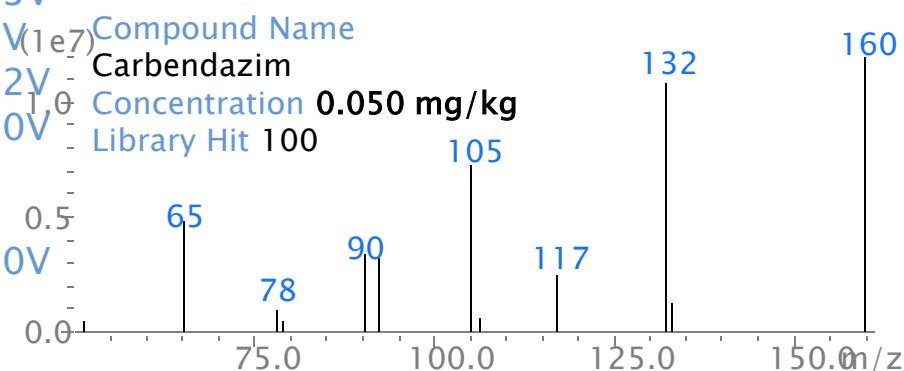
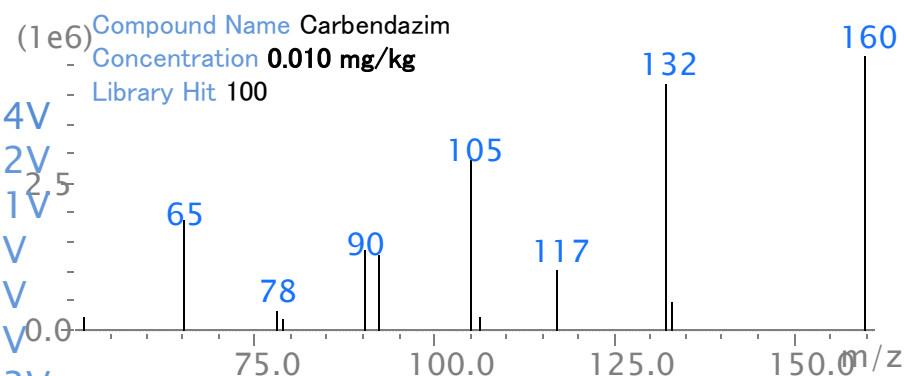
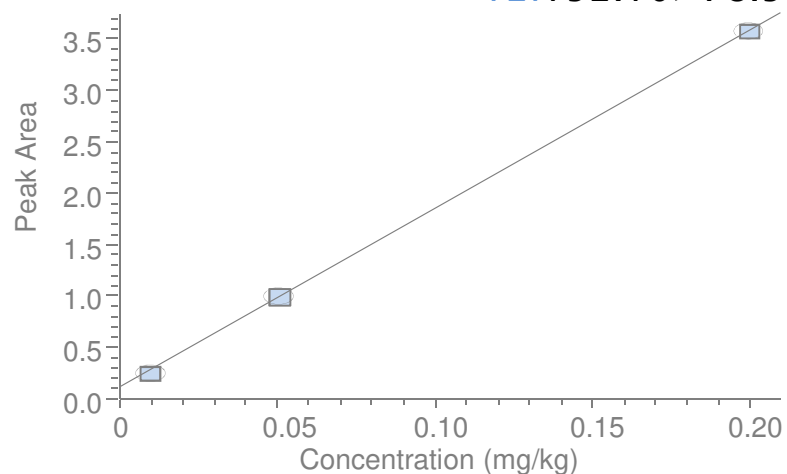
9:192.10>133.10 CE: -32V

10:192.10>51.10 CE: -60V

11:192.10>106.20 CE: -

42V

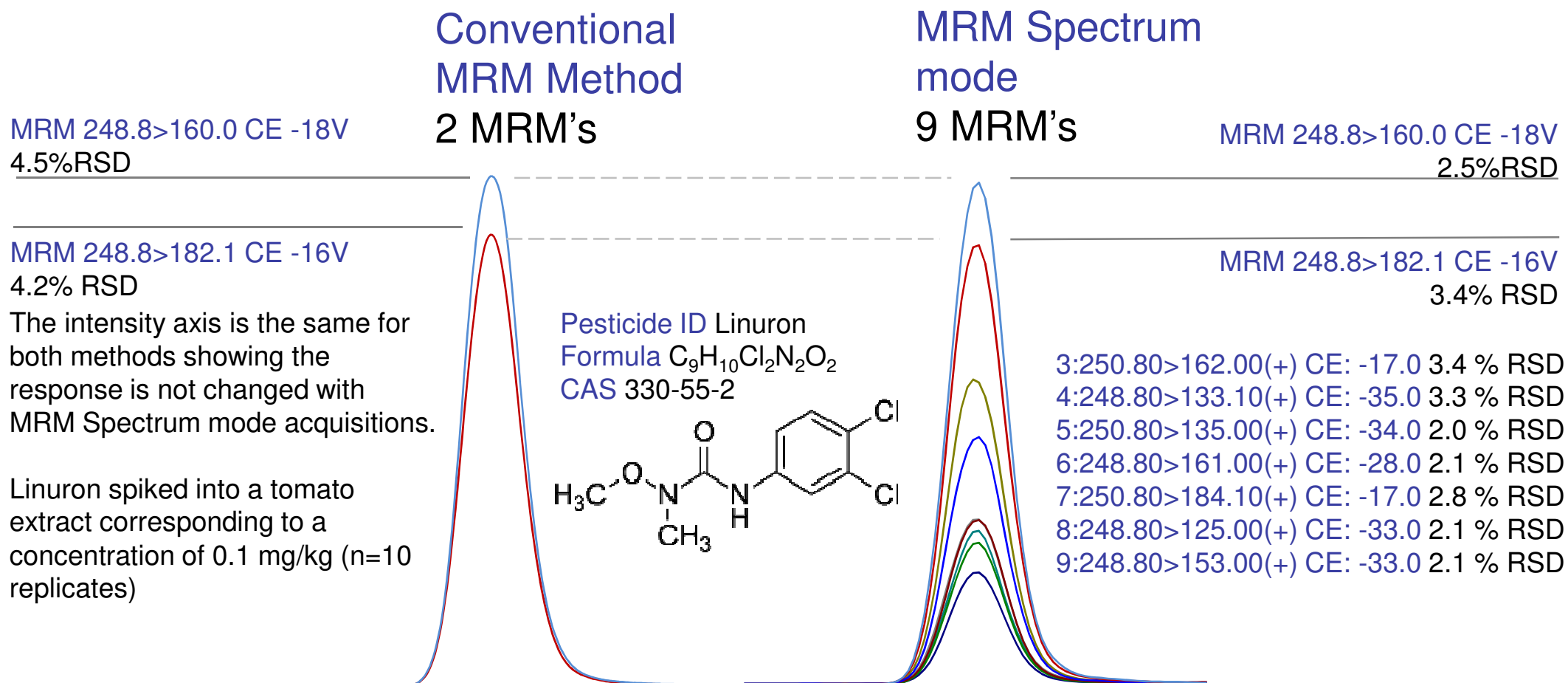
12:192.10>78.90 CE: -50V



MRM Spectrum mode advantages

MRM Spectrum mode

Same sensitivity and signal response as a conventional method; same reproducibility (n=10 injections)



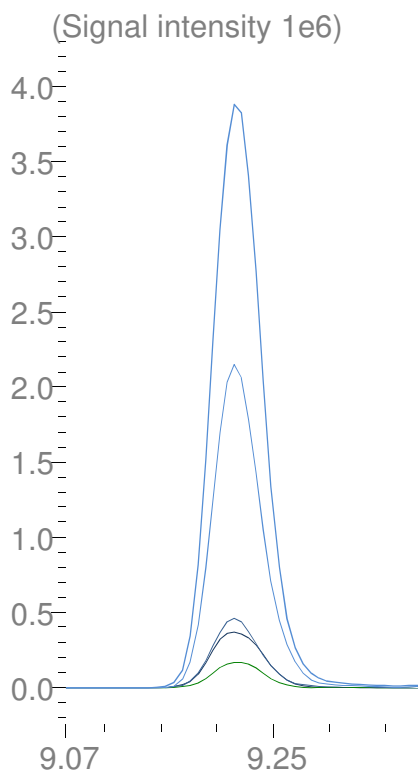
Works with complex matrices

MRM Spectrum mode

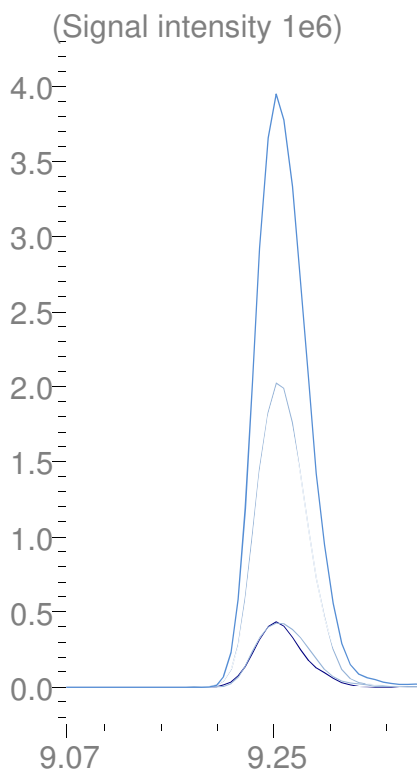
Works with polarity switching. Following example clearly shows that we can provide high data quality at fast polarity switching speeds.

Compound Name Bixafen
Polarity Negative ion

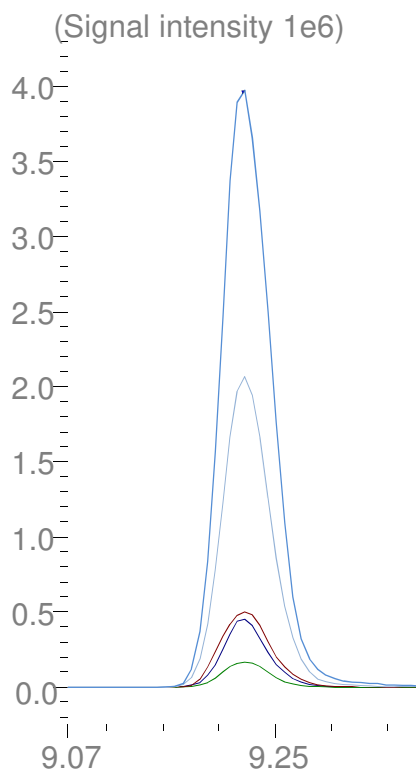
Switching speed 3 msec
%RSD 2.4



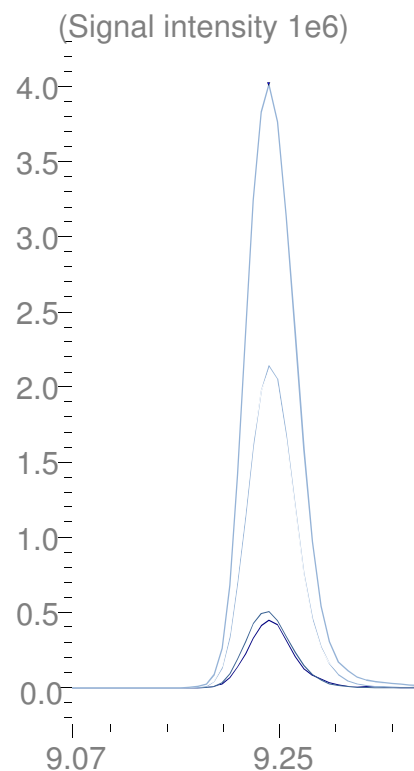
Switching speed 5 msec
%RSD 2.1



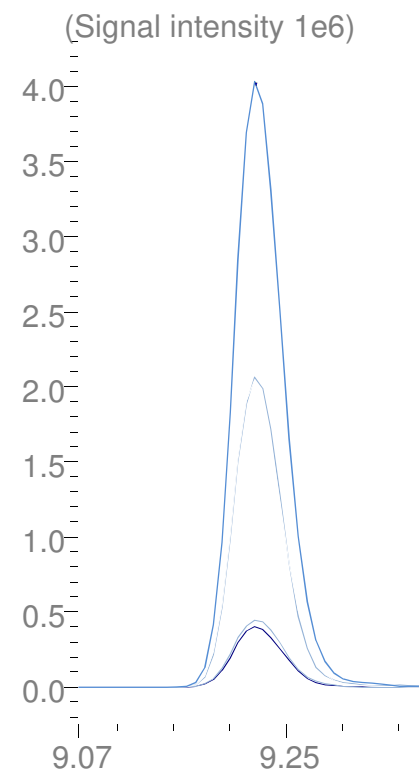
Switching speed 10 msec
%RSD 2.5



Switching speed 20 msec
%RSD 3.4



Switching speed 30 msec
%RSD 2.9



Routine pesticide screening

- MRM Spectrum mode can be used to quantify and identify pesticides with greater confidence without compromising the data quality
- It can help reduce false positive and false negative reporting in food safety
- All 1,291 MRM transitions were acquired throughout the MRM window. No 'triggering' of MRM transitions was necessary due to the short dwell times that were applied using the LCMS-8060. Therefore, MRM transitions can be swapped between qualifier and qualifier if needed and the peak shape of the additional MRM transitions can be assessed.
- The MRM Spectrum Mode method was setup using the Shimadzu Residual Pesticide Database v2.0 which contains >6,000 MRM transitions for >750 pesticides.

Nevertheless, never stop enjoying an old tannic wine of Bordeaux !

