

# Advanced Data Acquisition and Data Processing Workflows to Identify, Quantify and Confirm Pesticide Residues

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## Overview

Pesticides are widely used in agriculture to protect crops and to improve efficiency of production. Pesticide residues may pose a potential threat to human health. Modern analytical techniques, such as QuEChERS extraction followed by LC-MS/MS, allow screening for pesticides in a variety of food matrices.<sup>1-3</sup>

Here we present a new and powerful workflow to identify, quantify and confirm the presence of 400 pesticides utilizing generic QuEChERS extraction and LC-MS/MS analysis with the AB SCIEX QTRAP<sup>®</sup> 6500 system using the *Scheduled* MRM<sup>™</sup> Pro algorithm and Information Dependent Acquisition (IDA) of full scan MS/MS spectra. High confidence in identification and confirmation was achieved by automatically calculating the ratio of quantifier and qualifier ions and searching MS/MS spectral libraries in MultiQuant<sup>™</sup> and MasterView<sup>™</sup> software. Qualitative method performance was verified using guideline SANCO/12571/2013 guideline.<sup>4</sup>

## Introduction

Due to the high usage of pesticides in agriculture, there is a high probability that pesticide residues Pesticides are widely used in agriculture to protect crops and to improve efficiency of production. After application pesticide may remain on agricultural products or accumulate in the environment, posing a potential threat to human health. Consequently, government agencies, food producers and food retailers have the duty to ensure that pesticide residues occurring in food are below established maximum residue limits set by Codex Alimentarius, the European Union, the US EPA, or by the Japanese Ministry of Health, Labour and Welfare.

There is a demand for powerful and rapid analytical methods that can identify pesticides with high confidence in a broad range of food matrices and quantify them at low concentrations with good accuracy and reproducibility.

A new analytical workflow was developed to screen for 400 pesticides in fruit, vegetable, tea and spices utilizing generic QuEChERS extraction, UHPLC separation using a core-shell particle column, and MS/MS detection with the AB SCIEX QTRAP<sup>®</sup> 6500 system. The *Scheduled* MRM<sup>™</sup> Pro algorithm



was used to acquire ~800 MRM transitions to accurately quantify target pesticides and identify them based on the characteristic ratio of quantifier and qualifier ions. The *Scheduled* MRM<sup>™</sup> data were also used to automatically acquire full scan MS/MS spectra to allow data to be searched against spectral libraries. The data processing in MultiQuant<sup>™</sup> and MasterView<sup>™</sup> software was used as a confirmatory tool to enhance confidence in quantitative and qualitative results.

## Experimental

### Sample Preparation

A pesticide standard containing ~400 compounds was used for method development and sample analysis.

Store-bought food samples were extracted using a QuEChERS procedure based on the European standard method 15662.<sup>5</sup>

- 10 g of frozen homogenized sample
- Addition of water to increase the water content of the sample to approximately 10 g
- Addition of 10 mL acetonitrile and internal standard
- Extraction by vigorous shaking for 1 min
- Addition of Phenomenex roQ<sup>™</sup> QuEChERS kit buffer-salt mix (KS0-8909) and immediate vigorous shaking for 1 min

- Centrifugation for 10 min at 9000 rpm
- Transfer of a 1 mL aliquot of the sample extract into a tube containing Phenomenex roQ™ dSPE kit (KS0-8916, 8913, 8914 or 8915 depending on sample type)
- Cleanup by vigorous shaking for 30 sec
- Transfer of 100 µL of the cleaned sample extract into an autosampler vial
- 10x dilution with water prior LC-MS/MS analysis

Mix D of the SCIEX iDQuant™ kit for pesticide analysis, containing 20 compounds, was spiked into food samples and used to verify method performance for identification and confirmation.

## LC Separation

- Separation using a Phenomenex Kinetex Biphenyl (100 x 2.1 mm, 2.6u) column
- Gradient water/methanol with 5 mM ammonium formate with a total run time of 15 min (Table 1)
- Injection volume of 10 µL

**Table 1.** LC gradient conditions at a flow rate of 500 µL/min

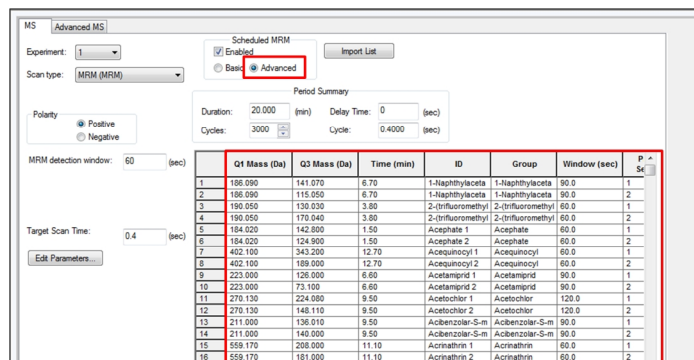
Step	Time	A (%)	B (%)
0	0.0	90	10
1	10	10.0	90
2	13	10	90
3	13.1	90	10
5	15	90	10

## MS/MS Detection

Samples were analyzed with two separate methods utilizing the AB SCIEX QTRAP® 6500 system with IonDrive™ Turbo V ion source using the electrospray ionization probe. The following gas settings were used: CUR 30 psi, Gas1 50 psi, Gas2 65 psi, CAD high.

The ion source temperature was set to 300°C to avoid degradation of thermally fragile pesticides such as Avermectin.

**Method 1:** Scheduled MRM™ Pro algorithm monitoring 2 transitions for each target pesticide (Figure 1)

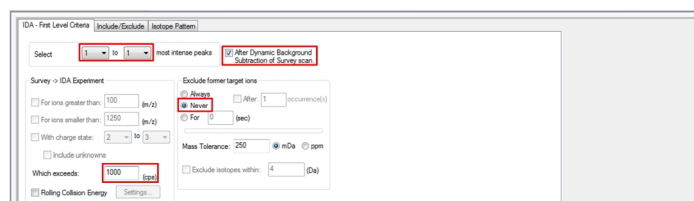


**Figure 1.** Acquisition method editor to build a method using the Scheduled MRM™ Pro algorithm

- Compound dependent detection window to match LC peak width and shape
- Compound dependent threshold for dynamic window extension and MRM-triggered MRM
- Target scan time of 0.4 sec to monitor ~800 transitions

**Method 2:** Scheduled MRM™-IDA-MS/MS to collect additional MS/MS information for identification (Figure 2)

- Information dependent acquisition of the most intense precursor ion detected in the MRM survey
- Dynamic background subtraction with a threshold of 1000 cps in methods without using an inclusion list (screening methods)
- Dynamic background subtraction with a threshold of >1000000 cps in methods when using an inclusion list, threshold of 100 cps for every compound in the inclusion list (confirmatory methods)



**Figure 2.** Acquisition method editor to build a method using (IDA)

MS/MS spectra were acquired in Enhanced Product Ion (EPI) scanning mode using a scan speed of 10000 Da/s. Dynamic fill time was used to achieve good quality spectra of compounds present at low and high concentrations. Highly characteristic MS/MS spectra were achieved using a collision energy (CE) of 35 V with collision energy spread (CES) of 15 V.

## Data Processing

MultiQuant™ software version 3.0 was used for quantitative analysis and automatic MRM ratio calculation. MasterView™ software version 1.1 was used for MS/MS library searching.

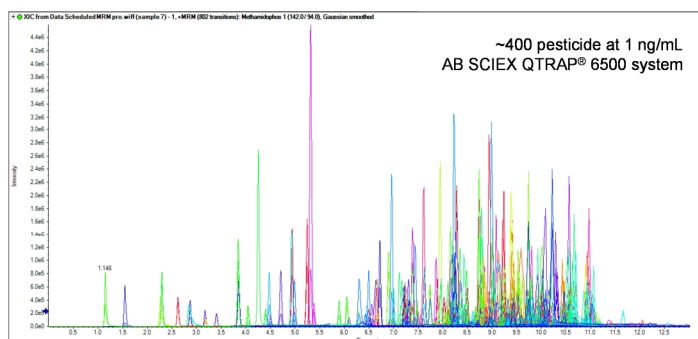
MS/MS spectra were searched against the MS/MS spectra were search against the iMethod™ Pesticide Library version 2.1.



## Results and Discussion

### Compound Coverage

An example chromatogram of a solvent standard at 1 ng/mL is shown in Figure 3.



**Figure 3.** Approximately 400 pesticides detected using 800 MRM transition with the *Scheduled MRM™* Pro algorithm

Approximately 800 MRM transitions were monitored using the *Scheduled MRM™* Pro algorithm. This allows quantitation and identification of 400 pesticides in a single LC-MS/MS run while using the ratio of quantifier and qualifier transitions. Further optimization of the gradient profile is planned to spread late eluting compounds more evenly through the chromatogram to extend the method to a total of 500 compounds (1000 MRM transitions).

The example chromatograms shown in Figure 4 highlight the advantage of setting compound dependent detection windows to match LC peak width and shape. Pesticides with wider peaks or partly separated isomers were detected using a longer window,

while narrow peaks were detected using a shorter window to enhance scheduling of transitions for best data quality.

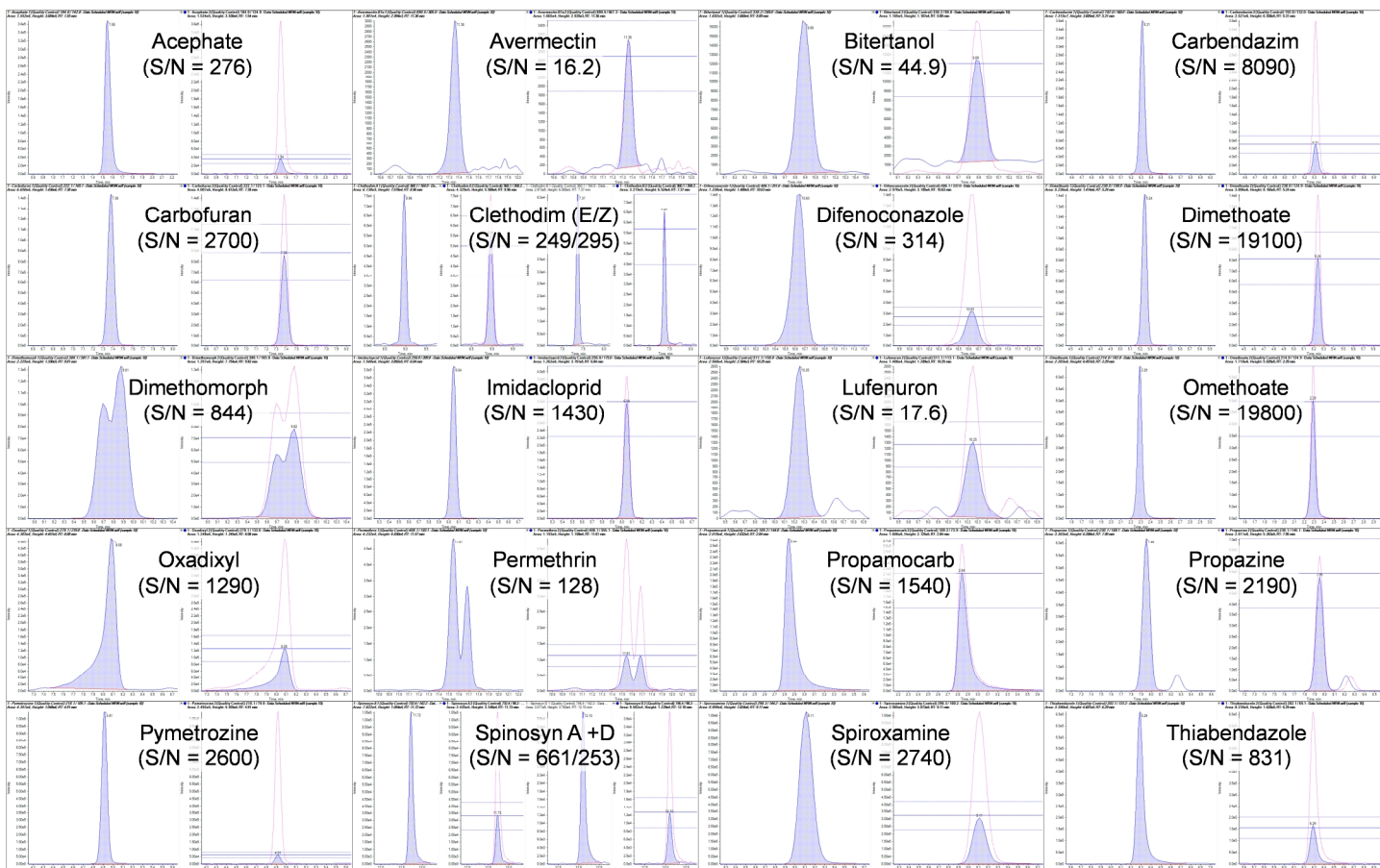
### Quantitative Results

Solvent standards were injected at a concentration ranging from 0.1 to 100 ng/mL. Example calibration lines are shown in Figure 5. Linear regression with 1/x weighting was used and points with accuracy values outside 80 to 120% were excluded. The coefficient of regression was typically higher than 0.99.

All target compounds had limits of quantitation (LOQ) of at least 1 ng/mL, for most compounds the estimated LOQ was much lower than 0.1 ng/mL (Signal-to-Noise, S/N >10). Example chromatograms and S/N at 1 ng/mL are shown in Figure 4 and Table 1.

**Table 1.** Signal-to-Noise (S/N) and Coefficient of Variation (%CV) for selected pesticides at a concentration of 1 ng/mL

Pesticide	S/N at 1 ng/mL	%CV at 1 ng/mL
<i>Acephate</i>	276	1.18
<i>Avermectin</i>	16.2	6.16
<i>Bitertanol</i>	44.9	6.12
<i>Carbendazim</i>	8090	1.70
<i>Carbofuran</i>	2670	1.52
<i>Clethodim E</i>	249	4.18
<i>Clethodim Z</i>	295	2.02
<i>Difenoconazole</i>	314	8.65
<i>Dimethoate</i>	19100	0.98
<i>Dimethomorph</i>	844	1.71
<i>Imidacloprid</i>	1430	0.49
<i>Lufenuron</i>	17.6	4.79
<i>Omethoate</i>	19800	1.22
<i>Oxadixyl</i>	1290	2.39
<i>Permethrin</i>	128	5.91
<i>Propamocarb</i>	1540	0.44
<i>Propazine</i>	2190	1.92
<i>Pymetrozine</i>	2600	1.66
<i>Spinosyn A</i>	661	3.10
<i>Spinosyn D</i>	253	4.47
<i>Spiroxamine</i>	2740	2.62
<i>Thiabendazole</i>	831	2.32



**Figure 4.** Quantifier and qualifier MRM transitions of selected pesticides with S/N at a concentration of 1 ng/mL, the MRM ratio tolerance of 30% is displayed in the MultiQuant™ software peak review (SANCO/12571/2013)

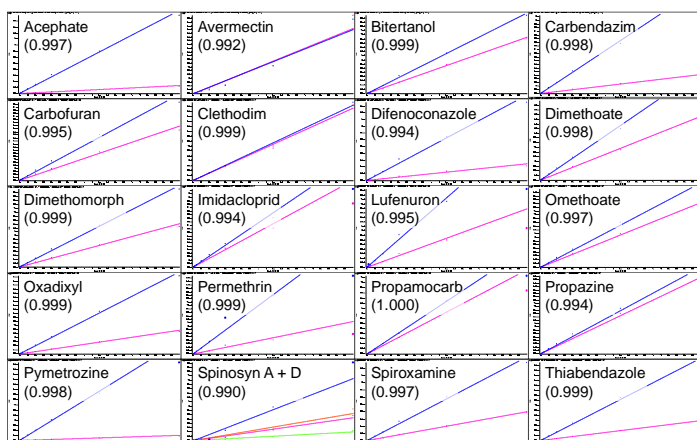
Replicate injections at 1 ng/mL (n=5) were used to evaluate repeatability. The results are summarized for selected compounds in Table 1.

As a result the developed method provides sufficient sensitivity to dilute matrix extracts by a factor of 10 or more while quantifying and identifying pesticides at 10 µg/kg.

### Qualitative Results

Compound identification is typically performed by retention time matching and calculating the ratio of quantifier and qualifier MRM transition. The ion ratio of unknown samples is compared to standard samples and tolerance levels are applied to decide if a result is positive. These tolerance levels are defined by a number of guidelines.<sup>4,6</sup>

MRM ratios were automatically calculated in MultiQuant™ software. The ratio of quantifier and qualifier transition in unknown samples is automatically compared to the average ratio of all included standard samples for compound identification. Tolerance levels are displayed in the peak review window (Figure 4). Here we used a generic tolerance of 30% following SANCO/12571/2013 guideline.

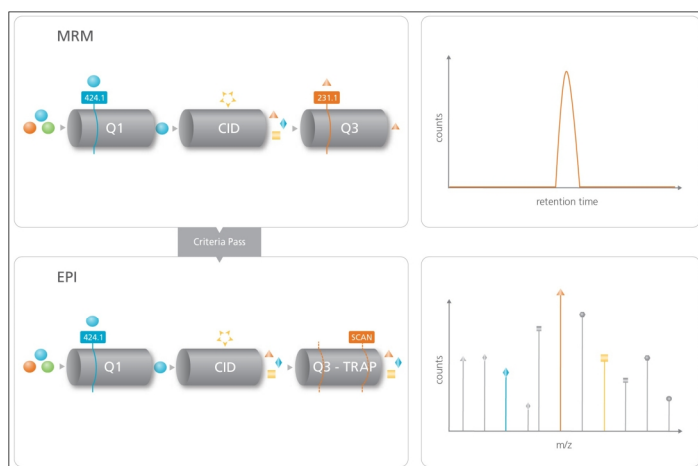


**Figure 5.** Calibration lines of selected pesticides from 0.1 to 100 ng/mL

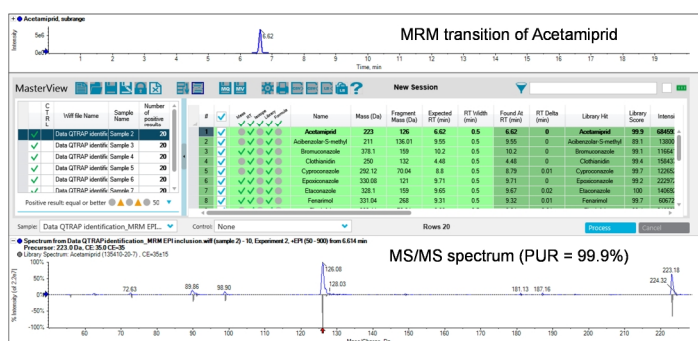


Despite the high selectivity of MRM detection, there is always a risk of false positive or negative findings due to interfering matrix signals. To increase confidence in identification or to confirm MRM ratio results, highly sensitive MS/MS spectra can be acquired on QTRAP® systems and searched against mass spectral libraries. Full scan MS/MS spectra contain more structural information of a detected compound resulting in a more confident identification.

Full scan spectra were acquired using and *Scheduled* MRM™-IDA-MS/MS method (Figure 6). This way quantitative (MRM peak area) and qualitative information (MRM ratio and MS/MS full scan spectrum) can be collected at the same time. Data processing was performed in MasterView™ software. A library PUR value of 70% or higher was used for positive identification.



**Figure 6.** Information Dependent Acquisition (IDA) of MS/MS spectra using an MRM survey scan on a QTRAP® system



**Figure 7.** Processing of *Scheduled* MRM™ and MS/MS data in MasterView™ software, compound identification is achieved through automatic retention time matching and MS/MS library searching

## Verification of Qualitative Method Performance

Mix D of the SCIEX iDQuant™ kit for pesticide analysis, containing 20 compounds, was spiked into carrot, grapes, grapefruit, red pepper, and spinach extract at 10 µg/kg.<sup>7</sup>

The results of identification based on retention time matching, MRM ratio comparison, and MS/MS library searching are summarized in Table 2. All 20 pesticides were confidentially identified in all 5 spiked samples. The average retention time error ranged from 0.008 to 0.024%, the average MRM ratio error from 5.09 to 6.30%, and the average MS/MS PUR from 95.9 to 98.5%.

**Table 2.** Pesticides identified in different spiked food samples based on retention time (RT) matching with a tolerance of 0.2 min, MRM ratio comparison, and MS/MS library searching for qualitative method validation

Pesticides in Carrot	RT (min)	RT Error	MRM Ratio	% Ratio Error	MS/MS PUR (%)
Acetamidol	6.63	0.01	0.20	1.7	97.7
Acibenzolar-S-methyl	9.56	0.01	0.35	6.5	62.7
Bromuconazole	10.20	0.00	0.16	9.2	99.5
Clothianidin	4.48	0.00	0.35	5.6	98.1
Cyproconazole	8.84	0.04	0.58	8.4	100.0
Epoxiconazole	9.73	0.02	0.35	5.2	95.6
Etaconazole	9.68	0.03	0.17	3.2	99.6
<b>Fenarimol</b>	<b>9.30</b>	<b>0.01</b>	<b>0.26</b>	<b>36.7</b>	<b>99.7</b>
Flutriafol	8.04	0.01	0.59	6.0	99.8
Imazalil	9.98	0.01	0.57	1.8	97.9
Imidacloprid	6.04	0.00	0.81	0.9	98.7
Metribuzin	6.97	0.01	0.43	2.6	100.0
Myclobutanil	9.04	0.00	0.76	7.5	99.5
Nitenpyram	4.38	0.00	0.86	3.2	94.3
Paclobutrazol	8.41	0.01	0.19	6.5	100.0
Pyrimethanil	8.57	0.00	0.53	3.2	99.5
Thiacloprid	7.43	0.01	0.11	3.7	99.8
Thiamethoxam	4.97	0.00	0.35	1.1	98.8
Triadimenol	8.46	0.00	0.38	0.4	100.0
Triticonazole	9.14	0.02	0.07	3.3	98.6
<b>Average</b>		<b>0.009</b>		<b>5.84</b>	<b>96.99</b>

Pesticide in Grapes	RT (min)	RT Error	MRM Ratio	% Ratio Error	MS/MS PUR (%)
Acetamiprid	6.64	0.02	0.20	1.3	98.0
Acibenzolar-S-methyl	9.59	0.04	0.39	4.9	96.1
Bromuconazole	10.23	0.03	0.13	7.5	98.6
Clothianidin	4.49	0.01	0.36	2.4	97.4
Cyproconazole	8.81	0.01	0.61	14.2	99.0
Epoxiconazole	9.75	0.04	0.33	0.2	74.6
Etaconazole	9.69	0.04	0.16	1.3	97.7
<b>Fenarimol</b>	<b>9.33</b>	<b>0.02</b>	<b>0.25</b>	<b>33.3</b>	<b>99.3</b>
Flutriafol	8.06	0.03	0.56	1.7	100.0
Imazalil	10.01	0.02	0.58	3.6	98.8
Imidacloprid	6.05	0.01	0.81	0.7	98.7
Metribuzin	6.98	0.02	0.43	3.2	100.0
Myclobutanil	9.05	0.01	0.78	11.0	100.0
Nitenpyram	4.39	0.01	0.85	1.3	95.2
Paclobutrazol	8.44	0.04	0.17	4.9	100.0
Pyrimethanil	8.60	0.03	0.51	7.8	99.5
Thiacloprid	7.44	0.02	0.12	10.7	99.8
Thiamethoxam	4.98	0.01	0.34	2.1	99.3
Triadimenol	8.50	0.04	0.39	2.3	99.2
Triticonazole	9.15	0.03	0.09	11.7	99.7
Average		0.024		6.30	97.55

Pesticide in Grapefruit	RT (min)	RT Error	MRM Ratio	% Ratio Error	MS/MS PUR (%)
Acetamiprid	6.63	0.01	0.20	0.0	99.4
Acibenzolar-S-methyl	9.53	0.02	0.40	5.1	80.0
Bromuconazole	10.22	0.02	0.14	5.2	99.8
Clothianidin	4.48	0.00	0.36	2.2	98.1
<b>Cyproconazole</b>	<b>8.77</b>	<b>0.03</b>	<b>0.57</b>	<b>6.9</b>	<b>50.3</b>
Epoxiconazole	9.70	0.01	0.34	2.3	99.5
Etaconazole	9.66	0.01	0.17	1.8	99.3
<b>Fenarimol</b>	<b>9.30</b>	<b>0.01</b>	<b>0.24</b>	<b>27.8</b>	<b>99.7</b>
Flutriafol	8.04	0.01	0.62	11.3	100.0
Imazalil	9.99	0.00	0.60	7.5	98.8
Imidacloprid	6.04	0.00	0.79	1.5	99.5
Metribuzin	6.96	0.00	0.46	10.4	100.0

Myclobutanil	9.05	0.01	0.72	1.5	99.6
Nitenpyram	4.38	0.00	0.84	0.5	95.6
Paclobutrazol	8.40	0.00	0.16	8.3	100.0
Pyrimethanil	8.56	0.01	0.55	0.5	99.5
Thiacloprid	7.42	0.00	0.11	1.7	100.0
Thiamethoxam	4.97	0.00	0.34	3.4	98.5
Triadimenol	8.45	0.01	0.36	6.3	99.7
Triticonazole	9.12	0.00	0.08	4.2	100
Average		0.008		5.42	95.87

Pesticide in Red Pepper	RT (min)	RT Error	MRM Ratio	% Ratio Error	MS/MS PUR (%)
Acetamiprid	6.63	0.01	0.20	0.2	99.5
Acibenzolar-S-methyl	9.55	0.00	0.41	8.3	71.4
Bromuconazole	10.20	0.00	0.14	5.0	99.1
Clothianidin	4.49	0.01	0.35	3.5	98.0
Cyproconazole	8.88	0.08	0.61	14.8	98.9
Epoxiconazole	9.72	0.01	0.35	6.7	96.5
Etaconazole	9.66	0.01	0.18	7.0	99.2
<b>Fenarimol</b>	<b>9.30</b>	<b>0.01</b>	<b>0.25</b>	<b>33.4</b>	<b>94.4</b>
Flutriafol	8.04	0.01	0.57	3.7	99.9
Imazalil	9.98	0.01	0.59	6.7	98.0
Imidacloprid	6.05	0.01	0.80	0.0	99.1
Metribuzin	6.97	0.01	0.42	1.7	100.0
Myclobutanil	9.04	0.00	0.70	1.6	99.8
Nitenpyram	4.39	0.01	0.84	1.0	95.9
Paclobutrazol	8.40	0.00	0.17	4.6	100.0
Pyrimethanil	8.57	0.00	0.54	1.9	99.5
Thiacloprid	7.43	0.01	0.12	4.3	100.0
Thiamethoxam	4.98	0.01	0.34	3.6	99.2
Triadimenol	8.45	0.01	0.36	6.8	100.0
Triticonazole	9.14	0.02	0.08	4.7	99.0
Average		0.012		5.98	97.37

Pesticide in Spinach	RT (min)	RT Error	MRM Ratio	% Ratio Error	MS/MS PUR (%)
Acetamiprid	6.61	0.01	0.20	0.0	99.6
Acibenzolar-S-methyl	9.57	0.02	0.34	8.9	95.5

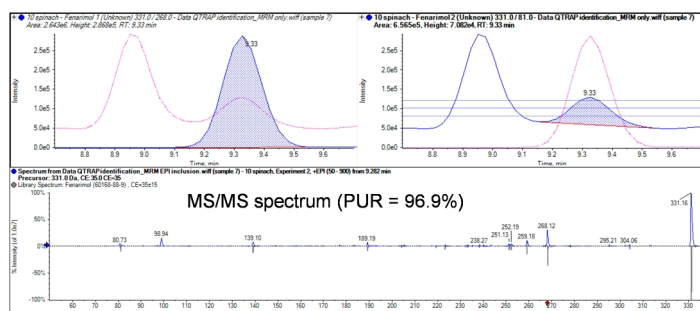
Bromuconazole	10.21	0.01	0.13	10.1	98.4
Clothianidin	4.47	0.01	0.36	1.3	98.7
Cyproconazole	8.75	0.05	0.54	1.3	99.7
Epoxiconazole	9.70	0.01	0.33	0.1	99.8
Etaconazole	9.67	0.02	0.17	0.7	89.6
<b>Fenarimol</b>	<b>9.31</b>	<b>0.00</b>	<b>0.25</b>	<b>32.0</b>	<b>96.9</b>
Flutriafol	8.03	0.00	0.56	1.4	99.4
Imazalil	9.99	0.00	0.63	13.1	98.8
Imidacloprid	6.03	0.01	0.82	2.0	97.9
Metribuzin	6.96	0.00	0.44	4.7	100.0
Myclobutanil	9.04	0.00	0.72	1.7	99.9
Nitenpyram	4.38	0.00	0.85	1.5	97.0
Paclobutrazol	8.42	0.02	0.18	1.4	100.0
Pyrimethanil	8.58	0.01	0.55	0.5	99.5
Thiacloprid	7.42	0.00	0.12	5.5	99.8
Thiamethoxam	4.96	0.01	0.34	2.6	99.3
Triadimenol	8.49	0.03	0.34	12.2	100.0
Triticonazole	9.12	0.00	0.08	1.0	100.0
Average		0.011		5.09	98.5

**Bold and green** = positive identification (RT error < 0.2 min, ratio error < 30%, MS/MS PUR > 70%)

**Bold and yellow** = questionable identification (MS/MS PUR < 70%),

**Bold and red** = no identification (ratio error > 30%)

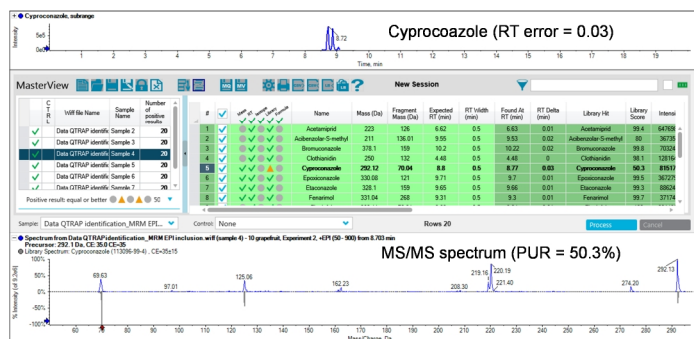
However, very few pesticides required confirmatory analysis since the identification criteria were slightly outside of tolerance levels.



**Figure 8.** Detection of Fenarimol in spiked spinach: the MRM ratio was slightly off the 30% tolerance due to high background and a closely eluting interfering matrix peak, but MS/MS library searching confirmed the presence of the detected pesticide.

For example Fenarimol was detected in all samples with matching retention time but the MRM ratio was outside or very close to the 30% tolerance due to high background and a closely eluting interfering matrix peak (Figure 8). But the analysis of a second sample extract to acquire MS/MS spectra confirmed the presence of Fenarimol with excellent library PUR well above 90% (94.4 to 99.7%).

Cyproconazole was identified in the grapefruit sample with matching retention time but the MS/MS PUR value was below the tolerance level (50.3%). Figure 9 shows the MS/MS review in MasterView™ software which helped to identify an isobaric matrix interference causing the low library search PUR. The analysis of a second sample extract confirmed the presence of Cyproconazole by MRM ratio matching (0.569 vs. theoretical 0.532).



**Figure 9.** Detection of Cyproconazole in grapefruit: the MS/MS library search resulted in a PUR value of 50.3% only, however, review of spectra revealed in isobaric matrix interference, the MRM ratio error of 6.9% further confirmed the presence of the pesticide.

These two data examples highlight the complementary nature of identification using MRM ratios and MS/MS library searching. Both methods, utilizing the *Scheduled MRMTM* Pro algorithm and *Scheduled MRMTM-IDA-MS/MS*, are suitable to quantify and identify pesticides in food samples. However, matrix interferences and high background can result in questionable identification. The analysis of a second sample extract using the alternative approach greatly enhances identification making it a viable tool for confirmation. Such a confirmation method is especially important if the target pesticide is not amenable to an orthogonal method, such as GC-MS.

## Application to Incurred Food Samples

Store-bought food samples were extracted using a QuEChERS procedure. Extracts were diluted 10x to minimize possible matrix effects and analyzed by LC-MS/MS using the two described methods utilizing the *Scheduled MRM™* Pro algorithm and the *Scheduled MRM™*-IDA-MS/MS approach.

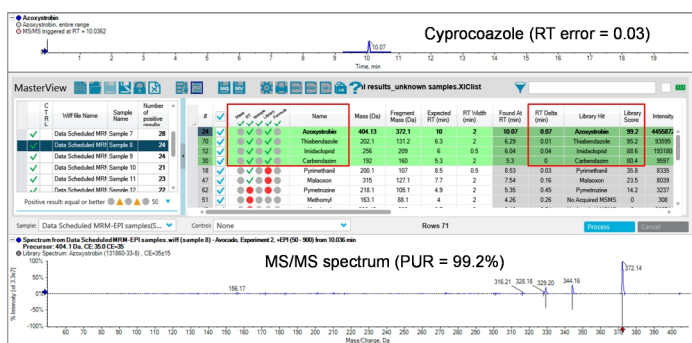
Results are summarized in Table 3.

**Table 3.** Pesticides identified in different incurred food samples based on retention time matching, MRM ratio comparison, and MS/MS library searching

Sample	Pesticide	Conc. (µg/kg)	RT Error	% Ratio Error	MS/MS PUR (%)
Avocado	Azoxystrobin	55.0	0.07	3.9	99.2
	Imidacloprid	6.2	0.01	0.6	95.2
Banana	Bifenthrin	26.8	0.12	9.4	73.0
	Fenpropimorph	12.2	0.08	4.6	99.7
	Imazalil	120	0.08	4.2	97.0
	Thiabendazole	37.3	0.00	0.7	100
Carrot	Linuron	14.3	0.07	1.9	95.1
Grapefruit	Fenbuconazole	5.1	0.05	9.8	75.4
	Imazalil	900	0.08	7.3	97.7
	Thiabendazole	269	0.01	2.3	100
Grapes 1	Fenhexamid	711	0.04	10.4	100
	<b>Pyrimethanil</b>	<b>226</b>	<b>0.06</b>	<b>32.8</b>	<b>99.4</b>
	Quinoxifen	5.9	0.02	7.8	99.4
	Trifloxystrobin	16.2	0.03	4.0	99.2
Grapes 2	Boscalid	15.9	0.07	8.9	78.7
	Fenhexamid	363	0.05	11.4	100
	Myclobutanil	14.2	0.05	0.86	70.7
	Pyrimethanil	687	0.07	28.2	99.5
	Spirotetramat metabolite	6.0	0.04	7.1	not in library
	Tebuconazole	7.1	0.33	11.6	75.4
	Thiabendazole	7.6	0.20	0.59	99.5
Lemon	Imazalil	981	1.00	0.8	98.8
Onion	no pesticides detected				
	Thiabendazole	3110		13.2	
Orange	Imazalil	1830		4.4	
Thiabendazole		3110		13.2	

Pepper 1	Acetamiprid	8.9	0.04	3.4	98.6
	Boscalid	9.8	0.06	7.2	82.8
	Clothianidin	6.0	0.00	7.6	87.2
	Imidacloprid	9.1	0.05	0.7	80.8
	Myclobutanil	17.3	0.03	9.0	86.4
	Pyriproxyfen	11.7	0.00	2.4	87.6
Pepper 2	Thiamethoxam	10.6	0.02	0.9	83.5
	Boscalid	47.6	0.06	4.2	87.2
	Pyraclostrobin	21.5	0.03	0.6	80.2
Spinach	<b>Boscalid</b>	<b>14.9</b>	<b>0.07</b>	<b>21.3</b>	<b>14.9</b>
	Dimethomorph	53.7	0.17	6.2	79.0
	Fenamidone	755	0.02	5.9	99.2
	Imidacloprid	217	0.04	0.8	98.0
	<b>Permethrin</b>	<b>1060</b>	<b>0.10</b>	<b>1.4</b>	<b>17.0</b>
Tomato	no pesticides detected				

Four pesticides were identified in the avocado samples based on retention time matching and MS/MS library searching. Confirmatory analysis and quantitation was performed using the *Scheduled MRM™* Pro method and MRM ratio calculation (Figure 9).

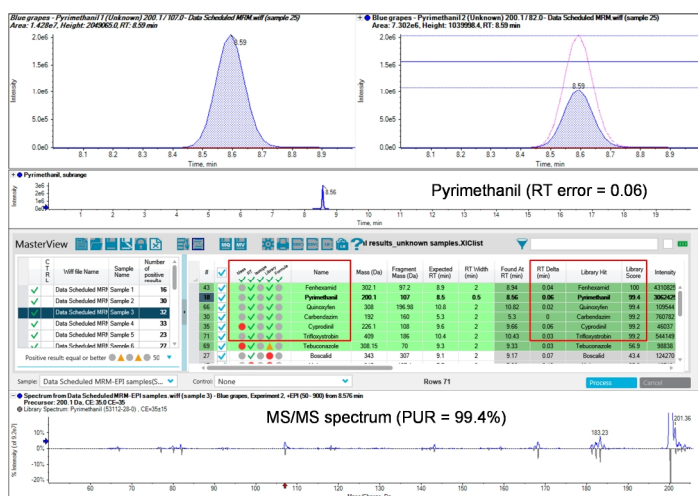


**Figure 9.** Identification of Azoxystrobin, Imidacloprid, Thiabendazole, and Carbendazim in an avocado sample based on retention time matching and MS/MS library searching, results were confirmed using MRM ratio calculation (note: Thiabendazole and Carbendazim were present below 5 µg/kg)

Four pesticides were identified and quantified in the grapes samples using the *Scheduled MRM™* Pro method. The example presented in Figure 10 shows the results for Pyrimethanil. It can be seen in the Peak Review window that the MRM ratio is outside the 30% tolerance.



We performed confirmatory analysis of a second sample extract using the *Scheduled MRM™*-IDA-MS/MS approach. Figure 10 shows the excellent MS/MS library match with a PUR 99.4% confirming the presence of Pymethanil.



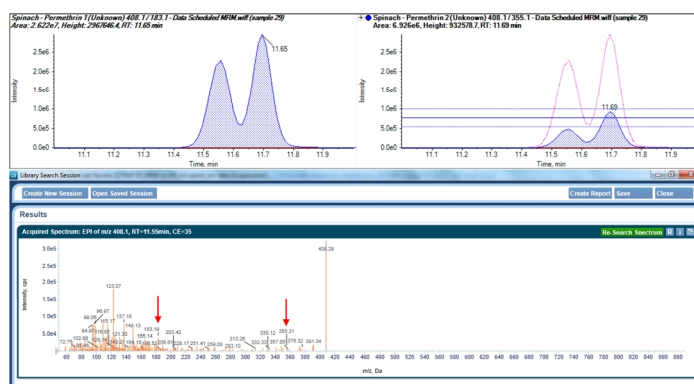
**Figure 10.** Fenhexamid, Pymethanil, Quinoxifen, and Trifloxystrobin were identified based on MRM ratios and quantified in a grapes sample, the MRM ratio of Pymethanil were slightly outside the 30% tolerance (top), however, second analysis using MS/MS library searching confirmed the presence of Pymethanil (bottom)

Figure 11 and 12 highlight the complementary nature of MRM ratio and MS/MS library searching for identification.



**Figure 11.** Boscalid was detected in a spinach samples with a concentration of 14.9 µg/kg, the ion ratio of 21.3 is inside the 30% tolerance (top), however, the MS/MS library searching with a PUR of 14.9% indicated strong matrix interference and suggest that Boscalid is not present in the sample (bottom)

Boscalid was detected in spinach. The ion ratio was inside the 30% tolerance, however, the MS/MS library searching with a PUR of 14.9% indicated strong matrix interference and suggested that Boscalid was not present in the sample.



**Figure 12.** Permethrin was detected in the spinach sample at a high concentration of 1060 µg/kg, the identification using MRM ratio was positive but the MS/MS library searching indicates strong matrix interferences, manual searching in LibraryView™ software confirms the presence the presence of both characteristic ions in the MS/MS spectrum, further confidence is gained through the presence of characteristic isomers in the LC profile

Permethrin was detected in the spinach sample at a high concentration of 1060 µg/kg (above the MRL of 50 µg/kg set by the EU<sup>8</sup>). MRM ratio and library searching are in disagreement for compound identification. Manual evaluation of the MS/MS spectrum in LibraryView™ software confirms the presence of both characteristic fragment ions in the MS/MS spectrum suggesting that Permethrin is present in the sample. The characteristic LC profile of Permethrin isomers further helps compound identification (Figure 12). Since the high level detected is a violation of the maximum residue level additional confirmation is recommend, which can be achieved by using an alternative LC separation setup and the acquisition of additional confirmatory MRM transitions using the *Scheduled MRM™* Pro algorithm.

## Summary

A QuEChERS and LC-MS/MS based method for the analysis of approximately 400 pesticides in food samples was developed.

The method used the AB SCIEX QTRAP® 6500 system utilizing the *Scheduled* MRM™ Pro algorithm and information dependent acquisition of full scan MS/MS spectra allowing quantitation and confident identification.

The method provide sufficient speed and sensitivity to quantify all ~400 pesticides at a concentration of 1 µg/kg in 10x diluted QuEChERS extract of food samples. Good linearity was observed for most compounds from 0.1 to 100 ng/mL with coefficient of variation typically well below 10%.

Qualitative method performance was verified by 20 compounds, into 5 different matrices at a concentration of 10 µg/kg. All compounds were confidentially identified in all samples using the dual method approach. Retention time errors observed were well below the 0.2 min tolerance. Very few pesticides required confirmatory analysis since the identification criteria were slightly outside of tolerance levels (MRM ratio tolerance of 30% or library PUR value of less than 70%). However, these results highlight the complementary nature of MRM ratios and MS/MS full scan offering a possibility for confirmatory analysis.

Last but not least store-bought food samples were analyzed. Automatic identification, quantitation, and confirmation were performed in MultiQuant™ and MasterView™ software.

## References

- <sup>1</sup> M. Anastassiades, et al.: 'Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce' J. AOAC Int. 86 (2003) 412-431
- <sup>2</sup> St. Lehotay: 'Determination of Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate: Collaborative Study' J. AOAC Int. 90 (2007) 485-520
- <sup>3</sup> J. Wong et al.: 'Development and Interlaboratory Validation of a QuEChERS-Based Liquid Chromatography-Tandem Mass Spectrometry Method for Multiresidue Pesticide Analysis' J. Agric. Food Chem. 58 (2010) 5897-5903
- <sup>4</sup> SANCO/12571/2013: 'Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed.'
- <sup>5</sup> CSN EN 15662: 'Foods of plant origin - Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE - QuEChERS-method' (2008)
- <sup>6</sup> 2002/657/EC: European Commission Decision 'concerning the performance of analytical methods and the interpretation of results'
- <sup>7</sup> A. Schreiber et al.: 'Using the iDQuant™ Standards Kit for Pesticide Analysis to Analyze Residues in Fruits and Vegetable Samples' Application Note AB SCIEX (2011) #3370211-01
- <sup>8</sup> [http://ec.europa.eu/sanco\\_pesticides](http://ec.europa.eu/sanco_pesticides)

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