Comprehensive Quantitation and Identification of Pesticides in Food Samples using LC-MS/MS with *Scheduled* MRM™, Fast Polarity Switching, and MS/MS Library Searching

André Schreiber and Yun Yun Zou
SCIEX, Concord, Ontario, Canada

Overview

Liquid Chromatography coupled to Tandem Mass Spectrometry (LC-MS/MS) is a widely used analytical tool for the screening of food residues and contaminants. Here we present a new and unique method using QuEChERS extraction, separation using a polar embedded C18 phase, and MS/MS detection with highly selective and sensitive Multiple Reaction Monitoring (MRM) on an SCIEX QTRAP® 5500 system. The *Scheduled* MRM algorithm was used to obtain the best data quality and combined with fast polarity switching to cover the broadest range of pesticides possible. In addition MS/MS spectra were acquired to enable compound identification with highest confidence based on mass spectral library matching.

Introduction

LC-MS/MS is a powerful analytical tool capable of screening samples for numerous compounds. MRM is typically used because of its excellent sensitivity, selectivity, and speed. As LC-MS/ MS technology continues to be adapted demands are made to detect and quantify an increasing number of compounds in a single run.

The development of generic extraction procedures, like QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) and LC methods using polar embedded C18 phases with good resolution and excellent peak shape made it possible to detect pesticides of a wide variety of compound classes and chemical properties in each sample.[1-3]

Modern LC-MS/MS systems make it possible to detect hundreds of pesticides and other food residues in a single run. The Turbo V™ source with Curtain Gas™ interface to reduce chemical noise, and the LINAC® collision cell to allow fast MS/MS scanning, are key technologies that make these highthroughput experiments possible. In addition, advanced software tools like the *Scheduled* MRM algorithm intelligently uses information of retention times to automatically optimize MRM dwell time of

each transition and total cycle time of the experiment resulting in highest data quality. To further increase confidence in analytical results QTRAP technology is used to automatically acquire fast and sensitive MS/MS spectra in Enhanced Product Ion (EPI) mode and search them against mass spectral libraries for compound identification. The information of the complete molecular fingerprint saved into EPI spectra significantly reduces the risk of false positive results.[4-6]

Additionally, for a comprehensive screening of pesticides it is necessary to employ both positive and negative Electrospray Ionization (ESI).

Here we present a new and unique LC-MS/MS method utilizing the *Scheduled* MRM algorithm in combination with fast polarity switching and acquisition of MS/MS spectra for compound identification. The method was successfully applied to quantify and identify pesticides in a number of QuEChERS extracts of fruit, vegetables, and spices.

Method Details

- Different fruit and vegetable samples were extracted using a modified QuEChERS procedure and diluted 10 to 50 times with water to optimize chromatographic peak shape and minimize possible matrix effects and interferences.

- The SCIEX iDQuant™ Standards Kit for Pesticide Analysis was used for method setup and preparation of calibration standards. Additional pesticides were added to cover all compounds of interest.
- LC separation was achieved on a Shimadzu UFLCXR system with a Restek Ultra Aqueous C18 3 µm (100x2.1 mm) column and a 15 min gradient of water and methanol with ammonium formate buffer at a flow rate of 0.5 mL/min. The injection volume was set to 10 µL.
- The SCIEX QTRAP® 5500 system was operated with Turbo V™ source and Electrospray Ionization (ESI) probe.
- A total of 386 transitions in positive and 56 transitions in negative polarity were monitored with an MRM pause time of 2 ms. Optimized transitions for all compounds were obtained through the MRM catalogue of the iMethod™ Test for Pesticide Screening version 2.1.
- The *Scheduled* MRM™ algorithm was used with an MRM detection window of 90 s and a target scan time of 0.3 s in Analyst® 1.6 Software
- A settling time of 50 ms was used for polarity switching.
- For increased confidence in compound identification EPI spectra at a scan speed of 10000 Da/s were acquired using a dynamic fill time for optimal MS/MS quality.
- EPI spectra were generated using standardized Collision Energy (CE) of ±35 V with Collision Energy Spread (CES) of 15 V to ensure a characteristic MS/MS pattern independently on compound's fragmentation efficiency. MS/MS spectra were search against the iMethod™ Pesticide Library version 2.1.
- MultiQuant™ 2.1 Software was used for quantitative data processing.

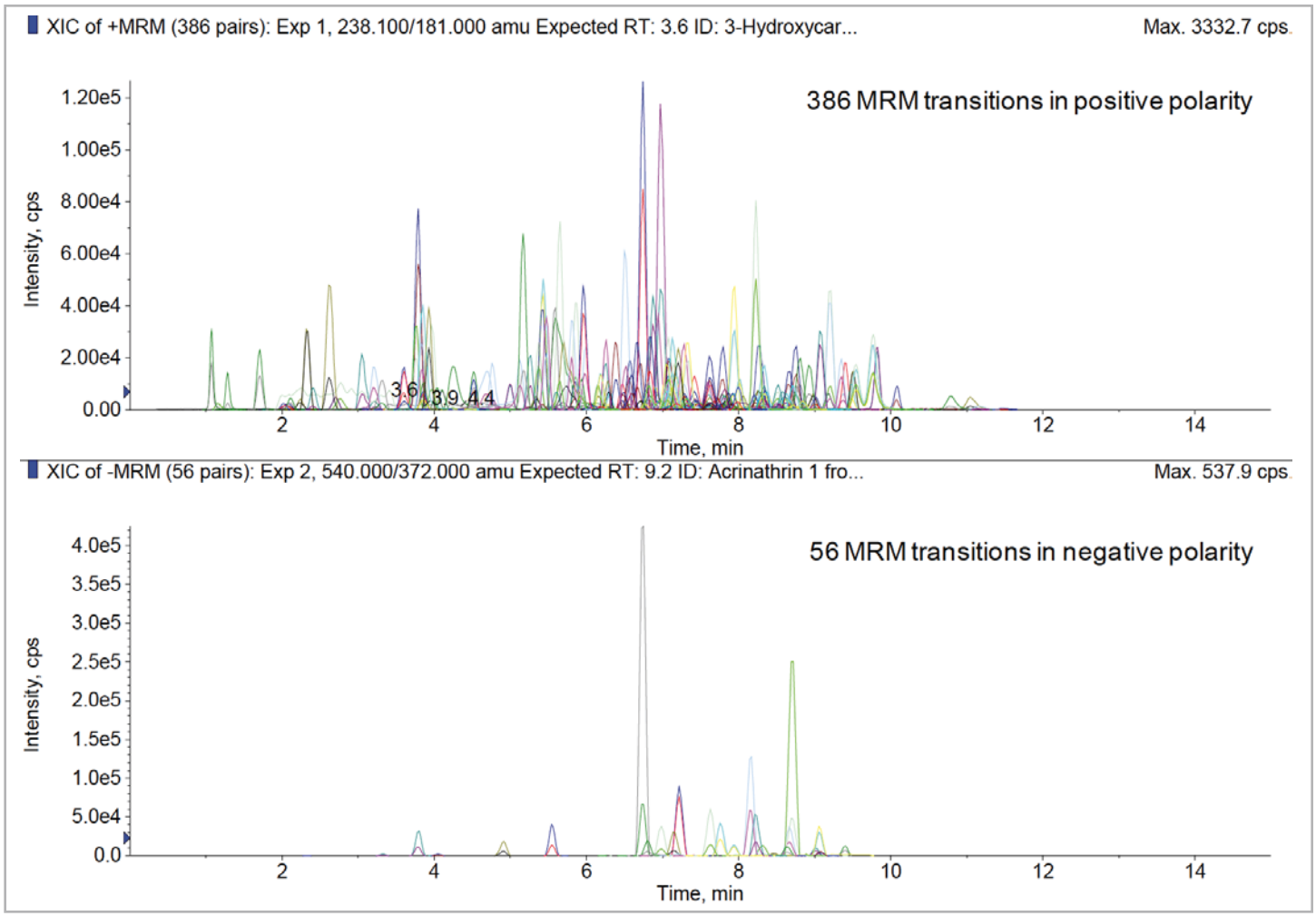

Figure 1. Detection of pesticides at a concentration of 1 ng/mL by monitoring 442 MRM transitions in positive and negative polarity using the *Scheduled* MRM algorithm and fast polarity switching

Results

Scheduled MRM™ with Fast Polarity Switching

The *Scheduled* MRM algorithm uses knowledge of the retention of each analyte to monitor the MRM transition only in a short time window. Thus at any one point in time, the number of concurrent MRM transitions are significantly reduced resulting in much higher duty cycles for each analyte. The software computes maximum dwell times for the co-eluting compounds while still maintaining the desired cycle time for best signal-to-noise (S/N), accuracy, and reproducibility. As a result *Scheduled* MRM allows the monitoring of many more MRM transitions in a single acquisition without compromising data quality (Figure 2).[4]

The enhanced version of the *Scheduled* MRM algorithm offered in Analyst® 1.6 software also allows to combine MRM scheduling with fast polarity switching to further extend the panel of compounds by covering substances with a wider range of chemical properties.

Easy Method creation

A key advantage of the *Scheduled* MRM algorithm is the ease with which powerful quantitative MRM acquisition methods can be created. The user is required to specify a few key parameters (Figure 3):[1]

- MRM transition: (Q1, Q3) and any compound dependent parameters in both polarities
- Expected retention time for each MRM transition
- MRM detection window must be wide enough to allow the MRM peak to stay entirely within the window across all injections
- Target scan time for each polarity to adjust the total cycle time
- MRM ID, like compound name, for easier data processing and reporting

The software algorithm then automatically builds an acquisition method that schedules the appropriate MRM transitions to be monitored and the required polarity switches at the appropriate times over the chromatographic analysis.

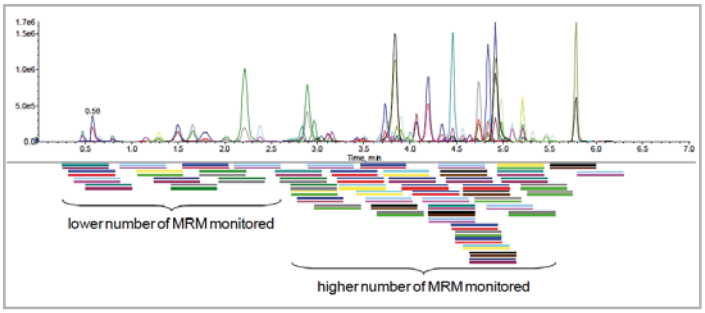

Figure 2. The *Scheduled* MRM Algorithm uses the knowledge of the elution of each analyte to monitor MRM transitions only in a short retention time window. This allows many more MRM transitions to be monitored in a single LC run, while maintaining maximized dwell times and optimized cycle time.

Good Chromatography is the Key to the Best LC-MS/MS Data using the Scheduled MRM Algorithm

The key to the highest order multiplexing and optimal MS/MS performance is high quality and highly reproducible LC separation.

One of the user inputs to the software to automatically create the *Scheduled* MRM method is the MRM detection window. This is an estimate of the LC peak width and chromatographic reproducibility expected, and should therefore reflect the time window around the supplied retention time which will contain the entire LC peak plus any shifts in chromatography. The narrower the peak widths and the more reproducible the elution, the tighter this MRM detection window can be and, thus, less concurrent MRM transitions are monitored. Reduced concurrency also means that higher dwell times will be used for each MRM, improving the data quality.

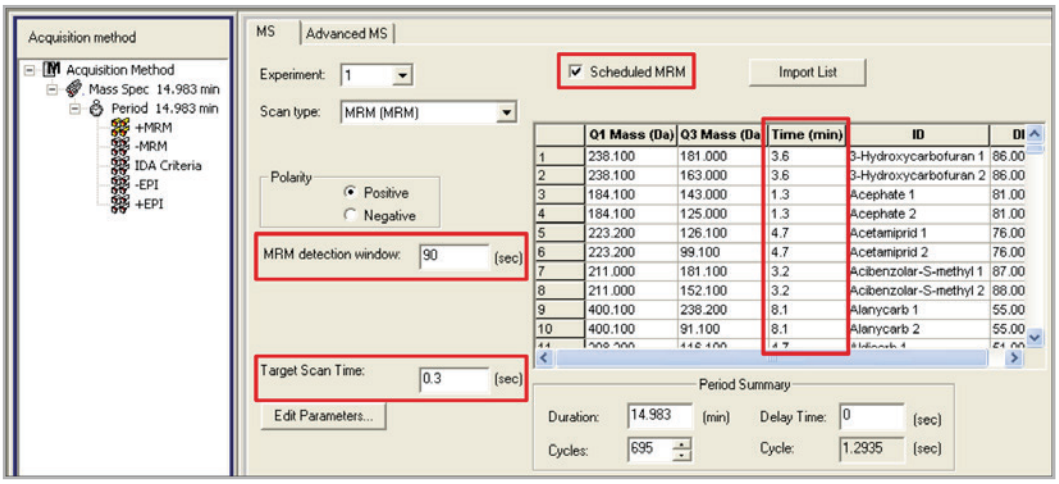

Figure 3. Acquisition method interface for *Scheduled* MRM, in addition to traditional MRM parameters, the user provides retention times of all analytes, an MRM detection window, and a Target scan time. The software then automatically designs and optimizes the *Scheduled* MRM acquisition method.

Quantitative Performance

The developed LC-MS/MS method delivered excellent quantitative data. Calibration standards were injected over the range of 0.1 to 100 ng/mL. For a maximum residue level of 10 µg/kg, the limit of quantitation (LOQ) will depend on the dilution factor of the extract. Here we used a dilution factor of 10x, 20x, or 50x, respectively, depending on the matrix to be analyzed. Therefore, an LOQ of at least 0.2 ng/mL was required for the 50x dilution. Example chromatograms of pesticides detected at 0.2 ng/mL using two MRM transitions are shown in Figures 4a-d.

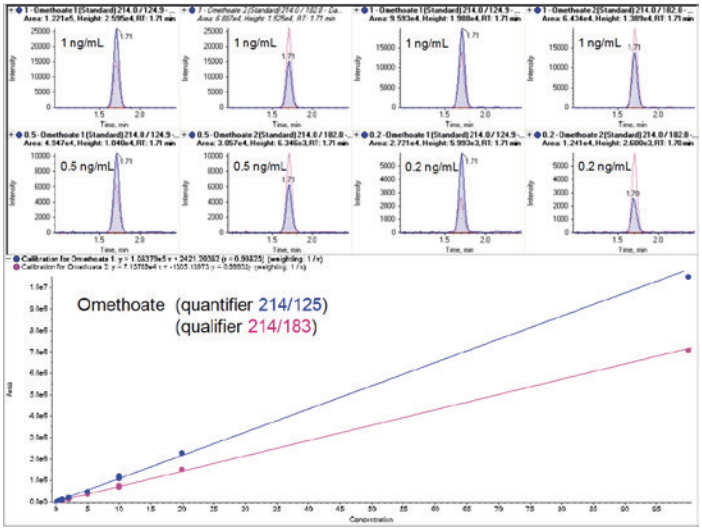

Figure 4a. Calibration lines of the quantifier and qualifier MRM transition of Omethoate from 0.1 to 100 ng/mL

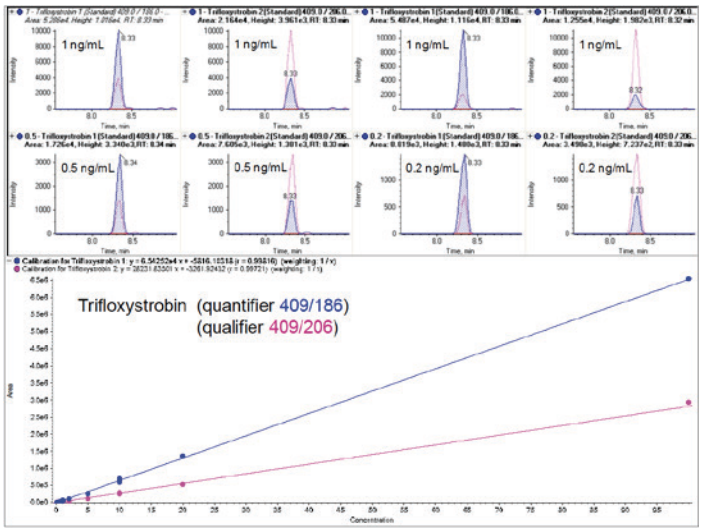

Figure 4b. Calibration lines of the quantifier and qualifier MRM transition of Trifloxystrobin from 0.1 to 100 ng/mL

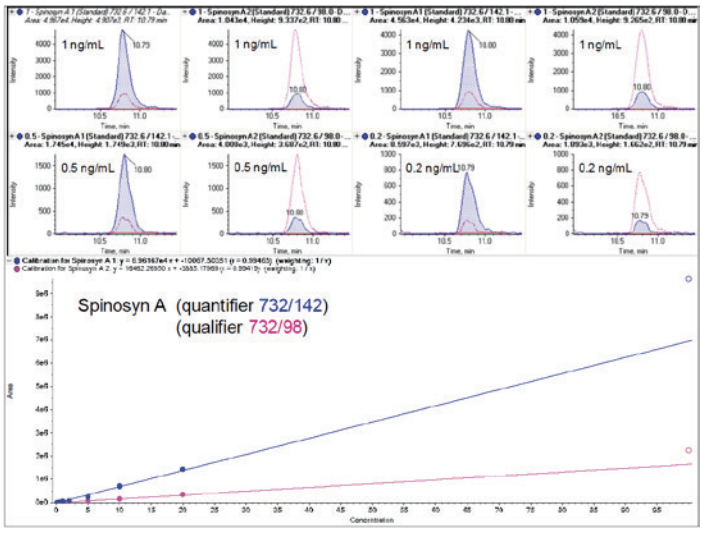

Figure 4c. Calibration lines of the quantifier and qualifier MRM transition of Spinosyn A from 0.1 to 100 ng/mL

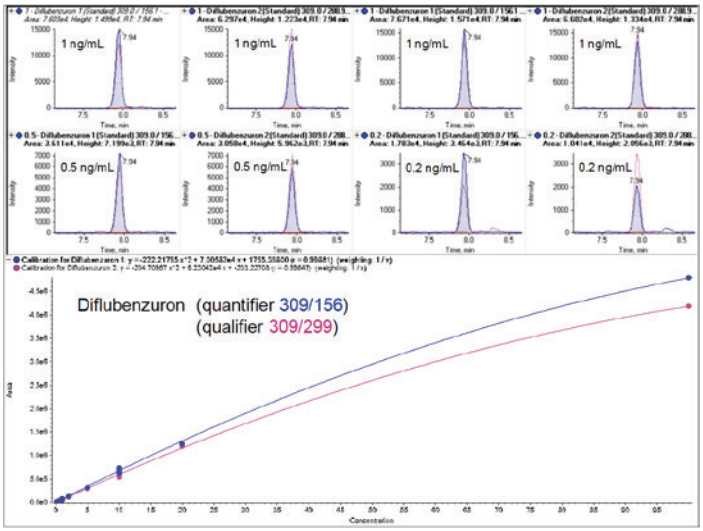

Figure 4d. Calibration curves of the quantifier and qualifier MRM transition of Diflubenzuron from 0.1 to 100 ng/mL

Calibration standards were injected from 0.1 to 100 ng/mL (Figure 4a-d). Accuracy between 80 and 120% were achieved for all targeted pesticides over the entire calibration range. Data points of the lowest or highest standards were excluded for a few pesticides with weak or strong ionization, respectively.

Reproducibility was investigated by repeat injections at 1 and 10 ng/mL (n = 5). The coefficients of variation (%CV) were typically found to be much below 10% for both MRM transitions.

These excellent quantitative results highlight the advantage of combining *Scheduled* MRM with fast polarity switching for a comprehensive multi-target quantitative screen.

Findings in Fruit and Vegetable Samples

The developed method was applied to the quantitation of pesticides in real food extracts. Example chromatograms are shown in Figures 5a-e. The findings are also summarized in Table 1.

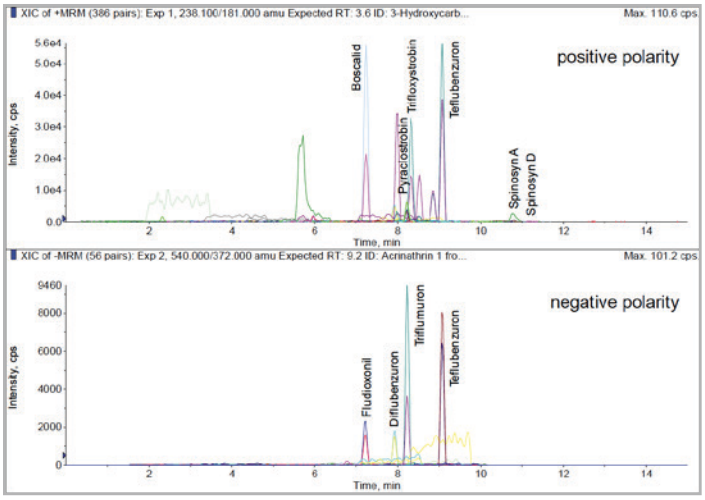

Figure 5a. Pear sample (extract 10x diluted) screened for pesticides using *Scheduled* MRM and fast polarity switching, identified and quantified pesticides are summarized in Table 1

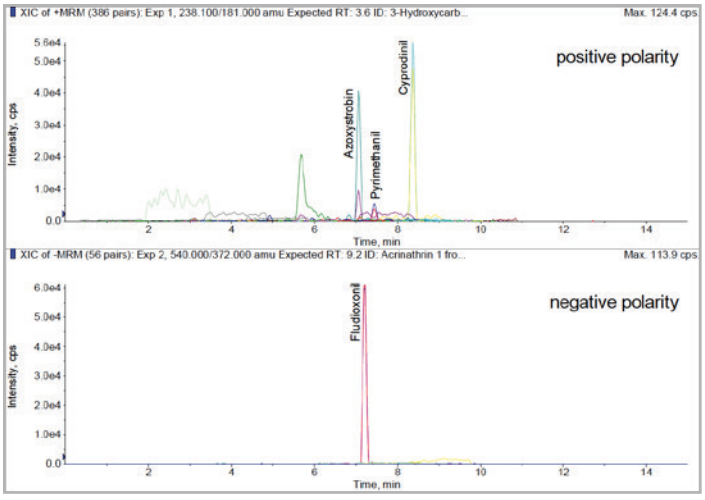

Figure 5b. Organic raspberry sample (extract 10x diluted) screened for pesticides using *Scheduled* MRM and fast polarity switching, identified and quantified pesticides are summarized in Table 1

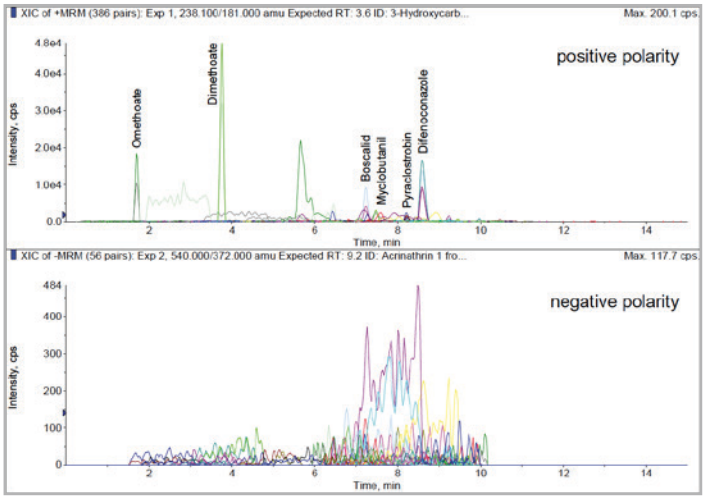

Figure 5c. Carrot sample (extract 10x diluted) screened for pesticides using *Scheduled* MRM and fast polarity switching, identified and quantified pesticides are summarized in Table 1

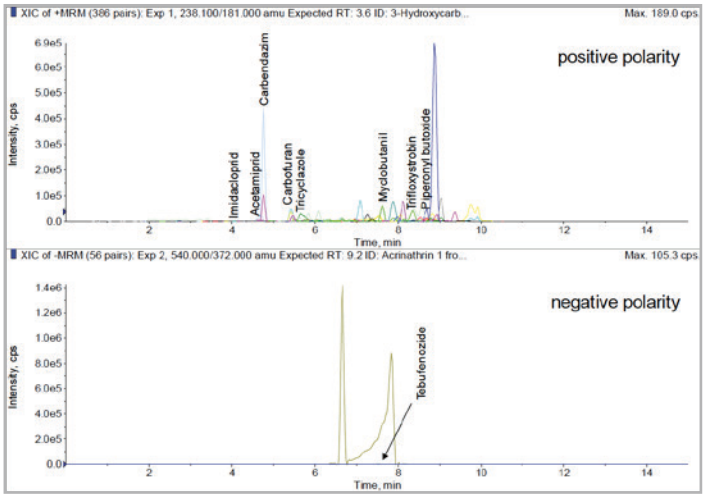

Figure 5d. Curry powder sample (extract 50x diluted) screened for pesticides using *Scheduled* MRM and fast polarity switching, identified and quantified pesticides are summarized in Table 1

Sample	Pesticide	Concentration (µg/kg)
Pear	Boscalid	150
	Diflubenzuron	1.3
	Pyraclostrobin	7.0
	Spinosyn A	7.3
	Spinosyn D	4.2
	Teflubenzuron	16
	Trifloxystrobin	32
	Triflumuron	1.3
Organic raspberry	Azoxystrobin	38
	Cyprodinil	71
	Fludioxonil	7.2
	Pyrimethanil	26
Carrot	Boscalid	26
	Difenoconazole	24
	Dimethoate	16
	Myclobutanil	11
	Omethoate*	8.5
	Pyraclostrobin	5.4
Curry powder	Acetamiprid	59
	Carbendazim	1300
	Carbofuran	51
	Imidacloprid	5.4
	Myclobutanil	960
	Piperonyl butoxide	39
	Tebufenozide	4.9
	Tricyclazole	45
	Trifloxystrobin	18
Raisin	Acetamiprid	20
	Azoxystrobin	21
	Boscalid	29
	Buprofezin	11
	Carbendazim	76
	Cyprodinil	1.7
	Fenpyroximate	8.7
	Fludioxonil	1.0
	Flufenoxuron	36
	Hexythiazox	10
	Imazalil	10
	Indoxacarb	58
	Metalaxyl	7.9
	Methoxyfenozide	11
	Myclobutanil	65
	Penconazole	17
	Propargite	100
	Pyrimethanil	417
	Quinoxyfen	10
	Tetraconazole	10
	Trifloxystrobin	14
** identified as false positive by MS/MS library searching*		

Table 1. Summary of pesticide findings in real samples above 1 µg/kg (findings above the MRL of 10 µg/kg are highlighted)

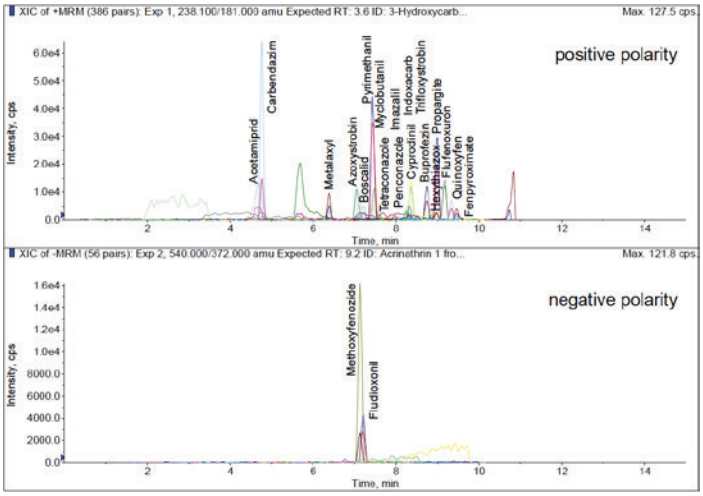

Figure 5e. Raisin sample (extract 20x diluted) screened for pesticides using *Scheduled* MRM and fast polarity switching, identified and quantified pesticides are summarized in Table 1

Sample data was processed using MultiQuant™ Software version 2.1 with the 'Multicomponent' query. Query files are customizable commands to perform custom querying of the result table. Here we used the 'Multicomponent' query to automatically calculate and compare MRM ratios for compound identification and to highlight concentrations above a specified maximum residue level. An example of the results and peak review after running the query file is shown in Figure 6.

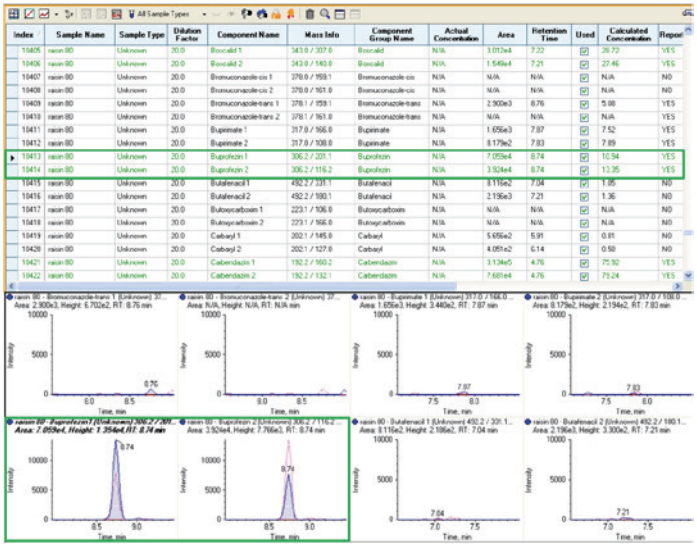

Figure 6. Results and peak review after running the 'Multicomponent' query in MultiQuant™ Software, shown here is an example from raisins, of pesticides detected above an MRL of 10 µg/kg and positively identified by automatic MRM ratio calculation (compare to Figure 5d and Table 1 for complete results).

Compound Identification using MS/MS Library Searching

Despite the high selectivity of MRM detection, there is always a risk of false positive findings due to interfering matrix signals. Typically a second MRM is monitored per analyte and the ratio of quantifier to qualifier transition is calculated for each unknown sample and compared to the MRM ratio of standards for identification. However, it has been reported that relying only on MRM ratios for identification can result in a significant number of false positive results for compound identification, especially if the targeted analytes have a low fragmentation efficiency (many low intensity product ions).[7-9]

For improved accuracy, identification can be performed using full scan MS/MS experiments and library searching to compare the unknown with a standard spectrum. Here MS/MS spectra acquired in the EPI mode of the QTRAP® 5500 system and mass spectral library searching were used to increase the confidence of detection. Example spectra and library search FIT values using a new and improved MS/MS library search algorithm are shown in Figure 7.

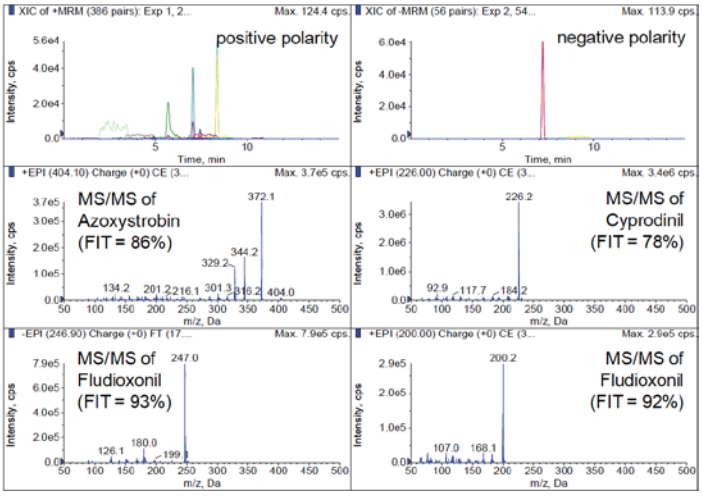

Figure 7a. Organic raspberry sample (extract 10x diluted) screened for pesticides with MS/MS library search results for additional confidence in compound identification

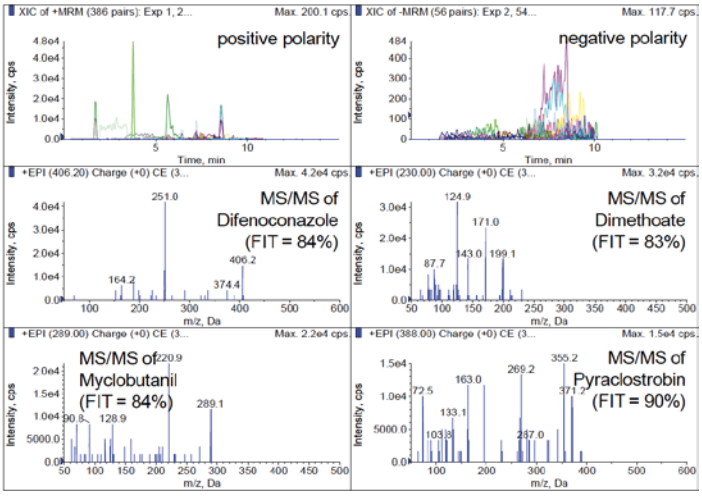

Figure 7b. Carrot sample (extract 10x diluted) screened for pesticides with MS/MS library search results for additional confidence in compound identification

The additional experiment carried out using MS/MS scanning and library searching allowed the identification of a false positive result for the carrot sample. Omethoate was not present in the sample, although the retention time and MRM ratio of Omethoate was identical to the found peak in the extract. Figure 8 shows a comparison of MRM chromatograms and MS/MS spectra.

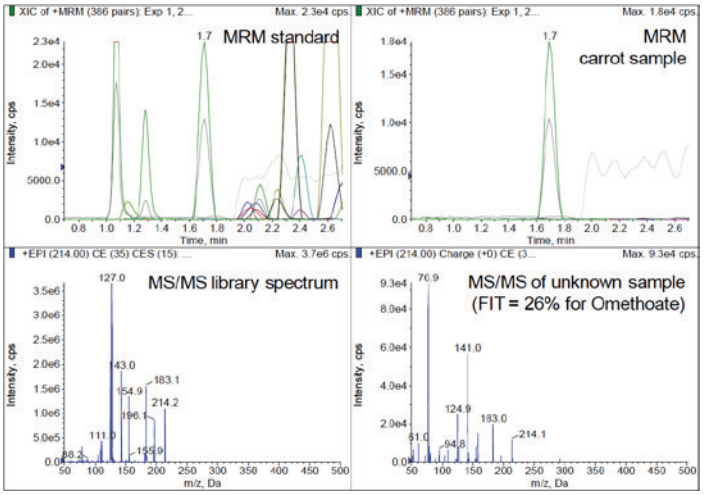

Figure 8. False positive finding identified by MS/MS library searching, standard and carrot sample have identical retention times of 1.7 min and MRM ratio of 0.6 but MS/MS spectra differ and the search results clearly prove the false positive

Summary

This new and unique LC-MS/MS method utilizing the *Scheduled* MRM algorithm in combination with fast polarity switching and acquisition of MS/MS spectra for compound identification has significant advantages. The method was successfully used to quantify and identify pesticides covering a broad range of chemical properties, including the acquisition of positive and negative polarity spectra.

The automatic method setup based on the *Scheduled* MRM algorithm resulted in excellent quantitative data. LOQ were measured for all pesticides at 0.1 ng/mL or below. This allows the dilution of sample extracts by up to 50x, significantly reducing matrix effects and interferences. Accuracies were typically found between 80 and 120% with %CV of less than 10%.

Different samples of fruits, vegetables, and spices were analyzed after QuEChERS extraction and dilution.

Results were processed using MultiQuant™ Software with the 'Multicomponent' query. This query automatically highlights findings above a user specified threshold (like the MRL) and when identification based on MRM ratio comparison was positive.

In addition full scan MS/MS spectra were acquired using the QTRAP® 5500 system. MS/MS spectra contain the complete molecular fingerprint of each analyte and searched against a spectral library reduce the possibility of false positive and negative results. This procedure helped to identify and correct a false positive finding in one of the samples.

References

1. M. Anastassiades, et al.: *J. AOAC Int. 86* (2003) 412-431
2. A. Schreiber and RebeccaWittrig.: *AgroFOOD 21* (2010) 16- 19
3. J. Wong et al.: J. *Agric. Food Chem. 58* (2010) 5897-5903
4. A. Schreiber and Nadia Pace.: Application Note SCIEX (2010) 1282310-01
5. K. von Czapiewski et al.: Application Note SCIEX (2011) 2110211-01
6. A. Schreiber et al.: Application Note SCIEX (2010) 1121010-01
7. M. J. M. Bueno at al.: *Anal. Chem. 79* (2007) 9372-9384
8. A. Schürmann et al.: *Rapid Commun. Mass Spectrom. 23* (2009) 1196-1200
9. M. Gros et al.: *Anal. Chem. 81* (2009) 898-912

RUO-MKT-02-2469-A 08 /2015

Headquarters
500 Old Connecticut Path, Framingham, MA 01701, USA
Phone 508-383-7800
sciex.com

International Sales
For our office locations please call the division headquarters or refer to our website at
sciex.com/offices