Comprehensive Quantitation and Identification of Pesticides in Food Samples Using the SCIEX UltraLC 100 and the SCIEX QTRAP® 4500 System

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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 method using QuEChERS extraction, separation using the SCIEX UltraLC 100 system with a Phenomenex Synergi™ 2.5u Fusion-RP column, and the SCIEX QTRAP 4500 System. The mass spectrometer was operated in highly selective and sensitive Multiple Reaction Monitoring (MRM) mode. 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 evolve, demands in the food testing industry to detect and quantify an increasing number of compounds in a single run are becoming more prevalent.

Generic extraction procedures, like QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) and ultra high performance LC systems combined with polar embedded C18 phases with small particles, providing 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]

State-of-the-art LC-MS/MS systems make it possible to detect hundreds of pesticides and other food residues in a single run.

The SCIEX UltraLC 100 is a UHPLC system designed specifically for use with SCIEX mass spectrometers sustaining pressure of up to 18000 psi at any flow rate up to 5 mL/min. It contains a unique injector valve to maximize column life time, a side port injector needle for increased ruggedness, and a programmable needle wash to greatly reduce carry-over.

The SCIEX QTRAP 4500 system combines the legendary sensitivity, reproducibility, and accuracy of the 4000 series with the speed and trapping capabilities of the QTRAP 5500 system. The Turbo V™ source and Curtain Gas™ interface provide exceptional robustness and successfully reduce chemical noise. The advanced eQ™ electronics and Qurved LINAC® collision cell were designed for unparalleled speed of MRM detection and fast polarity switching for comprehensive multi-component analysis.

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.

Here we present a new LC-MS/MS method utilizing the Ultra LC 100 and the QTRAP 4500 system using 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 QuEChERS extracts of fruit and juice samples.

Method Details

- Different fruit samples were extracted using Restek QuEChERS kits (Q110, Q210 and Q213) and diluted 5 times with water to optimize chromatographic peak shape and minimize possible matrix effects and interferences. Juice samples were injected directly after centrifugation and 5x dilution. The injection volume was set to 10 µL.
- The SCIEX iD*Quant*™ 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 the SCIEX UltraLC 100 with a Phenomenex Synergi-Fusion 2.5u 50x2 mm column and a fast gradient of water and methanol with 10 mM ammonium formate buffer at a flow rate of 0.5 mL/min.
- The new SCIEX QTRAP® 4500 System was operated with Turbo V™ source and Electrospray Ionization (ESI) probe.
- Approximately 500 MRM transitions were monitored in both positive and negative polarity. 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 in combination with fast polarity switching using Analyst® 1.6.1 Software.
- For increased confidence in compound identification, EPI spectra were acquired at a scan speed of 10000 Da/s using dynamic fill time for best spectral quality and Collision Energy Spread (CES) to ensure a characteristic MS/MS pattern independent of the 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.

Results

Sensitivity, Reproducibility, Linearity and Accuracy

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 data quality.[3] Combining *Scheduled* MRM™ with fast polarity switching further allows extending the target list of pesticides while maintaining throughput.

An example chromatogram of a solvent standard at 1 ng/mL is shown in Figure 1. Approximately 500 MRM transitions were monitored in both polarities throughout the entire chromatographic run. The total target cycle time of 0.7 sec ensures the collection of at least 12 data points across the LC peak resulting in excellent accuracy and reproducibility.

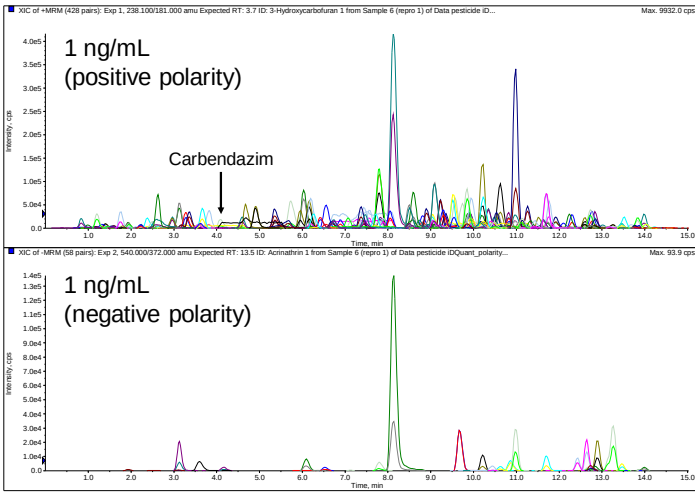

Figure 1. Comprehensive pesticide screening using the *Scheduled* MRM™ algorithm and fast polarity switching, ~500 MRM transition were detected with a total target cycle time of 0.7 sec

Figure 2 shows example chromatograms of 10 repeat injections at 1 ng/mL of early to late eluting pesticides in both polarities. The %CV values of 10% or less highlight the speed and effectiveness of *Scheduled* MRM combined with fast polarity switching. The developed method enables quantitation of all target pesticides with an LOD of at least 1 ng/mL and, thus, allowing sample extract dilution to minimize possible matrix effects.

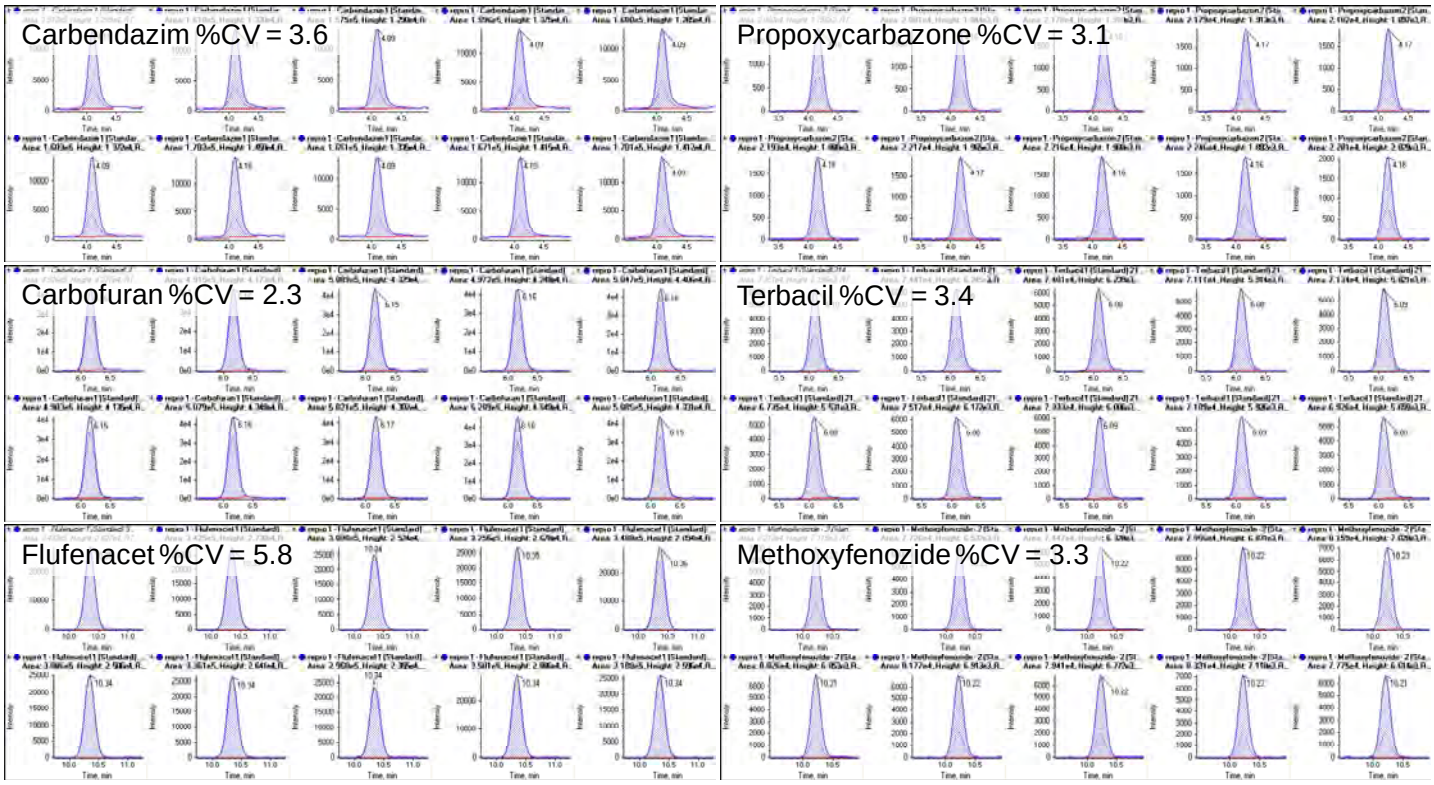

Figure 2. Repeat injections of pesticides at a concentration of 1 ng/mL detected in positive (left) and negative (right) polarity in a single run using *Scheduled* MRM™ and fast polarity switching (Carbendazim and Propoxycarbazone at 4.1 min, Carbofuran and Terbacil at 6.1 min, and Flufenacet and Methoxyfenozide at 10.3 min)

Linearity was obtained for most pesticides over 4 orders of magnitude (0.1-100 ng/mL). An example calibration line of Carbendazim is shown in Figure 3. Both MRM transitions have a regression coefficient of > 0.999 with accuracies between 97 and 109%.

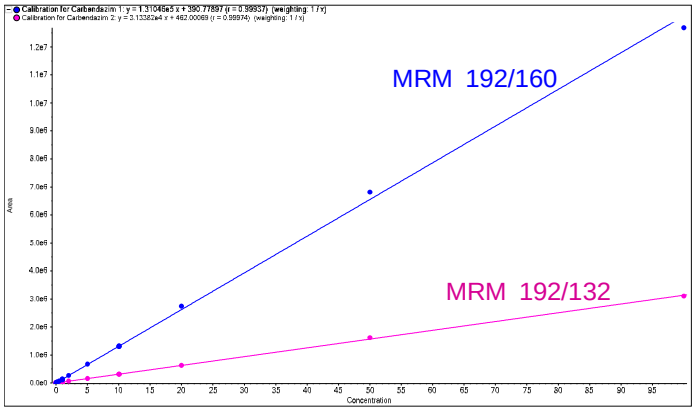

Figure 3. Calibration lines of both MRM transitions of Carbendazim

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.

Findings in Fruit and Vegetable Samples

The developed method was applied to the quantitation and identification of pesticides in real food extracts. QuEChERS extracts of fruits and vegetables were diluted 5x prior LC-MS/MS analysis. Juice samples were injected directly after centrifugation and 5x dilution.

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. The 'Multicomponent' query automatically calculates and compares MRM ratios for compound identification and highlights concentrations above a user specified maximum residue level. An example of the results and peak review after running the query file is shown in Figure 4.

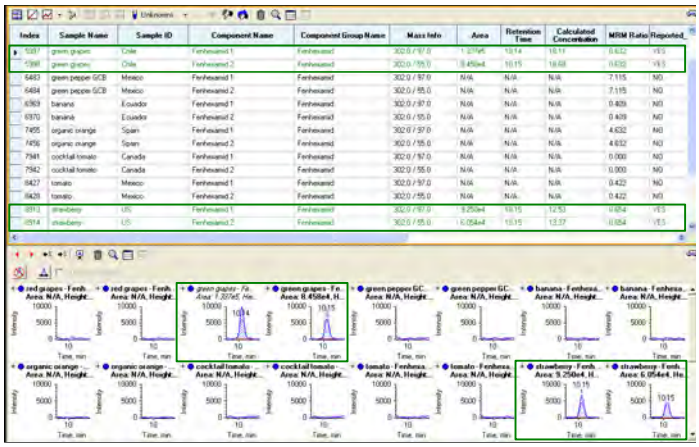

Figure 4. Automatic reporting of pesticides using the 'Multicomponent' query in MultiQuant™ software: Fenhexamid was positively identified using MRM ratio calculation in two samples and quantified in green grapes at 18.1 µg/kg and in strawberry at 12.5 µg/kg, respectively.

Example chromatograms of analyzed samples are shown in Figures 5a-e. The findings are also summarized in Table 1.

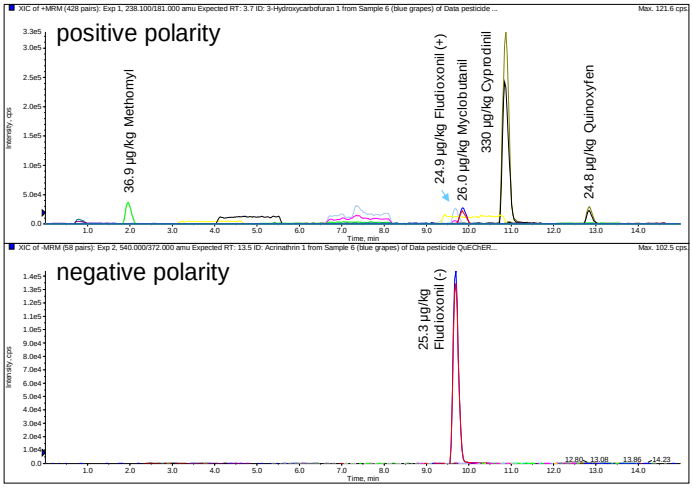

Figure 5a. Pesticides identified and quantified in a red grape sample

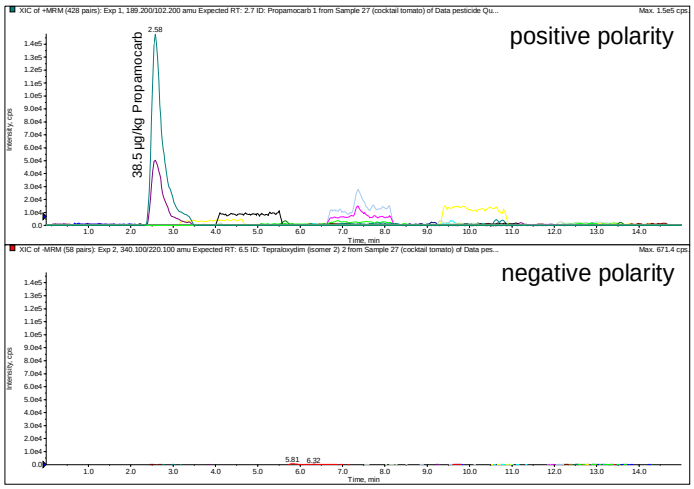

Figure 5b. Pesticides identified and quantified in a cocktail tomato sample

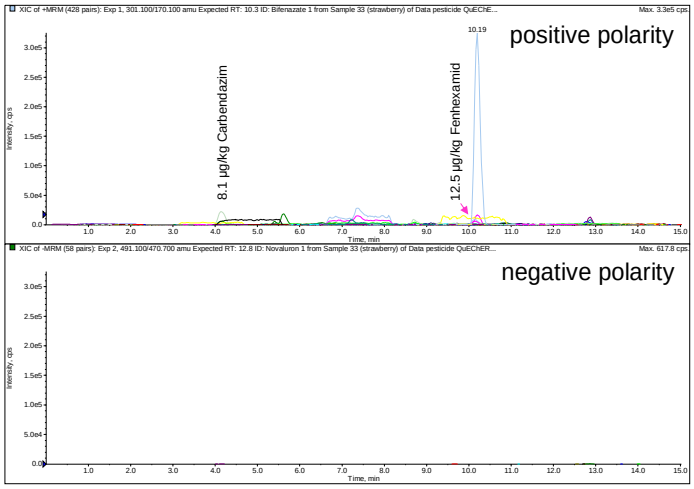

Figure 5c. Pesticides identified and quantified in a strawberry sample

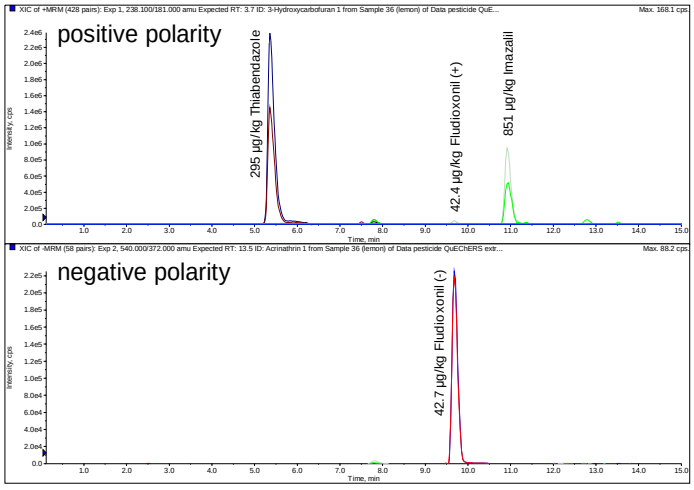

Figure 5d. Pesticides identified and quantified in a lemon sample

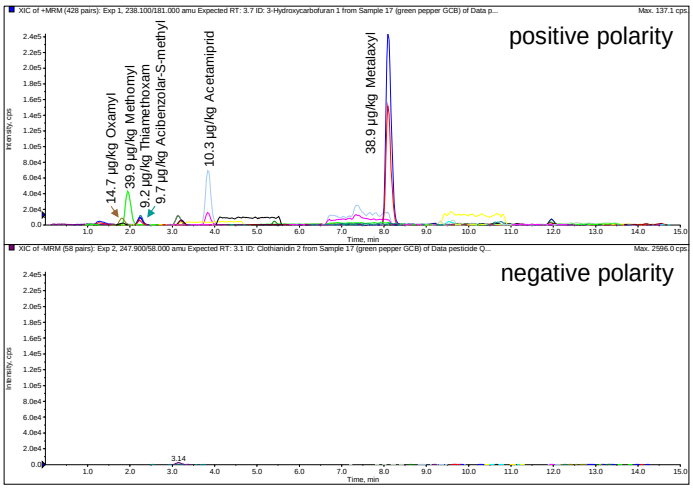

Figure 5e. Pesticides identified and quantified in green pepper sample

Table 1. Summary of pesticide findings in store bought food and orange juice samples above a concentration of 5 µg/kg

Sample	Pesticide	Concentration (µg/kg)
Red grapes	Cyprodinil	330
	Fludioxonil	24.9
	Methomyl	36.9
	Myclobutanil	26.0
	Quinoxyfen	24.8
Cocktail tomato	Propamocarb	38.5
Strawberry	Carbendazim	8.1
	Fenhexamid	12.5
Lemon	Fludioxonil	42.4
	Imazalil	851
	Thiabendazole	295
Green pepper	Acetamiprid	10.3
	Acibenzolar-S-methyl	9.7
	Metalaxyl	38.9
	Methomyl	39.9
	Oxamyl	14.7
	Thiamethoxam	9.2
Banana	Imazalil	40.7
	Thiabendazole	18.5
Clementine	Imazalil	1250
Green grapes	Boscalid	10.8
	Fenhexamid	18.1
	Imidacloprid	32.0
	Myclobutanil	7.2
	Quinoxyfen	12.5
Organic orange	no pesticides detected above 5 µg/kg	
Raspberry	Azoxystrobin	35.5
	Cyprodinil	71.0
	Fludioxonil	7.2
	Pyrimethanil	22.7
Red pepper	Flutriafol	44.0
Tomato	Difenoconazole	61.0
	Buprofezin	97.8
Orange juice 1	Carbendazim	13.0 ng/mL
Orange juice 2	Carbendazim	67.0 ng/mL

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.

To increase confidence in identification full scan MS/MS experiments can be performed, and unknown spectra can be searched against mass spectral libraries. Here MS/MS spectra acquired in the EPI mode of the QTRAP® 4500 system were searched against the iMethod™ pesticide library (version 1.1). Example spectra and library search FIT values to identify Carbendazim in orange juice samples and Cyprodinil and Fludioxonil in a raspberry sample are shown in Figures 6 and 7. These examples highlight that MS/MS library searching increases confidence in identification, especially if the targeted analytes have low fragmentation efficiency (many low intensity product ions).

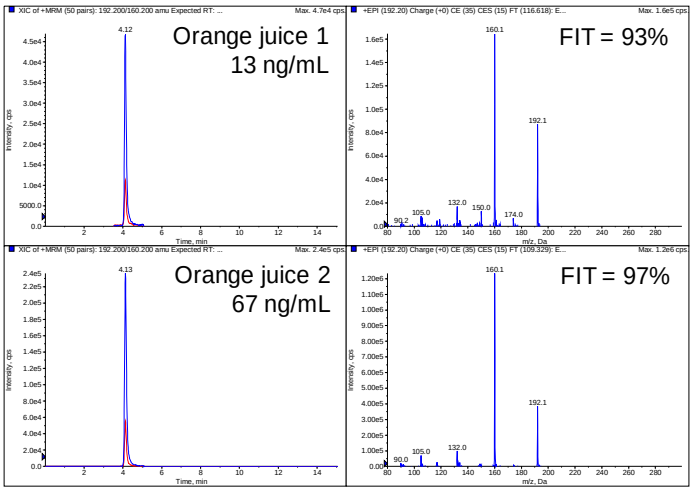

Figure 6. Identification of Carbendazim in two orange juice samples using MS/MS library searching: The samples were injected directly after 5x dilution, and FIT values above 90% clearly confirm the identity of Carbendazim.

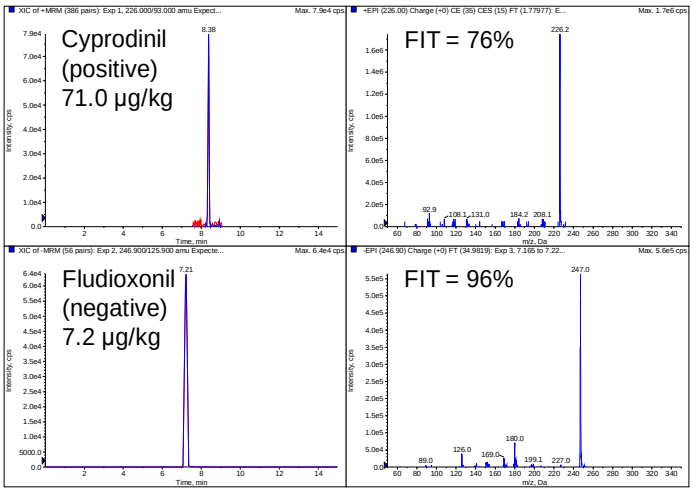

Figure 7. Identification of Cyprodinil and Fludioxonil in a raspberry sample using MS/MS library searching: The samples were injected after QuEChERS extraction and 5x dilution, and MS/MS spectra were acquired in positive polarity and negative polarity.

Summary

This new and unique LC-MS/MS method using the SCIEX UltraLC 100 and QTRAP® 4500 system 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 to significantly reduce possible matrix effects and interferences. Accuracies were typically found between 80 and 120% with %CV of less than 10%.

Different food and juice samples were analyzed after QuEChERS extraction and dilution to minimize possible matrix effects.

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

In addition full scan MS/MS spectra were acquired using the QTRAP 4500 system. MS/MS spectra contain the complete molecular fingerprint of each analyte, and when searched against a spectral library, reduce the possibility of false positive and negative results.

References

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2 J. Wong et al.: J. Agric. Food Chem. 58 (2010) 5897-5903 3

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