Analysis of Pesticides in Food Samples Using the AB SCIEX Triple Quad™ 3500 System

André Schreiber
AB SCIEX Concord, Ontario (Canada)

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 LC-MS/MS allow the screening for hundreds pesticide residues in food samples quickly, efficiently, and with excellent sensitivity and selectivity to meet global food trade guidelines and regulations.[1-3]

Mass spectrometers are typically considered to be expensive and complex instruments. However, the AB SCIEX Triple Quad™ 3500 system, combined with an extensive compound MRM catalog, provides labs with robust and reliable mass spec technology and method starting points, at an affordable price.

Here we present a method using QuEChERS extraction with Phenomenex roQ kits, filtration with Thomson filter vials, separation using a Kinetex Biphenyl 2.6u (50 x 2.1mm) column, and the AB SCIEX Triple Quad™ 3500 system. The mass spectrometer was operated in highly selective and sensitive Multiple Reaction Monitoring (MRM) mode. The *Scheduled* MRM™ Pro algorithm was used to obtain the best data quality. Compound identification and quantitation was achieved by monitoring two MRM transitions for each pesticide. The MRM ratio was automatically evaluated in MultiQuant™ software.

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.

Generic extraction procedures, like QuEChERS, ultra high performance LC systems combined with core-shell particle columns, 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. 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 AB SCIEX Triple Quad™ 3500 system takes the best features of the API 3200™ system and enhances them with

modern engineering and electronics. The proven design of Turbo V™ source and Curtain Gas™ interface provide exceptional robustness and ruggedness. The advanced eQ™ electronics and the curved LINAC® collision cell were designed for ultra-fast speed of MRM detection and fast polarity switching for comprehensive multi-component analysis.

Advanced software tools like the *Scheduled* MRM™ Pro 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 best data quality. Two MRM transitions were monitored for each pesticide to use the ratio of quantifier and qualifier ion for compound identification.

Experimental

- The SCIEX i*DQuant*™ standards kit for pesticide analysis was used for method setup and preparation of calibration standards.[4]
- Store-bought fruit and vegetable samples were extracted using Phenomenex roQ QuEChERS kit buffer-salt mix and dSPE kits following the European standard method 15662.[5]
- Extracts were diluted 5 times with water in Thomson filter vials, filtered using the 0.45 µm PVDF membrane and directly

placed into the autosampler for LC-MS/MS analysis. The injection volume was set to 2 µL.

- LC separation was achieved using a Phenomenex Kinetex Biphenyl 2.6u (50 x 2.1mm) column and a fast gradient of water and methanol with 5 mM ammonium formate buffer at a flow rate of 0.5 mL/min (see Table 1 for the gradient profile).

Table 1. Gradient conditions used for the separation of pesticides

Step	Time (min)	A (%)	B (%)
0	0.0	90	10
1	0.5	90	10
2	2.0	70	30
3	9.0	40	60
4	11.0	20	80
5	12.0	5	95
6	15.0	5	95
7	16.0	90	10
8	20.0	90	10

- The AB SCIEX Triple Quad™ 3500 system was operated with Turbo V™ source and Electrospray Ionization (ESI) probe set to 400°C.
- Approximately 400 MRM transitions were monitored in positive polarity. Optimized transitions for all compounds were obtained through the MRM catalogue of the iMethod™ application for Pesticide Screening version 2.1.
- The *Scheduled* MRM™ Pro algorithm was used with a target cycle time of 0.5 sec and compound dependent detection windows and thresholds (Figure 1).

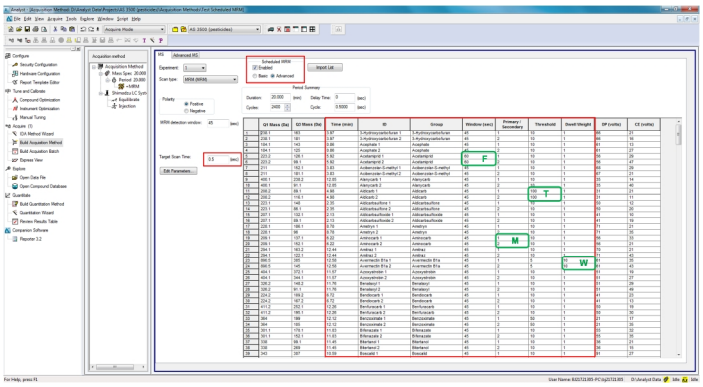

Figure 1. *Scheduled* MRM™ Pro algorithm allowing: Flexible Window Width (F), Dynamic Window Extension (T), MRM-triggered MRM (M, T), Dwell Time Weighting (W)

- MultiQuant™ software version 3.0 was used for quantitative and qualitative data processing.

Results and Discussion

Sensitivity, Reproducibility, Linearity and Accuracy

Chromatograms of a solvent standard at 10 ng/mL analyzed using the API 3200™ and Triple Quad™ 3500 are shown in Figure 2. An average gain in sensitivity of 3x was observed.

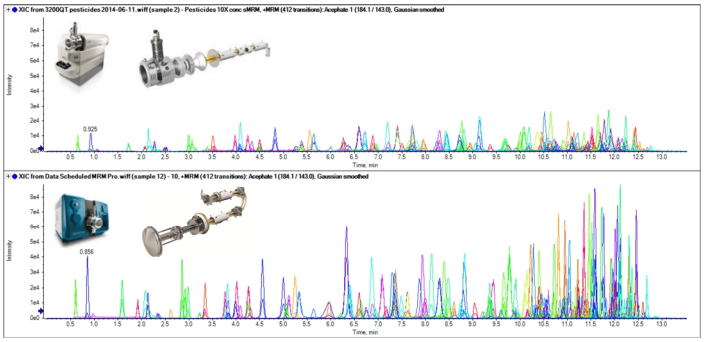

Figure 2. Sensitivity comparison of a 10 ng/mL standard analyzed using the API 3200™ system (top) and AB SCIEX Triple Quad™ 3500 system (bottom) with an average sensitivity gain of 3x

Most pesticides were detectable at a concentration below 1ng/mL and all pesticides had a limit of detection (LOD) of 2 ng/mL or lower. Example chromatograms at a concentration of 5 ng/mL are shown in Figure 3. The achieved sensitivity allows sample extract dilution by 5x to minimize possible matrix effects.

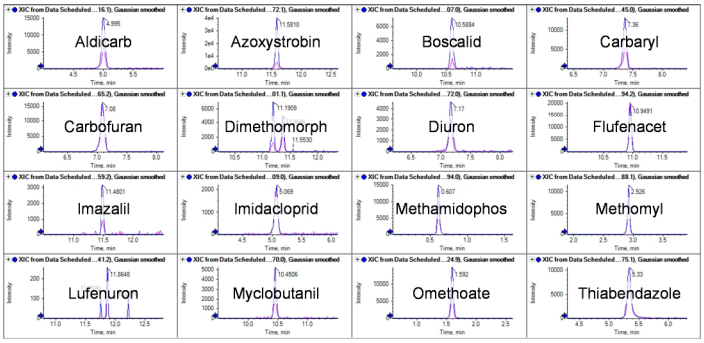

Figure 3. Sensitivity of selected pesticides detected at a concentration of 5 ng/mL using the Triple Quad™ 3500 system

Linearity was obtained over 3 to 4 orders of magnitude for most pesticides with accuracies between 80 and 120%. Data points of

the lowest or highest standards were excluded for a few pesticides with weak or strong ionization, respectively. Repeatability was studied at 1 and 10 ng/mL (n=5). The coefficient of variation (%CV) was typically below 10%.

An example calibration line of Acephate is shown in Figure 4. Both MRM transitions had a regression coefficient of > 0.998 and excellent repeatability of 2.9 and 3.2% at 1 and 10 ng/mL respectively (n=5).

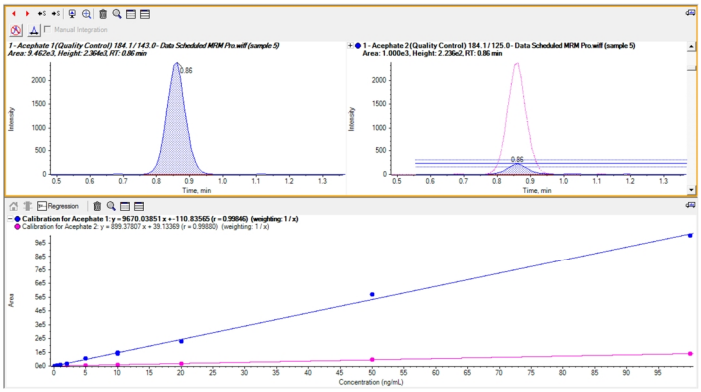

Figure 4. Peak review quantifier-qualifier ratio of Acephate at 1 ng/mL and calibration line from 0.1 to 100 ng/mL with %CV of 2.9% and 3.2% at 1 and 10 ng/mL, respectively, and.

Findings in Fruit and Vegetable Samples

The developed method was applied to the quantitation and identification of pesticides in real food extracts. Different dispersive SPE kits of Phenomenex (roQ KS0-8913, 8914, 8915, 8916) were used for sample cleanup depending on the type of matrix following the European standard method 15662. Extracts were diluted 5 times with water to minimize possible matrix effects. The diluted extracts were filtered using the Thompson 0.45 µm PVDF membrane and directly placed into the autosampler for LC-MS/MS analysis.

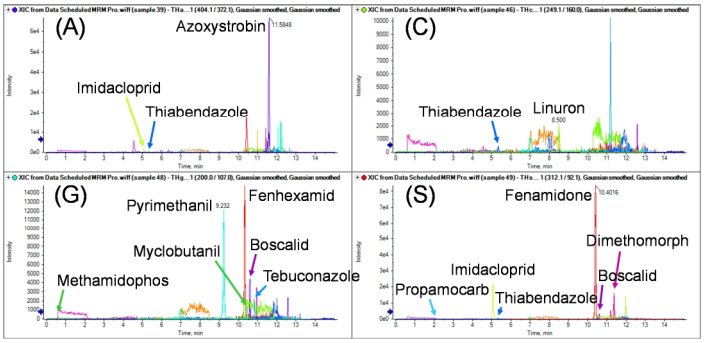

Figure 5. Detection of pesticides in filtered QuEChERS extracts of avocado (A), carrot (C), grapes (G), and spinach (S)

Example chromatograms of different type of food samples with detected compounds are presented in Figure 5. Qualitative and quantitative results are summarized in Table 2. Compound identification was based on the criteria of SANCO/12571/2013 (retention time tolerance of ± 0.02 min and maximum tolerances for ion ratios ± 30%). All quantitative and qualitative results were automatically calculated in MultiQuant™ software (Figure 6).[6]

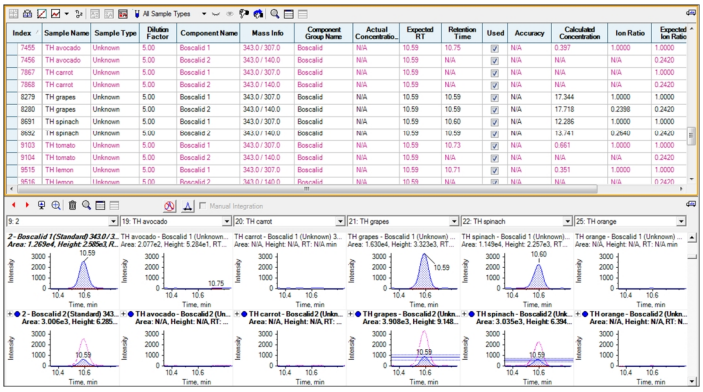

Figure 6. Quantitation and identification based on MRM ratios in MultiQuant™ software, the example shows the side-by-side peak review for Boscalid with positive findings in grapes and spinach samples

Table 2. Summary of pesticide findings in store bought food above a concentration of 1 µg/kg

Sample	Pesticide	Concentration (µg/kg)	RT Error (min)	MRM Ratio (Expected)
Avocado	Azoxystrobin	55.0	0.01	0.146 (0.126)
	Imidacloprid	6.2	0.03	0.823 (0.818)
	Thiabendazole	2.9	0.06	1.035 (0.820)
Carrot	Linuron	14.3	0.00	0.613 (0.742)
	Thiabendazole	5.3	0.04	0.995 (0.820)
Grapes	Boscalid	17.3	0.00	0.240 (0.242)
	Fenhexamid	363	0.04	0.973 (1.053)
	Methamidophos	1.2	0.01	0.873 (0.698)
	Myclobutanil	14.2	0.02	0.811 (0.830)
	Pyrimethanil	687	0.05	0.482 (0.435)
	Tebuconazole	7.1	0.03	0.030 (0.261)
Grapefruit	Imazalil	899	0.07	0.410 (0.348)
	Imidacloprid	1.3	0.03	1.052 (0.993)
	Thiabendazole	7.6	0.03	0.812 (0.820)
Lemon	Imazalil	981	0.06	0.266 (0.348)
	Thiabendazole	7.6	0.04	0.782 (0.820)
Orange	Imazalil	1830	0.06	0.282 (0.348)
	Thiabendazole	>3000	0.04	0.812 (0.820)
Spinach	Boscalid	12.3	0.00	0.264 (0.242)
	Dimethomorph	53.7	0.08	0.537 (0.541)
	Fenamidone	755	0.01	0.749 (0.672)
	Imidacloprid	217	0.03	0.907 (0.993)
	Propamocarb	3.1	0.06	0.260 (0.336)
	Thiabendazole	3.6	0.05	0.917 (0.820)

Improving data acquisition quality with Scheduled MRM Pro algorithm

Figures 7 and 8 show results of pesticides detected in food samples to explain different features of *Scheduled* MRM™ Pro algorithm.

The detection window can be set differently for each compound depending on LC peak width and potential retention time shifts. This allows a more effective scheduling of MRM transitions resulting in better data quality. The example in Figure 7 shows Boscalid detected with a window of 45 sec, while the window of Dimethomorph was set to 120 sec to detect both isomers together.

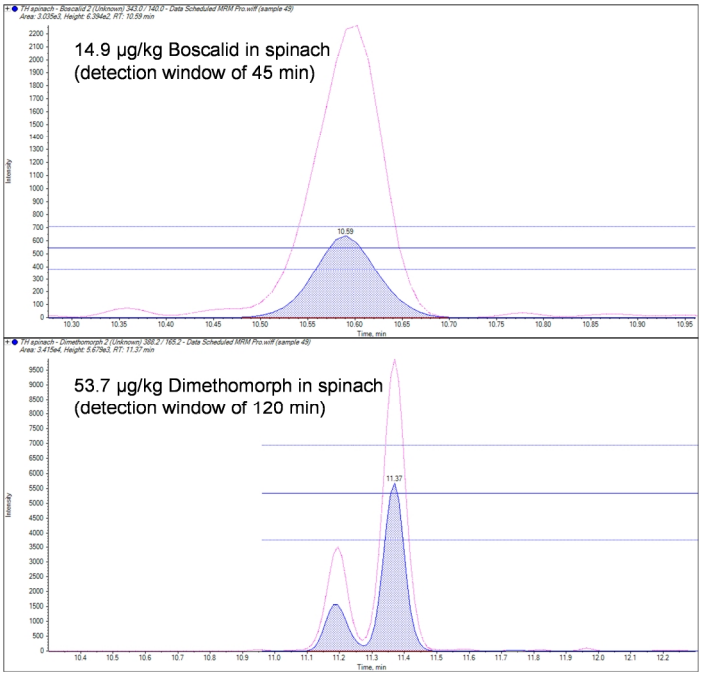

Figure 7. Examples of using the Flexible Window Width in a *Scheduled* MRM™ Pro method: the window for Boscalid was set to 45 sec and Dimethomorph was detected using a wider window to detect both isomers together

The *Scheduled* MRM™ Pro algorithm also allows automatic triggering of qualifier MRM transitions when a quantifier transitions is present (Figure 8). This feature further optimizes the MRM scheduling. The threshold is also used to automatically extend the detection window if an MRM signal is still present at the end of the default detection window.

Figure 8 shows an example of dynamic window extension for the detection of Thiabendazole in an orange sample. The sample contained Thiabendazole at more than 3000 µg/kg resulting in peak tailing. The automatic extension of the detection window enabled to capture the complete peak area for accurate quantitation and identification based on the MRM ratio.

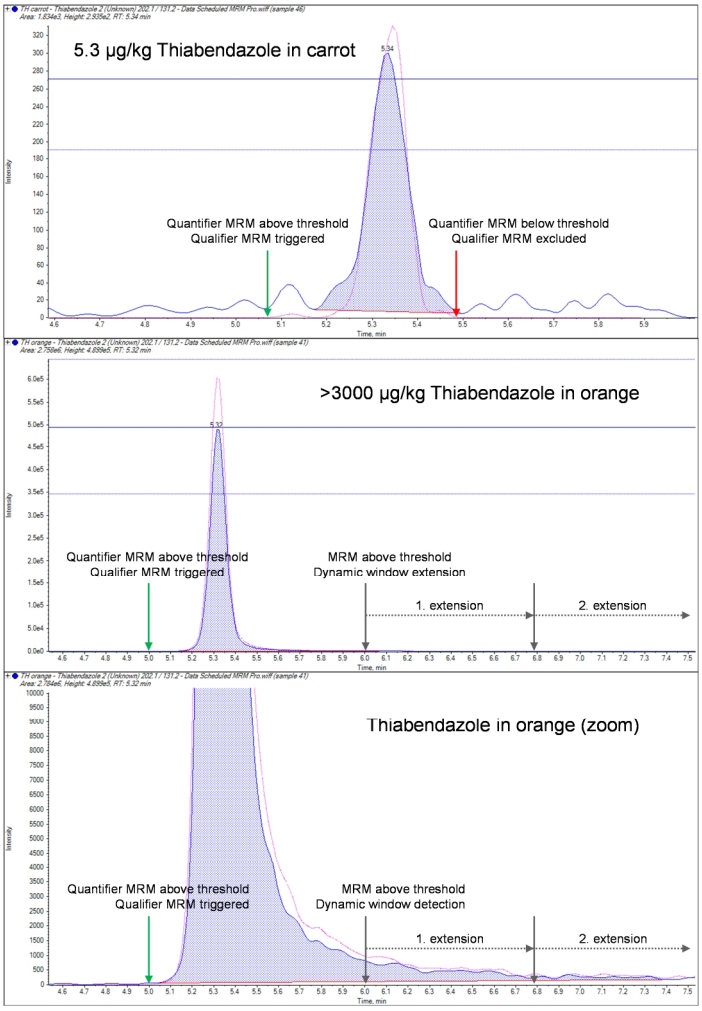

Figure 8. Examples of MRM-triggered MRM and Dynamic Window Extension: the qualifier MRM transition is automatically triggered when the quantifier MRM transitions exceeds the threshold set in the *Scheduled* MRM™ Pro method, the detection window is automatically extended if the MRM signal is above the threshold at the end of the detection window

Summary

A new LC-MS/MS method for the identification and quantitation of pesticides was developed and successfully applied to fruit and vegetable samples.

Samples were extracted using a QuEChERS protocol following the European standard method 15662 with Phenomenex roQ kits. Sample extracts were diluted 5x to minimize potential matrix effects and filtered using Thomson filter vials. The AB SCIEX Triple Quad™ 3500 system operated in MRM mode and utilizing the *Scheduled* MRM™ Pro algorithm was used for detection. Two MRM transitions were monitored for each analyte and the ratio of quantifier and qualifier transition was used for identification.

Qualitative and quantitative data processing was performed in MultiQuant™ software. Criteria of SANCO/12571/2013 were used for identification. All pesticides had an LOD of 2 ng/mL or lower and good linearity of 3-4 orders of magnitude with repeatability well below 10%.

References

1 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

2 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

3 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

4. A. Schreiber et al.: 'Using the iD*Quant*™ Standards Kit for Pesticide Analysis to Analyze Residues in Fruits and Vegetable Samples' Application Note AB SCIEX (2011) #3370211-01

5 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)

6 SANCO/12571/2013: 'Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed.'

Publication number: 10641314-01

Headquarters
500 Old Connecticut Path, Framingham, MA 01701 USA
Phone 508-383-7700
www.sciex.com

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