Routine Targeted Quantitation and Identification of Pesticide Residues using Triple Quadrupole LC-MS/MS and Advanced Scheduling of MRM Transitions

AB SCIEX

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, in combination with generic extraction methods, such as QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe), allow the screening for pesticide residues in a food samples.[1-3]

Here we present a new method using QuEChERS extraction with Phenomenex roQ™ kits, separation using a Kinetex™ Biphenyl 2.6u (50 x 2.1mm) column, and the new 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 particles 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 new 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 unparalleled 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 iD*Quant*™ standards kit for pesticide analysis was used for method setup and preparation of calibration standards.
- 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.[4]
- 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).
- The new AB SCIEX Triple Quad™ 3500 system was operated with Turbo V™ source and Electrospray Ionization (ESI) probe set to 400 ºC.
- 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).
- MultiQuant™ software version 3.0 was used for quantitative and qualitative data processing.

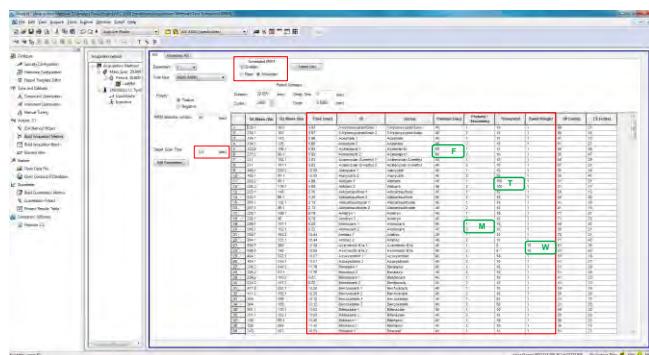| Step	Time (min)	A/B (%)
0	0.0	90/10
1	0.5	90/10
2	2.0	70/30
3	9.0	40/60
4	11.0	20/80
5	12.0	5/95
6	15.0	5/95
7	16.0	90/10
8	20.0	90/10

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

RESULTS

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. Most pesticides were detectable at a concentration below 1ng/mL and all pesticides had a limit of detection 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.
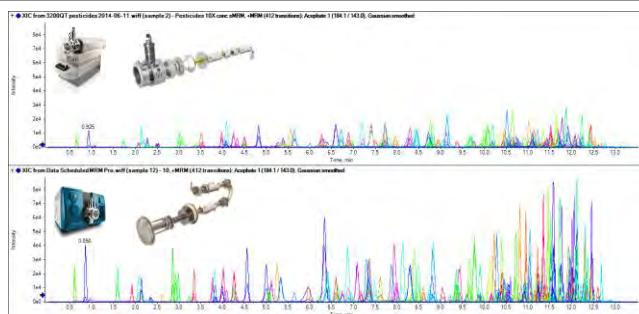
Figure 2. Sensitivity comparison of a 10 ng/mL standard analyzed using the API 3200™ system (top) and Triple Quad™ 3500 system (bottom) with an average sensitivity gain of 3x
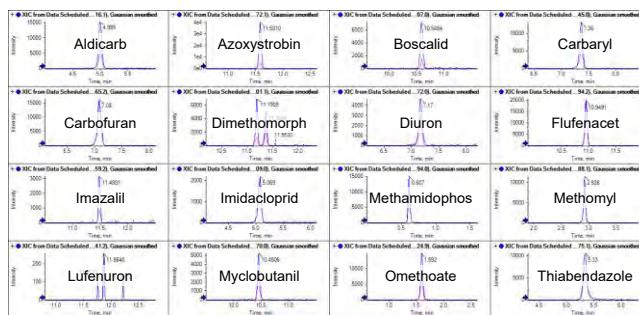
Figure 3. Sensitivity of selected pesticides detected at a concentration of 5 ng/mL using the Triple Quad™ 3500 system
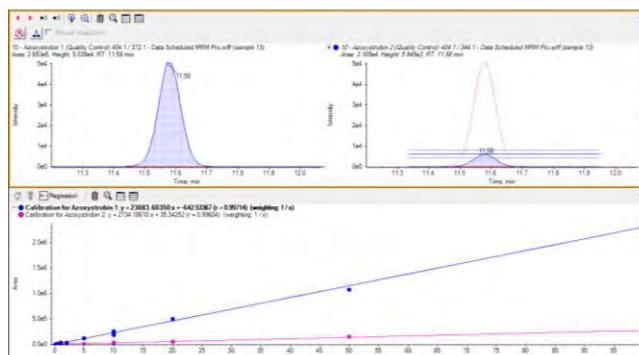
Figure 4. Calibration line of Azoxystrobin from 0.1 to 100 ng/mL and quantifier-qualifier ratio for compound identification

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 quality results. The example in Figure 5 shows Boscalid detected with a window of 45 sec, while the window of Dimethomorph was set to 120 sec 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 6). This feature further optimizes the MRM scheduling resulting in an increase of data quality. 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 6 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 using the MRM ratio.
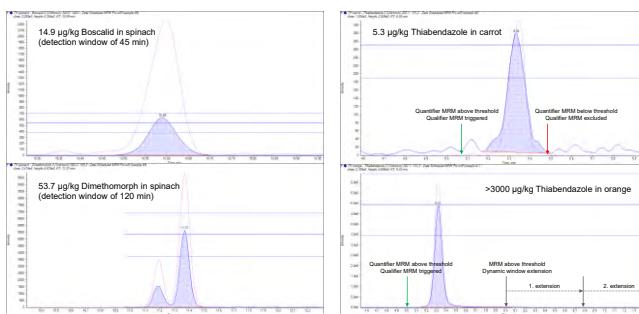
Figure 5. Examples of using the Flexible Window Width in a *Scheduled* MRM™ Pro method: the window for Boscalid was set to 45 sec and Dimethomorph was detected using a wider window to detect both isomers together

Figure 6. 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

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.

Example chromatograms of different type of food samples with detected compounds are presented in Figure 5. Quantitative results are presented in the Table below. All quantitative and qualitative results were automatically calculated in MultiQuant™ software (Figure 7).
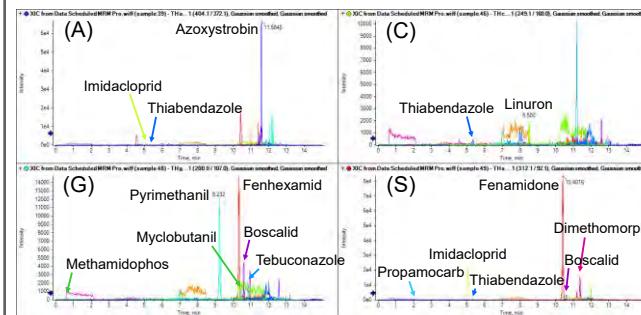
Figure 7. Detection of pesticides in filtered QuEChERS extracts of avocado (A), carrot (C), grapes (G), and spinach (S)
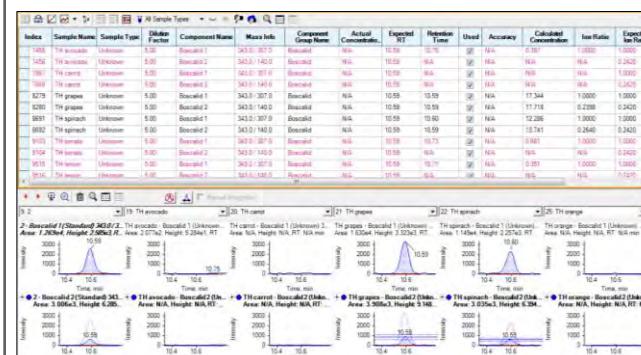
Figure 8. Quantitation and identification based on MRM ratios in MultiQuant™ software, the example shows the side-by-side peak review for Boscalid with positive findings in grapes and spinach samples

Sample	Pesticide	Concentration (µg/kg)	MRM Ratio (Expected Ratio)
Avocado	Azoxystrobin	55.0	0.146 (0.126)
	Imidacloprid	6.2	0.823 (0.818)
	Thiabendazole	2.9	1.035 (0.820)
Carrot	Linuron	14.3	0.613 (0.742)
	Thiabendazole	5.3	0.995 (0.820)
Grapes	Boscalid	17.3	0.240 (0.242)
	Fenhexamid	363	0.973 (1.053)
	Methamidophos	1.2	0.873 (0.698)
	Myclobutanil	14.2	0.811 (0.830)
	Pyrimethanil	687	0.482 (0.435)
	Tebuconazole	7.1	0.030 (0.261)
Orange	Imazalil	1830	0.282 (0.348)
	Thiabendazole	>3000	0.812 (0.820)
Spinach	Boscalid	12.3	0.264 (0.242)
	Dimethomorph	53.7	0.537 (0.541)
	Fenamidone	755	0.749 (0.672)
	Imidacloprid	217	0.907 (0.993)
	Propamocarb	3.1	0.260 (0.336)
	Thiabendazole	3.6	0.917 (0.820)

SUMMARY

The AB SCIEX Triple Quad™ 3500 system was used for multi-pesticide quantitation and identification in food samples. The developed method combined QuEChERS extraction, LC using a Phenomenex Kinetex™ Biphenyl column and selective and sensitive detection using the Scheduled MRM Pro algorithm. Compound identifcation was achieved using automatic calculation of MRM ratios.

REFERENCES

1 M. Anastassiades, et al.: J. AOAC Int. 86 (2003) 412-431
2 St. Lehotay: J. AOAC Int. 90 (2007) 485-520
3 J. Wong et al.: J. Agric. Food Chem. 58 (2010) 5897-5903
4 CSN EN 15662: (2008)

TRADEMARKS/LICENSING