Technical Overview

Robustness of the Agilent Ultivo Triple Quadrupole LC/MS for Routine Analysis in Food Safety

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Abstract

This Technical Overview demonstrates the robustness of an Agilent 1290 Infinity II LC system coupled to an Agilent Ultivo Triple Quadrupole LC/MS for the routine analysis of pesticides in food. Technological innovations within the Ultivo LC/MS result in an instrument with a much smaller footprint, while maintaining the performance found in many larger MS systems.

Robustness and reproducibility are shown over a 5-day period during which the Ultivo LC/MS system was exposed to more than 1,500 avocado extract samples spiked with pesticides. For the majority of analytes, we demonstrated less than 5 % peak area RSD for both the raw peak areas, and calculated concentrations of 20 ng/g down to the maximum residue level (MRL) of 10 ng/g.

Figure 1. Agilent Ultivo Triple Quadrupole LC/MS integrated into the Agilent 1290 Infinity II LC.

Introduction

Quantification of pesticides in food matrices is specifically challenging due to the presence of matrix components. These analyses require the use of sensitive, robust analytical techniques for routine day-in and day-out monitoring of contaminants. The most common analytical approach is to use triple (tandem) quadrupole mass spectrometers in multiple reaction monitoring (MRM) mode. However, the analytical performance of these systems can degrade over time as nonvolatile sample matrix components accumulate in the ion source and transfer optics. As a result, frequent cleaning may be required to maintain optimal performance.

Innovations such as VacShield, Cyclone Ion Guide, Vortex Collision Cell, Virtual Pre/Post-Filters, and Hyperbolic Quads maximize quantitative performance, and enhance instrument reliability and robustness, resulting in greater uptime. An Agilent Ultivo Triple Quadrupole LC/MS reduces user intervention for system maintenance, making it easy for the nonexpert MS user to operate and maintain.

Agilent MassHunter software simplifies data acquisition, method setup, data analysis, and reporting. Quant-My-Way, a new addition to MassHunter Quantitative analysis software, provides the option for users to streamline data analysis by customizing their unique workflows. This all results in the fastest acquisition-to-reporting time possible, increasing lab productivity and confidence.

This Application Note demonstrates the robustness of the Ultivo LC/MS for the quantification of common pesticides within a relatively complex food matrix.

Experimental

Reagents and Chemicals

All reagents and solvents were HPLC or LC/MS grade. Acetonitrile and methanol were purchased from Honeywell (Morristown, NJ, USA). Ultrapure water was produced with a Milli-Q Integral system equipped with a LC-Pak Polisher and a 0.22-µm point-of-use membrane filter cartridge (EMD Millipore, Billerica, MA, USA). LC/MS-grade formic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA). Submix 5 from the Agilent comprehensive pesticide mixture (p/n 5190-0551), and the basic checkout mix from the Agilent LC TOF/QTOF/QQQ Pesticide Test Mixture (p/n 5190-0469) were diluted with ACN to create 10 ppm pesticide working solutions for spiking the QuEChERS extracts.

Sample Preparation

Organic avocado was obtained from a local grocery store, and extracted according to the EN 15662 QuEChERS protocol using Agilent BondElut QuEChERS kits (p/n 5982-5650). The extracts were further cleaned with the Agilent Bond Elut EMR—Lipid dSPE kit (p/n 5982-1010), followed by a polishing step (p/n 5982-0102). The final extracts were diluted 2:5 with water, and spiked with pesticide working solutions in the following manner:

- Submix 5 was spiked into the total volume of QuEChERS extract at a final concentration of 20 ng/g.
- An aliquot of the submix 5-spiked extract was also spiked with the basic checkout mix at 100 ng/g, and further diluted to 50 ng/g, 20 ng/g, 10 ng/g, and 5 ng/g with the submix 5-spiked extract to create the calibration samples. QC samples were prepared in a similar manner to create 20 ng/g, 15 ng/g, and 10 ng/g levels.

Equipment

Separation was carried out using an Agilent 1290 Infinity II UHPLC system consisting of an Agilent 1290 Infinity II High Speed Pump (G7120A), an Agilent 1290 Infinity II Multisampler (G7167B), and an Agilent 1290 Infinity II Multicolumn Thermostat (G7116B). The UHPLC was coupled to an Agilent Ultivo Triple Quadrupole LC/MS equipped with an Agilent Jet Stream electrospray ionization source. Agilent MassHunter Acquisition (Ver. C.01.00), and Agilent MassHunter Quantitative Analysis (Ver B.08.00) software was used for data acquisition and analysis.

Results and Discussion

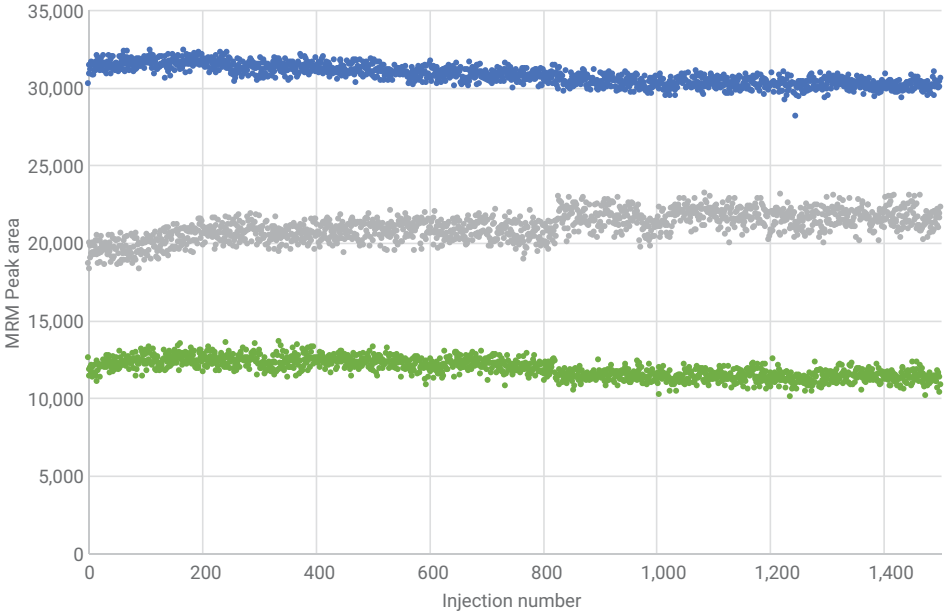
MRM Signal Stability

Figure 2 demonstrates the raw MRM signal response without correction or normalization for three compounds in the pesticide submix 5 spiked at 20 ng/g in avocado matrix. The data represent 1,500 injections acquired over a 5-day period of uninterrupted data acquisition. Excellent peak area stability was observed.

Instrument conditions

Agilent 1290 Infinity II LC System	
Column	Agilent Poroshell EC-C18, 2.1 × 50 mm, 2.7 µm
Guard column	Agilent Poroshell EC-C18, 2.1 × 5 mm, 2.7 µm
Column temperature	40 °C
Injection volume	5 µL
Multisampler temperature	4 °C
Needle wash	10 seconds in wash port (1:1 methanol/water)
Mobile phase	A) 0.1 % formic acid in water B) 0.1 % formic acid in acetonitrile
Flow rate	0.600 mL/min
Gradient program	Time A (%) B (%) 0.00 95 5 0.20 95 5 1.90 40 60 2.00 5 95 2.40 5 95 2.41 95 5 5.00 95 5

Agilent Ultivo Tandem Quadrupole Mass Spectrometer	
Ion source	Agilent Jet Stream ESI
Polarity	Positive
Gas temperature	300 °C
Drying gas flow (nitrogen)	11 L/min
Sheath gas temperature	400 °C
Sheath gas flow (nitrogen)	12 L/min
Nebulizer pressure (nitrogen)	35 psi
Capillary voltage	3,500 V
Nozzle voltage	0 V
Scan type	MRM
Q1/Q2 resolution	Unit (0.7 amu)
Total number of MRMs	38
Dwell time	12 ms
Divert valve	0–0.85 minutes to waste 0.85–5.00 minutes to MS



Compound	%RSD (n = 1,500)
Carbendazim	2.1
Methomyl	4.2
Diuron	5.1

Figure 2. MRM peak area stability for three compounds over 1,500 injections.

Reproducibility of Quantification

Routine laboratories are most concerned with accurate quantification over large worklists of samples. As a common practice, calibration curves are run intermittently between sample groups to account for instrument drift. To best emulate a similar injection worklist, Figure 3 depicts the experimental design for 34 sample groups, where each group contains a calibration curve and three QC levels. Table 1 shows the RSD values for calculated concentrations of 10 pesticides at three QC levels (20 ng/g, 15 ng/g, and 10 ng/g) spanning the 1,530 injections. The 10 ng/g level (MRL for most pesticides) had an average compound %RSD of 3.0, based on the calculated concentration. Note that the calculated concentration %RSD values only marginally improved the raw peak area %RSD values, attesting to the excellent peak area stability previously described.

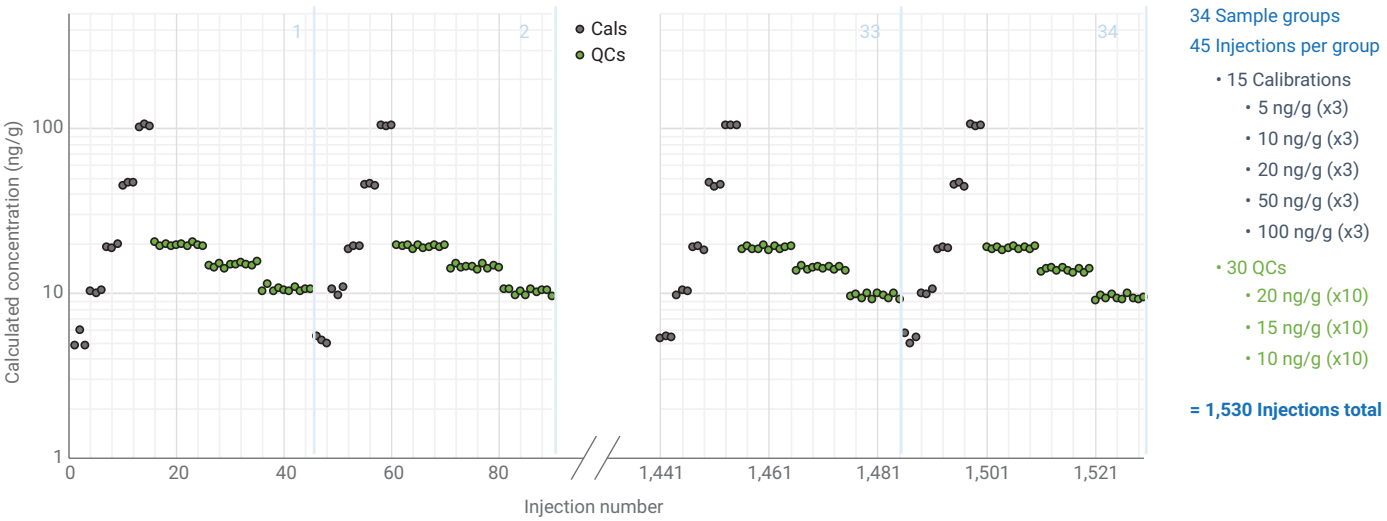


Figure 3. Experimental design for quantification repeatability. Calculated concentrations for thiabendazole are plotted for sample groups 1–2 (first 90 injections) and 3–4 (last 90 injections).

Table 1. Reproducibility of MRM peak area and calculated concentration values for three QC levels spanning 1,530 injections.

	Raw area RSD (n = 340 each level)			Calculated concentration RSD (n = 340 each level)		
Compound	10 ng/g	15 ng/g	20 ng/g	10 ng/g	15 ng/g	20 ng/g
Thiabendazole	4.7 %	4.6 %	4.4 %	4.3 %	3.4 %	2.4 %
Imazapyr	5.6 %	5.7 %	5.2 %	2.1 %	1.9 %	1.7 %
Dimethoate	3.4 %	3.5 %	3.4 %	3.0 %	3.0 %	2.8 %
Metoxuron	3.0 %	2.9 %	3.0 %	2.7 %	2.8 %	2.8 %
Imazalil	1.9 %	1.7 %	1.6 %	1.8 %	1.5 %	1.4 %
Carbofuran	3.2 %	3.2 %	3.1 %	2.6 %	2.5 %	2.4 %
Atrazine	3.1 %	3.2 %	2.8 %	1.5 %	1.6 %	1.4 %
Metosulam	3.5 %	3.3 %	3.0 %	3.0 %	2.8 %	2.5 %
Metazachlor	3.7 %	6.5 %	3.4 %	2.1 %	5.7 %	2.0 %
Molinate	8.0 %	6.9 %	6.2 %	6.5 %	5.2 %	4.8 %
Average	**4.1 %**	**4.1 %**	**3.6 %**	**3.0 %**	**3.1 %**	**2.4 %**

Figure 4 shows repeatability of calibration consistency. The R^2 values for most of the calibration curves were better than 0.99 with linear fitting and 1/x weighting.

Conclusion

The food safety industry requires robust analytical platforms for the routine, high-throughput analysis of pesticides in complex food matrices.

This Application Note demonstrates that the Agilent 1290 Infinity II UHPLC coupled to the Agilent Ultivo Triple Quadrupole LC/MS:

- Exhibits low MRM peak area variation over 5 days of uninterrupted data acquisition
- Maintains quantification accuracies at low-level pesticide limits specified by regulatory agencies

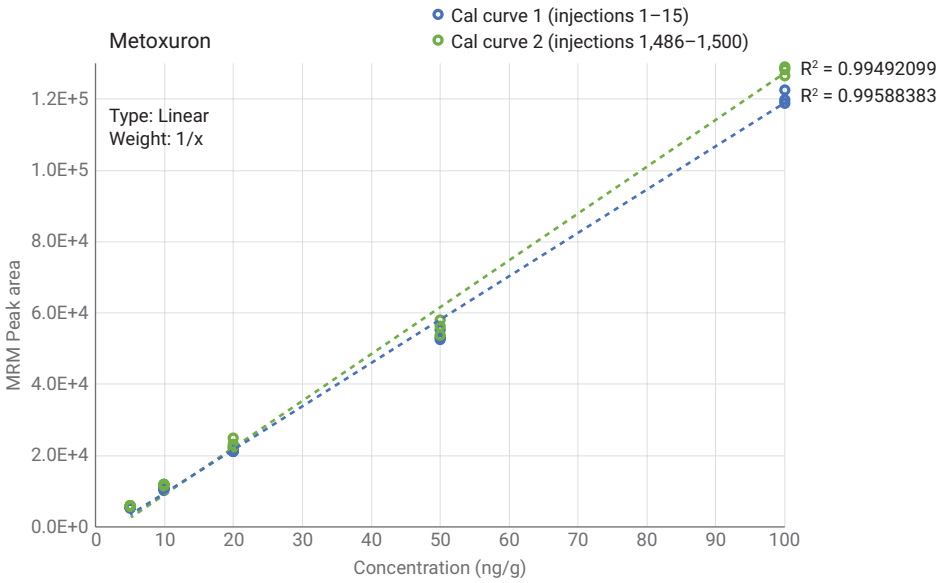


Figure 4. Demonstration of accuracy stability. Shown is an overlay of two metoxuron calibration plots corresponding to the first and last sample groups of the 1,530 injections of pesticide-spiked avocado extract.

References

1. A Robustness Study for the Agilent 6470 LC-MS/MS Mass Spectrometer, *Agilent Technologies Application Note*, publication number 5991-8004.
2. Robustness of an Agilent 6470 Triple Quadrupole Mass Spectrometer for Analysis of Pharmaceuticals in Plasma Matrices, *Agilent Technologies Application Note*, publication number 5991-5953.

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