Application Note

Multiresidue Pesticides Screening and Quantitation in Complex Food Matrices Using an Agilent Ultivo Triple Quadrupole LC/MS

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Abstract

This Application Note describes a UHPLC/MS/MS-based multiresidue method for the determination of more than 250 pesticides and their metabolites in food samples. The method benefits from the:

- Increased chromatographic resolution of the Agilent 1290 Infinity II UHPLC system
- Versatile ionization capabilities of the Agilent Jet Stream ionization source
- Innovations housed in the Agilent Ultivo triple quadrupole LC/MS system that reduce instrument footprint, yet maintain analytical sensitivity

The method has been applied to the analysis of pesticide residues in complex matrices such as avocado and black tea.

Our results demonstrate that most of the compounds can be accurately quantified below maximum residue limits (MRLs) in matrices with excellent precision. The Ultivo LC/TQ is an ideal fit-for-purpose choice for routine pesticide residue testing due to its much smaller footprint and ease-of-maintenance.

Introduction

The screening and quantitation of pesticide residues in food is one of the most important and most demanding applications in food safety. Regulatory agencies have set maximum residue limits (MRLs) for hundreds of pesticides and their metabolites in food[1]. Most MRLs are set at low part-per-billion (ng/g) levels, posing significant challenges to screen and quantify hundreds of analytes in complex matrices simultaneously. Therefore, reliable analytical instruments and methods are required to quantify pesticide residues with confidence. Typically, multiresidue methods based on LC/MS/MS or GC/MS/MS are used. The criteria for the identification of pesticide residues and requirements for method validation and quality control procedures for quantitation are specified in guidance documents such as SANTE/11813/2017[2].

The Agilent Ultivo triple quadrupole LC/MS is designed to address many challenges faced by routine production labs, especially in the environmental and food safety arenas. Innovative technologies within the Ultivo LC/TQ allowed us to reduce its overall footprint, while maintaining the comparable performance level of much larger MS systems. Innovations such as the Cyclone Ion Guide, Vortex Collision Cell, and Hyperbolic Quads not only maximize quantitative performance in a small package, but also enhance instrument reliability and robustness, promoting greater uptime. With VacShield, the Ultivo reduces the need for user intervention for system maintenance, making system operation and maintenance manageable for nonexpert users. Agilent MassHunter Software simplifies data acquisition, method setup, data analysis, and reporting, resulting in the fastest acquisition-to-reporting time, increasing lab productivity.

This Application Note demonstrates the screening and quantitation of 251 pesticides in food samples. An Agilent 1290 Infinity II UHPLC system was coupled to the Ultivo LC/TQ system operated with dynamic MRM mode with fast polarity switching.

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 using 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). Formic acid was from Fluka (Sigma-Aldrich Corp., St. Louis, MO, USA), and ammonium formate solution (5 M) was from Agilent (p/n G1946-85021). Pesticides were included in the Agilent comprehensive pesticide mixture (p/n 5190-0551). The eight submixes of the comprehensive pesticide mixture were combined and further diluted with acetonitrile to a final pesticide working solution containing 251 pesticides at a concentration of 10 µg/mL. This solution was used for spiking the QuEChERS extracts. Six calibration samples in a concentration range from 1 ng/g to 100 ng/g were prepared (1, 5, 10, 20, 50, and 100 ng/g).

Sample Preparation

Organic black tea, avocado, broccoli, and orange were obtained from local grocery stores. Samples were extracted according to the citrate buffered QuEChERS protocol using Agilent BondElut QuEChERS kits (p/n 5982-5650). Ten grams of homogenized fruit and vegetables or 2 g of tea were weighed into 50-mL polypropylene tubes and extracted with 10 mL of acetonitrile while shaking vigorously. Prior to extraction, the tea samples were wetted with 8 mL of ultrapure water. Raw extract of orange was cleaned up by dispersive SPE (dSPE) containing primary secondary amine (p/n 5982-5058). Broccoli extract was cleaned using the dSPE kit, which uses graphitized carbon for pigment removal (p/n 5982-5256). Black tea extract was cleaned using the dispersive kit containing graphitized carbon for high-pigment samples (p/n 5982-5356CH). Avocado extract was cleaned using Agilent EMR—Lipid (p/n 5982-1010) followed by a polishing step (p/n 5982-0101). Nonorganic broccoli, orange, and avocado extracts were prepared in the same way as their organic counterparts.

Instruments

Separation was carried out using an Agilent 1290 Infinity II UHPLC system consisting of:

- Agilent 1290 Infinity High Speed Pump (G7120A)
- Agilent 1290 Infinity II Multisampler with sample cooler (G7167B)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)

The UHPLC system was coupled to an Agilent Ultivo triple quadrupole LC/MS system equipped with an Agilent Jet Stream electrospray ionization source.

Method

Table 1 summarizes the 1290 Infinity II UHPLC conditions. Table 2 lists the Ultivo Triple Quadrupole parameters and Agilent Jet Stream (AJS) ESI source parameters. Analysis was carried out with positive and negative electrospray ionization with dynamic multiple reaction monitoring (dMRM).

Data were evaluated using Agilent MassHunter Quantitative Analysis Software B.09 with the Quant-My-Way feature. Linear calibration curves were generated using 1/x weighting.

Table 1. Agilent 1290 Infinity II UHPLC parameters.

Parameter	Value
Column	Agilent ZORBAX RRHD Eclipse Plus C18 3.0 × 150 mm, 1.8 µm (p/n 959759-302)
Column temperature	45 °C
Injection volume	2 µL
Autosampler temperature	6 °C
Mobile phase	A) 4.5 mM ammonium formate + 0.5 mM ammonium fluoride + 0.1 % formic acid in water B) 4.5 mM ammonium formate + 0.5 mM ammonium fluoride + 0.1 % formic acid in methanol
Flow rate	0.45 mL/min
Gradient program	Time (min) B % 0 2 0.5 2 1 50 4 65 16 100 18 100 18 1 2
Stop time	20
Post time	1.5

Table 2. Agilent Ultivo Triple Quadrupole and Agilent Jet Stream source parameters.

Parameter	Value
Ion mode	Positive and negative ESI with Agilent JetStream
Scan type	Dynamic MRM
Drying gas temperature	250 °C
Drying gas flow	11 L/min
Sheath gas temperature	350 °C
Sheath gas flow	12 L/min
Nebulizer pressure	40 psi
Capillary voltage	3,500 (pos/neg)
Nozzle voltage	300 V (pos); 1,000 V (neg)
Cycle time	650 msec
Total MRM	883
Minimum dwell time	5.45 ms
Maximum dwell time	215.84 ms
MS1 and MS2 resolution	Unit

Results and Discussion

Method Sensitivity

The AJS ionization source parameters were optimized using Agilent MassHunter Source Optimizer software to obtain the highest abundance for most target compounds. Compound-dependent parameters such as fragmentor voltage and collision energy were transferred from an existing method on an Agilent 6460 LC/TQ without further optimization to the Ultivo LC/TQ for this study.

Figure 1 illustrates that the signal response for 251 pesticides in black tea extract at 5 ng/g was quite good. Most of the compounds were detected at this concentration, which corresponds to ½ MRL or 1 ng/mL. The innovations housed within the Ultivo are critical for sensitive detection.

Due to the good signal response, the majority of the compounds were detected at 1 ng/g in matrices (corresponding to 1/10 MRL) with an accuracy of 80–120 % for at least four replicates. Additional analytes could be detected at higher concentrations (Figure 2). One compound was detected in both positive and negative ionization modes, and counted as two individual measurements.

Method Precision

The Cyclone Ion Guide, Vortex Collision Cell, and Hyperbolic Quads make the ion transmission consistent across a broad *m/z* range. This consistency renders excellent analytical precision. Six injections were carried out at each concentration level. The %RSD was calculated for each compound at the lowest concentration at which it could be quantified with an accuracy of 80–120 % for at least four out of six replicates. Most compounds had a %RSD <10 % (Figure 3).

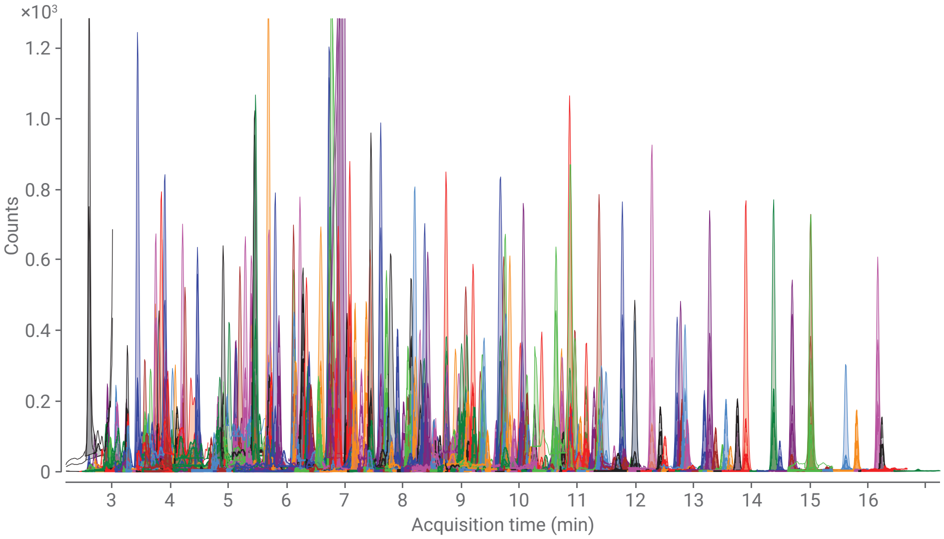

Figure 1. Chromatograms of 251 pesticides spiked into black tea at 5 ng/g (corresponding to a concentration of 1 ng/mL).

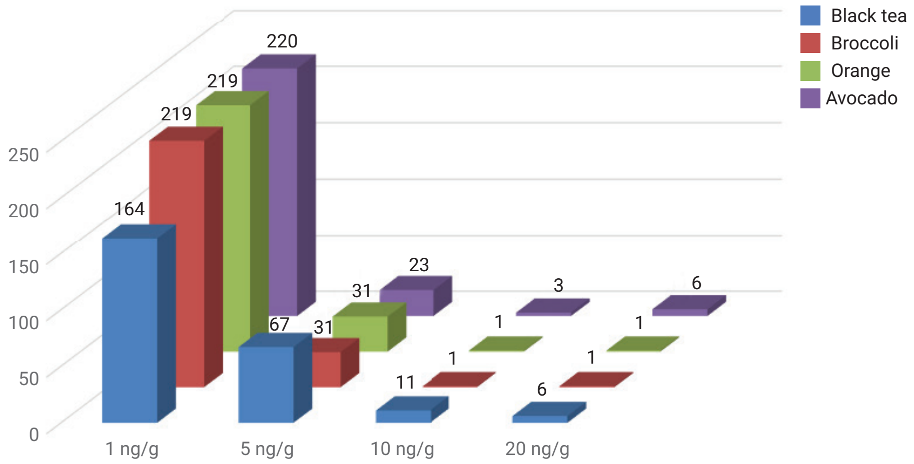

Figure 2. The number of compounds that could be accurately quantified at 1 ng/g. Additional compounds could be quantified at higher concentrations.

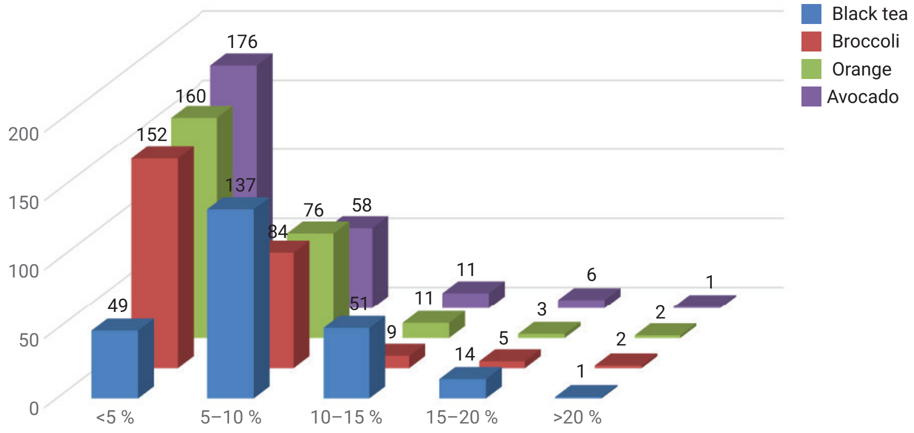

Figure 3. The %RSD distribution in different matrices. The %RSD was calculated at the lowest concentration at which the analytes could be accurately quantified.

Real World Samples

Nonorganic avocado, broccoli, and orange samples were processed in the same way as their organic counterparts. Most of the calibration curves had a R^2 >0.99, allowing accurate quantitation of samples. No pesticides could be detected in avocado, while three and seven pesticides were detected in orange and broccoli, respectively, at or above the MRL (Figures 4 and 5). Dimethomorph, azoxystrobin, and mandipropamid in broccoli were detected at concentrations exceeding the range of the calibration curve, at concentrations of 592 ng/g, 493 ng/g, and 154 ng/g, respectively. The dotted lines indicate the expected qualifier range according to calibration standards in the graphs of Figures 4 and 5. All pesticides in the samples were detected with high confidence. It is evident that some contaminated foods have reached consumers. It is a daunting task for industry and food testing labs to work together to safeguard our foods through technological innovations.

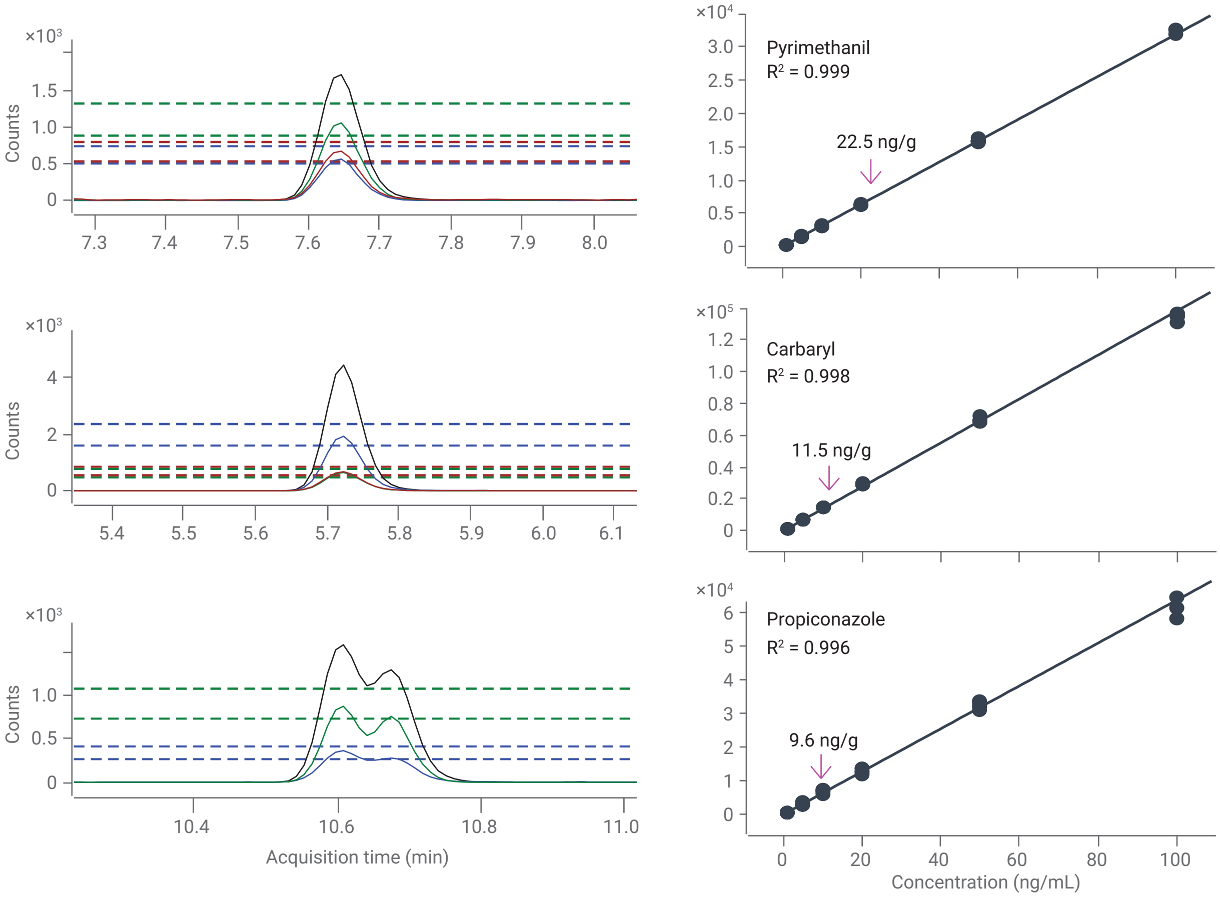

Figure 4. Pesticides detected at or above MRLs in nonorganic orange

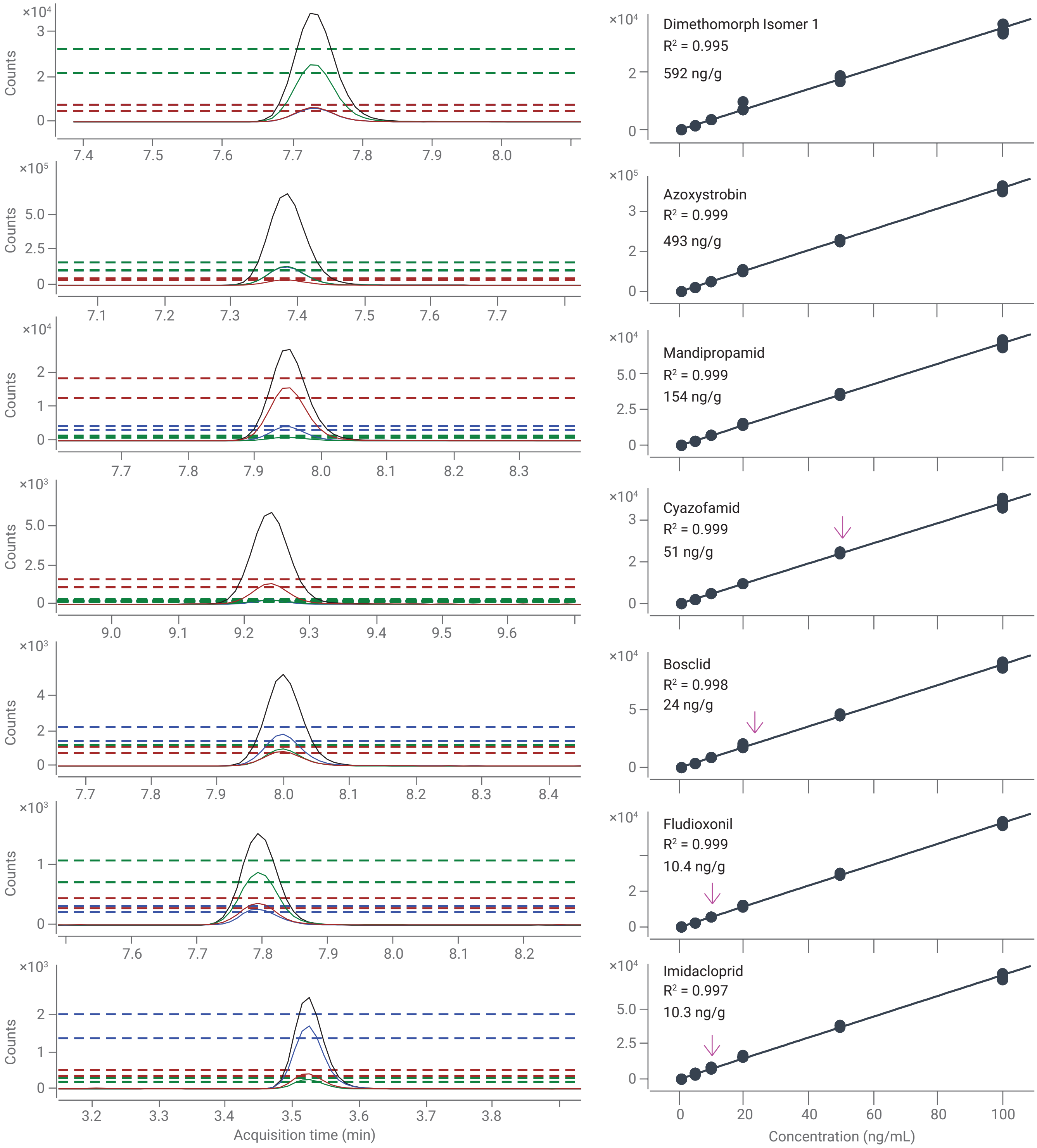

Figure 5. Pesticides detected at or above MRLs in nonorganic broccoli.

Conclusions

An Agilent Ultivo Triple Quadrupole LC/MS system fits the purpose of multiresidue pesticides analysis in complex food matrices. Technological innovations within the UltivoTriple Quadrupole LC/MS system provide the robust detection and easy maintenance that suits routine production labs with enhanced capabilities for nonexpert LC/MS users. Quant-My-Way streamlines data analysis based on users' needs, and results in faster sample-to-report time. Moreover, Agilent total workflow solutions that include sample preparation, databases, and reporting facilitate fast method development and validation in food safety analyses.

References

1. Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin (including amendments as of 18 March 2008) and complying with regulation (EC) 1107/2009.
2. Guidance document on analytical quality control and method validation procedures for pesticides residues and analysis in food and feed, SANTE/11813/2017, 21-22 November 2017. https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2017-11813.pdf

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