Application Note

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Method Development and Evaluation for Multiresidue Pesticide analysis in Foods Using the 6475 Triple Quadrupole LC/MS System

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Abstract

This application note describes the development and evaluation of a comprehensive LC/MS/MS method for over 500 pesticide analyses in three food matrices (wheat, olive oil, and black tea). The work was completed using the Agilent 6475 triple quadrupole LC/MS (LC/TQ) system coupled with the Agilent 1290 Infinity II Bio LC system and MassHunter Workstation 12.0.

The Agilent 6475 triple quadrupole LC/MS instrument contains hardware and software improvements to the legacy systems:

- Artificial Intelligence (AI)-based tuning and calibration
- Active system monitoring with early maintenance feedback (EMF)
- Reflexive reinjection logic with intelligent reflex (iReflex)
- Maintenance and method development automation
- Enhanced MS1 and MS2 resolution with narrow isolation mode
- Adherence to 21 CFR Part 11 and Annex 11 compliance guidelines

Introduction

Pesticides are integral for protecting crops and are necessary in most growing environments to obtain high product yields. Screening and reporting levels of pesticide residues remaining in or on commodities are required by many regulatory bodies, e.g., the US-EPA and the European Commission.[1,2] Major challenges for pesticide analysis in food include many pesticides from various compound classes, diverse complex food matrices, matrix effects, low concentrations of target analytes, and so on.

This application note describes the development and evaluation of an LC/MS/MS method for over 500 pesticides and pesticide metabolites quantitation in three food matrices. These matrices include wheat (high starch content), olive oil (high oil content), and black tea (difficult matrix). The analysis was performed using the Agilent 6475 triple quadrupole LC/MS system (LC/TQ) coupled with Agilent MassHunter Workstation software 12.0. An Agilent 1290 Infinity II Bio LC system and an Agilent Jet Stream (AJS) electrospray ion source were used with the LC/TQ system. The instrument was operated in a dynamic multiple reaction monitoring (dMRM) mode.

Matrix-matched calibration curves were generated using food extract samples postspiked with pesticide standards from 0.1 to 50 µg/L. Matrix effects on analyte response were studied at 10 µg/kg, showing that most analytes were recovered within 70 to 120%. The system robustness was also tested using replicate injections (n = 300) of black tea extract spiked at 10 µg/kg. All the results demonstrate the excellent analytical sensitivity, precision, accuracy, and robustness of the 6475 LC/TQ system for food analysis.

Experimental

Reagents and standards

Pesticide standards include standard mixtures purchased from Agilent (part number 5190-0551 and part number CUS-00004663), and over 200 single pesticide standard solutions purchased from Agilent or AccuStandard. Organic wheat, olive oil, and black tea were purchased from a local grocery store.

All reagents and solvents were HPLC or LC/MS grade. 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).

Sample preparation

All the pesticide standards were combined and diluted with acetonitrile to a final working solution containing more than 500 pesticides at a concentration of 1 µg/mL (1 ppm). This solution was used for spiking food extracts.

For wheat extract preparation, 2 g of wheat powder were wet in 8 mL of water, vortexed and soaked for 15 minutes. For black tea extract preparation, 2 g of tea samples were wetted with 8 mL of water and incubated for 2 hours at room temperature. Then 10 mL of acetonitrile was added into wheat and black tea samples, respectively. Each sample tube was vortexed for 1 minute. For olive oil extract preparation, 5 grams of olive oil was vigorously vortexed with 10 mL acetonitrile for 2 minutes. Then, 6 mL of water was added and further vortexed for 2 minutes. One pouch of Agilent EN extraction salts and one ceramic homogenizer (part number 5982-5650CH) was added into each sample extract tube. The tubes were vigorously shaken for 10 minutes and subjected to centrifugation at 3,000xg for 5 minutes at 4 °C. Supernatants from the three food samples were collected, respectively.

Wheat supernatants were cleaned with the universal dispersive SPE (dSPE) tubes (part number 5982-0028). Black tea supernatants were cleaned with the Agilent QuEChERS dSPE for high pigment EN (part number 5982-5356). Olive oil supernatants were cleaned with the Captiva EMR–Lipid 6 mL cartridges (part number 5190-1004) under mild pressure.

The final extracts were spiked with the pesticide working solutions at nine concentrations ranging from 0.1 to 50 µg/L to generate matrix-matched standard samples. The calibration curves were established considering the dilution factor of 1:5 introduced for wheat and black tea extract, and the dilution factor of 1:2 for olive oil extract. The final levels of calibration curves were from 0.5 to 250 µg/kg for wheat and black tea extract, and the levels were from 0.2 to 100 µg/kg for olive oil extract. All matrix-matched standard samples were prepared immediately before injection and measured with six technical replicates.

In addition, the pesticide working solutions were also spiked into acetonitrile at corresponding levels for matrix effect analysis. A bulk of black tea extract spiked with pesticide standards at 10 µg/kg was also prepared for system robustness analysis.

Equipment

Sample separation was performed using the Agilent 1290 Infinity II Bio LC system consisting of the following modules:

- 1290 Infinity II Bio High-Speed Pump (G7132A)
- 1290 Infinity II Bio Multisampler with thermostat (G7137A)
- 1290 Infinity II Multicolumn Thermostat (G7116B)

The LC system was coupled to the Agilent 6475 triple quadrupole LC/MS (G6475AA) equipped with the Agilent Jet Steam electrospray ion source (G1958-65638). Agilent MassHunter Workstation software 12.0 was used for data acquisition.

Methods

The LC/MS conditions and parameters are provided in Table 1 and 2. MRMs for pesticides were either transferred from a method previously optimized on the 6470 LC/TQ, or newly optimized with the new optimizer on the 6475 LC/TQ. For calibration curve analysis, linear fitting with origin ignored and 1/x weighting was used.

Results and discussion

Method transfer

To develop a large panel of pesticide screening method, we started from an existing pesticide screening method in the lab, which was previously developed on the 6470 LC/TQ system. The method was directly opened and executed on the new 6475 LC/TQ system with MassHunter 12.0 software. To examine if the original MRM parameters for each analyte including fragmentor voltage and collision energy need reoptimization, the dMRM method was reoptimized on the 6475 LC/TQ system. The two methods (before and after reoptimization) were performed and compared by overlaying their total ion chromatograms (TICs). Figure 1 shows almost identical chromatograms acquired from the two methods, demonstrating easy method transfer from Agilent legacy instruments to the 6475 LC/TQ, which does not need refinement.

Chromatography

Table 1. 1290 Infinity II Bio LC method.

1290 Infinity II Bio LC System	
Parameter	**Value**
Column	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 150 mm, 1.8 µm (part number 959759-902)
Sampler Temperature	4 °C
Mobile Phase	A) 5 mM ammonium formate + 0.1% formic acid in water B) 5 mM ammonium formate + 0.1% formic acid in methanol
Flow Rate	0.4 mL/min
Injection Volume	2 µL
Gradient Program	**Time (min) B (%)** 0.00 5 3.00 30 17 100 20.00 100
Post Time	3 min

Mass spectrometry

Table 2. LC/TQ parameters.

6475 Triple Quadrupole Mass Spectrometer	
Parameter	**Value**
Ion Source	Agilent Jet Stream (AJS) source
Polarity	Positive and negative
Gas Temperature	200 °C
Drying Gas	11 L/min
Nebulizer	35 psi
Sheath Gas	350 °C
Sheath Gas Flow	12 L/min
Capillary Voltage	+3,500 V, -3,000 V
Nozzle Voltage	0 ±V
MS1/MS2 Resolution	Unit/Unit
Cycle Time	500 ms
Total MRMs	1,003
Min/Max Dwell	1.12 ms/248.28 ms

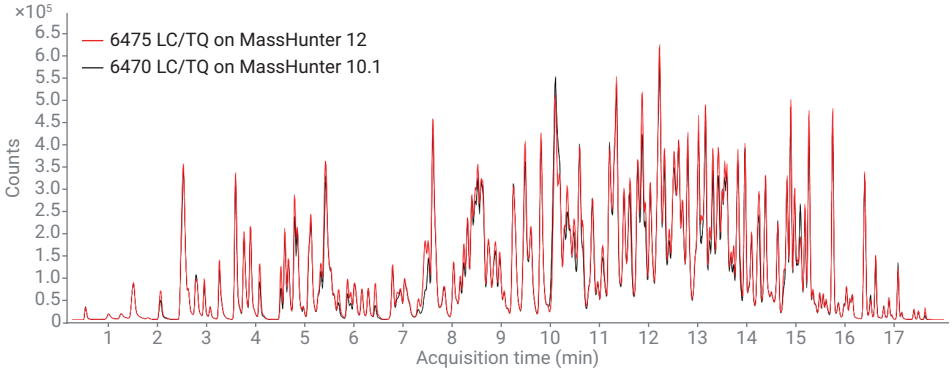

Figure 1. Overlaid TICs acquired by pesticide screening methods optimized on 6470 and 6475 LC/TQ, respectively.

Development of dynamic MRM method

To further extend the pesticide screening method, new pesticide compounds were added and optimized on the 6475 LC/TQ system. In MassHunter 12 software, a new optimizer option is available for compound optimization. This feature can resolve simple compound mixture and multiplex the compound optimization process based on chromatography separations on the analytical column. If multiple chromatography peaks were detected for one targeted precursor, e.g., isomers, isobaric compounds, or background interference, those peaks will be labeled with their corresponding retention time (RT) and optimized independently (Figure 2). In this study, pesticide standard mixtures containing new compounds were optimized using this new option to ensure more accurate MRM optimization. The ion source parameters were optimized with the new source optimizer in MassHunter 12, which provides an enhanced weighted optimization algorithm.

The final developed dMRM method contains 1,003 MRM covering over 500 pesticides (Figure 3). The LC gradient was left unchanged to maintain data analysis consistency in the lab, which led to many concurrent MRMs with RT approximately 13 minutes.

Figure 4 shows the overlaid MRM chromatograms for all the targeted pesticides postspiked at 1 µg/L in wheat matrix extract using the final comprehensive dMRM method. Most compounds were baseline separated showing symmetric sharp peaks. High MS signal counts were observed for most pesticides even for the compounds acquired with short dwell time (<2 ms). All these results demonstrate the high analytical sensitivity of the 6475 LC/TQ system for complex food analysis.

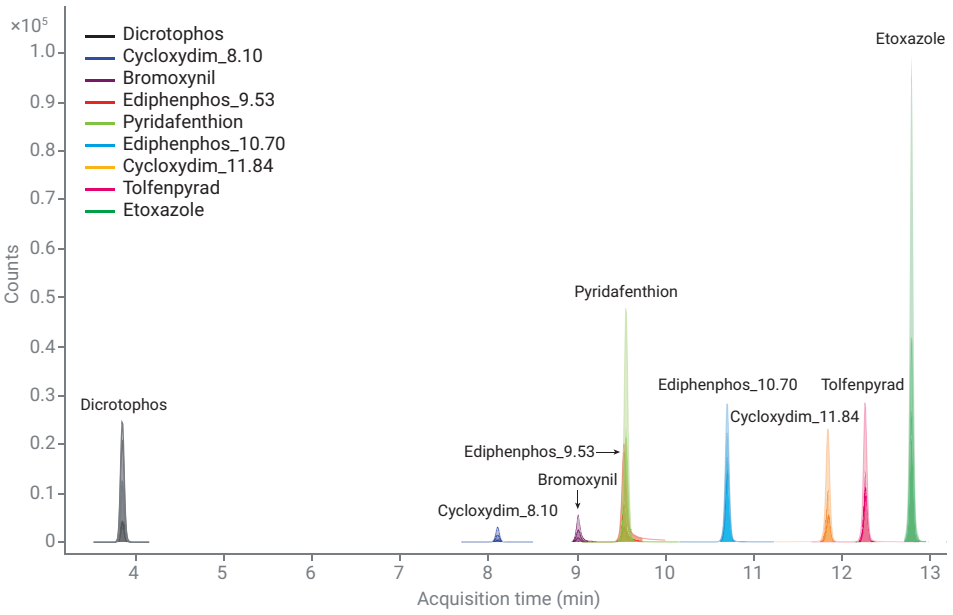

Figure 2. Representative MRM chromatograms acquired with a method optimized with a compound mixture using the new optimizer software.

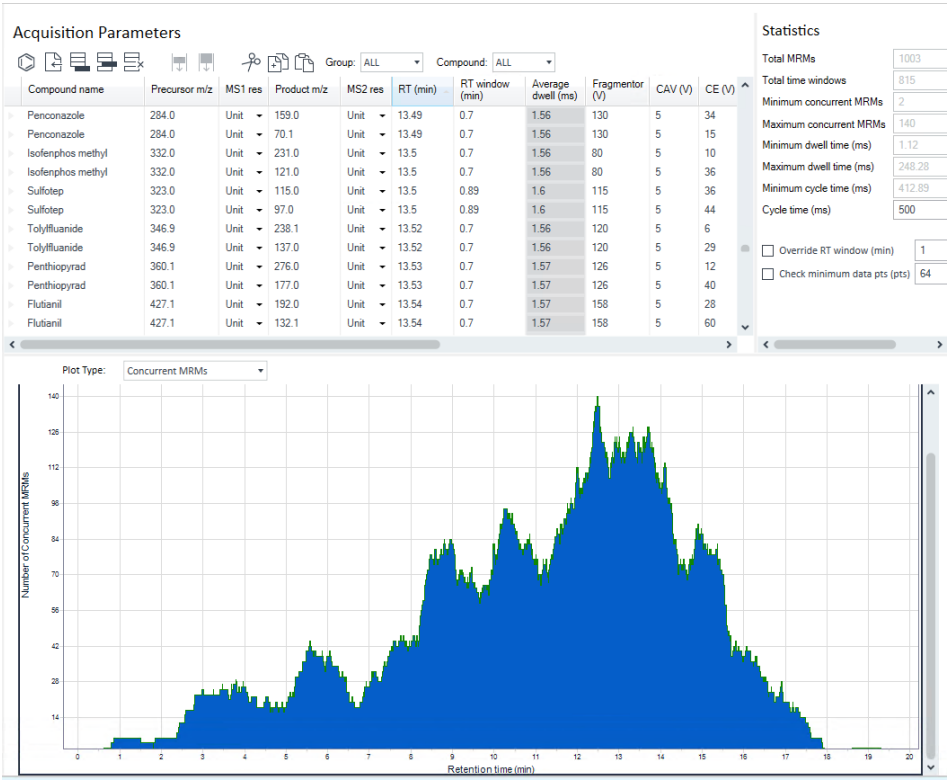

Figure 3. Comprehensive pesticide dMRM method shown in MassHunter 12 illustrating efficient management of more than 1,000 MRMs.

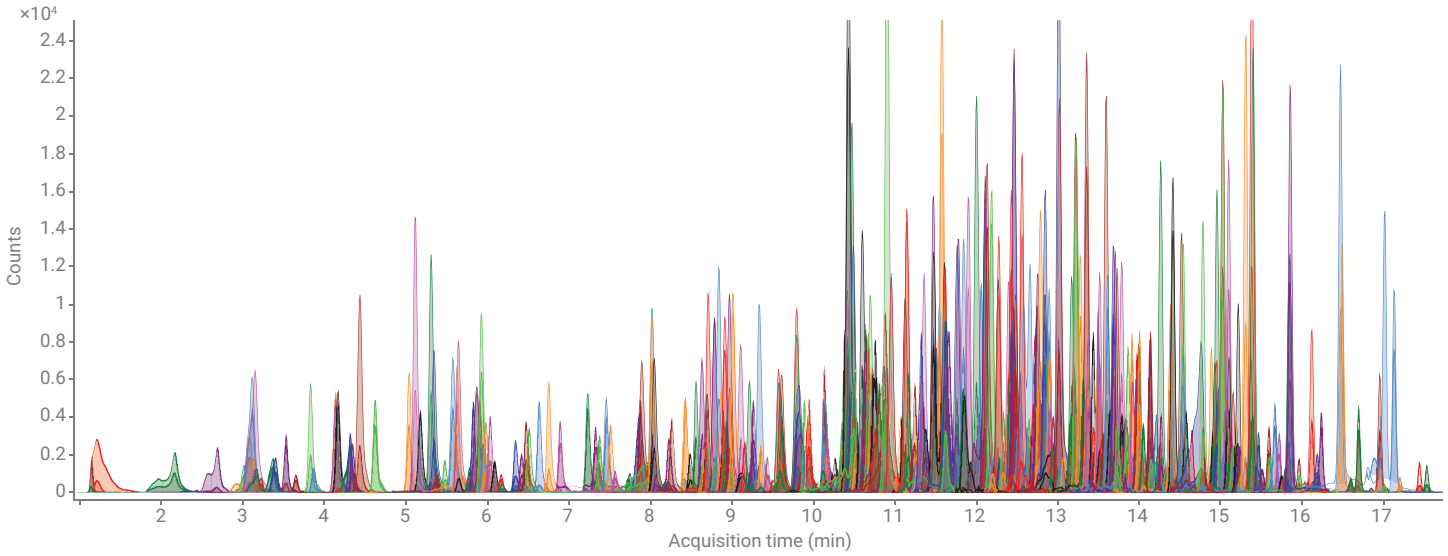

Figure 4. Overlaid MRM chromatograms of all targeted pesticides spiked at 1 µg/L in wheat matrix extract.

Calibration curve analysis

The multiresidue pesticide measurements were evaluated in wheat, olive oil, and black tea matrix by generating matrix-matched calibration curves with replicate injections (n = 6) for each standard sample.

Quantitation performance was summarized:

- Excellent linearity for matrix-matched calibration curves within the tested levels: correlation coefficients (R^2) were higher than 0.99 for 92% pesticides in wheat, 94% pesticides in olive oil, and 92% pesticides in black tea.
- Excellent quantification sensitivity: most pesticides show a lower limit of quantification (LLOQ) below the default MRL of 10 µg/kg in the three food matrices as shown in Figure 5 (LLOQs defined as the lowest tested level with accuracy within 80 to 120% and RSD <20%).[3]
- Calibration curves of four representative pesticides in black tea are shown in Figure 6. The curves include two pesticides in positive polarity (sulfotep and penthiopyrad) and two pesticides in negative polarity (flubendiamide and fipronil sulfone), which were all acquired with low average dwell time (<2 ms).

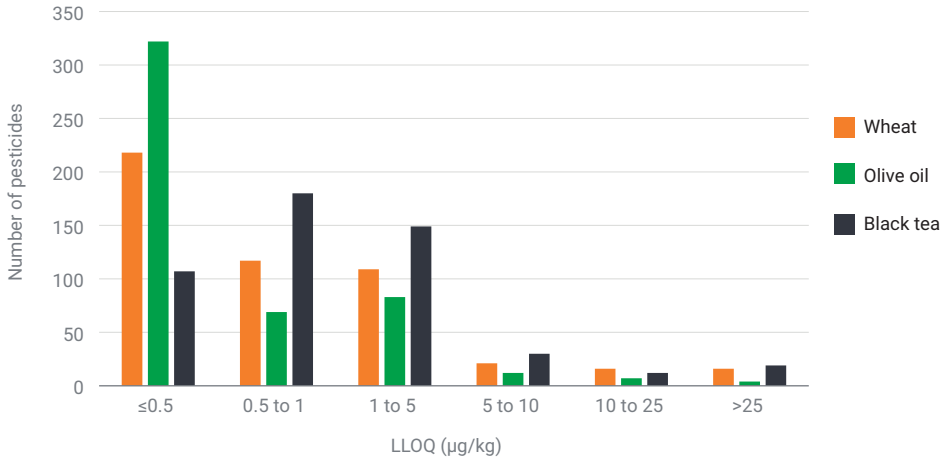

Figure 5. Distribution of LLOQs for pesticides spiked in wheat, olive oil and black tea.

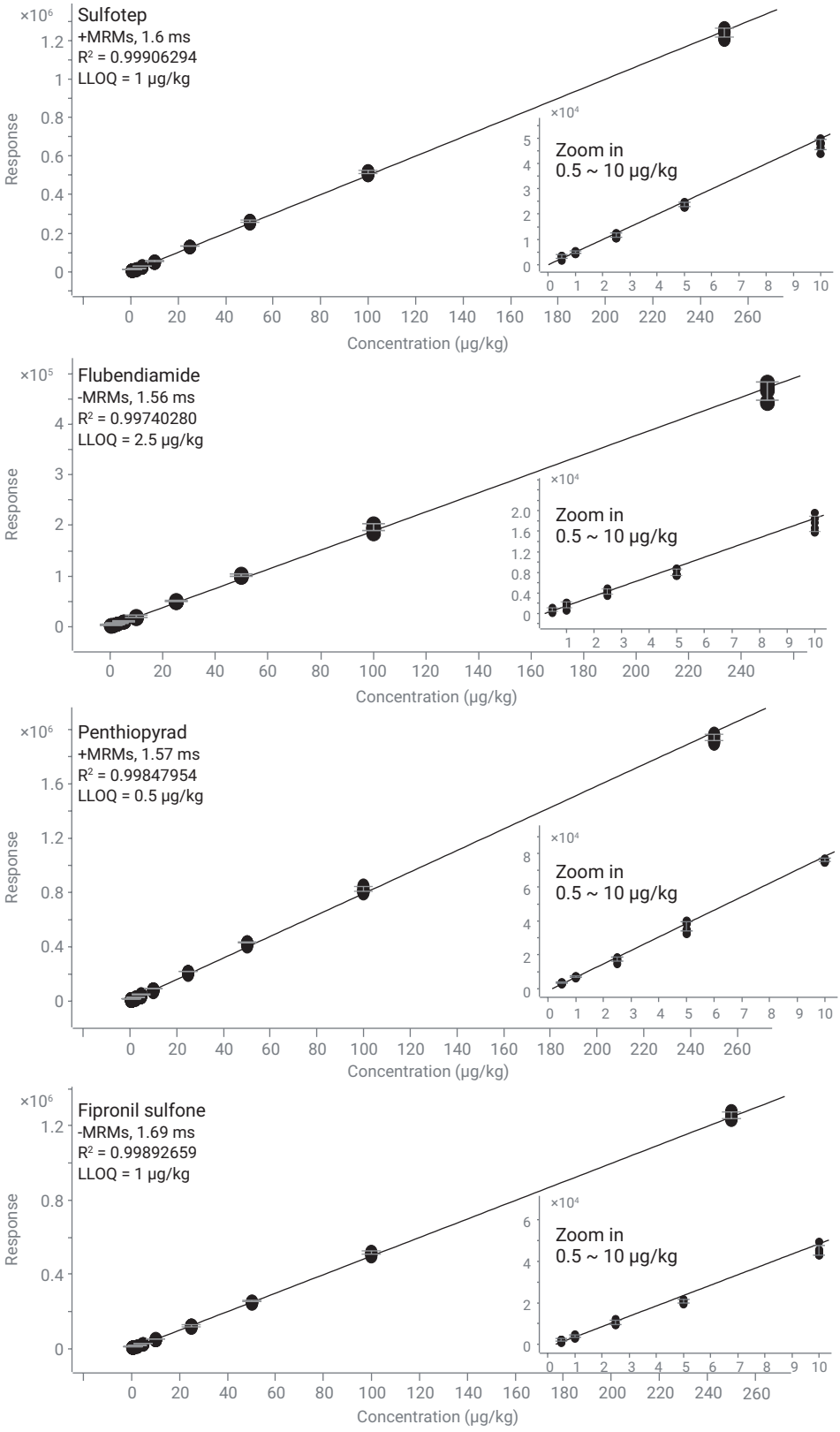

Figure 6. Calibration curves of four representative pesticides spiked in black tea extract.

Recoveries in food matrices

To evaluate matrix effects (ion suppression or enhancement), recoveries were calculated by comparing the response of pesticides in the matrix against the corresponding response in neat solvent. The comparison was done at the default MRL of 10 µg/kg (Figure 7). The majority of pesticides demonstrate signal recovery between 70 to 120%, which is the acceptable criteria suggested by the SANTE method validation guideline.[1]

Robustness test

To test the robustness of the 6475 LC/TQ system, replicate injections (n = 300) of black tea matrix spiked with pesticide standards at 10 µg/kg were carried out using the comprehensive pesticide LC/MS/MS method. The TICs of every 50 injections were overlaid showing excellent reproducibility in terms of both LC separation and overall MS signal response (Figure 8A). The instrument ran continuously for about 115 hours (approximately 5 days). Also, four representative pesticides were selected to evaluate MS signal stability. The evaluation included one early eluting pesticide (cyromazine, RT = 1.25 min) and three pesticides (penthiopyrad, fipronil sulfone, and penconazole) eluting within the time window with the most concurrent MRMs. Figure 8B shows MS responses for these four pesticides with their %RSD ranging from 3.7 to 5.2% (n = 300), including both positive and negative MRMs. All these results demonstrate the outstanding robustness of the 6475 LC/TQ system for complex food sample analysis.

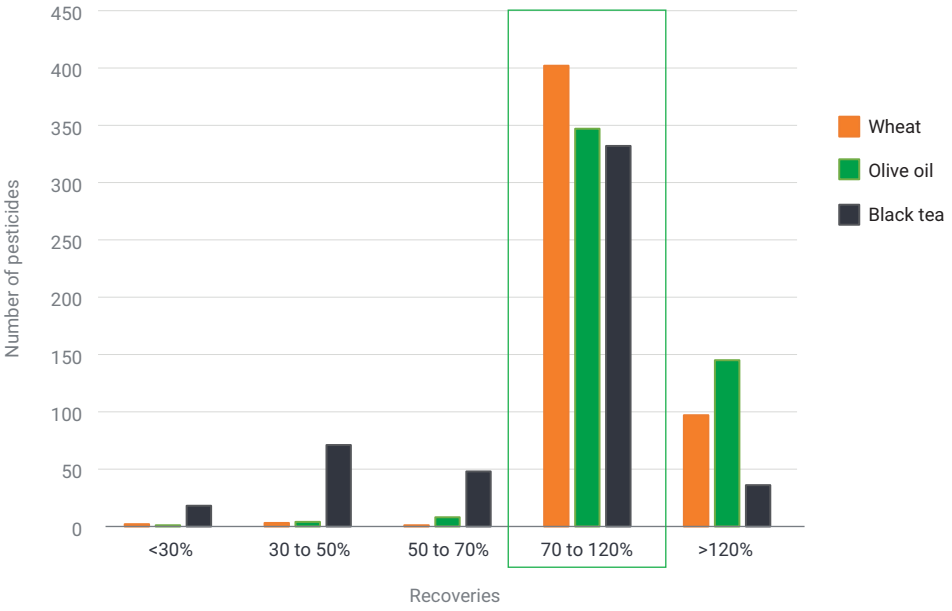

Figure 7. Histogram of response recoveries for pesticides spiked into wheat, olive oil, and black tea at 10 µg/kg. The green box denotes acceptable recoveries according to SANTE specification.

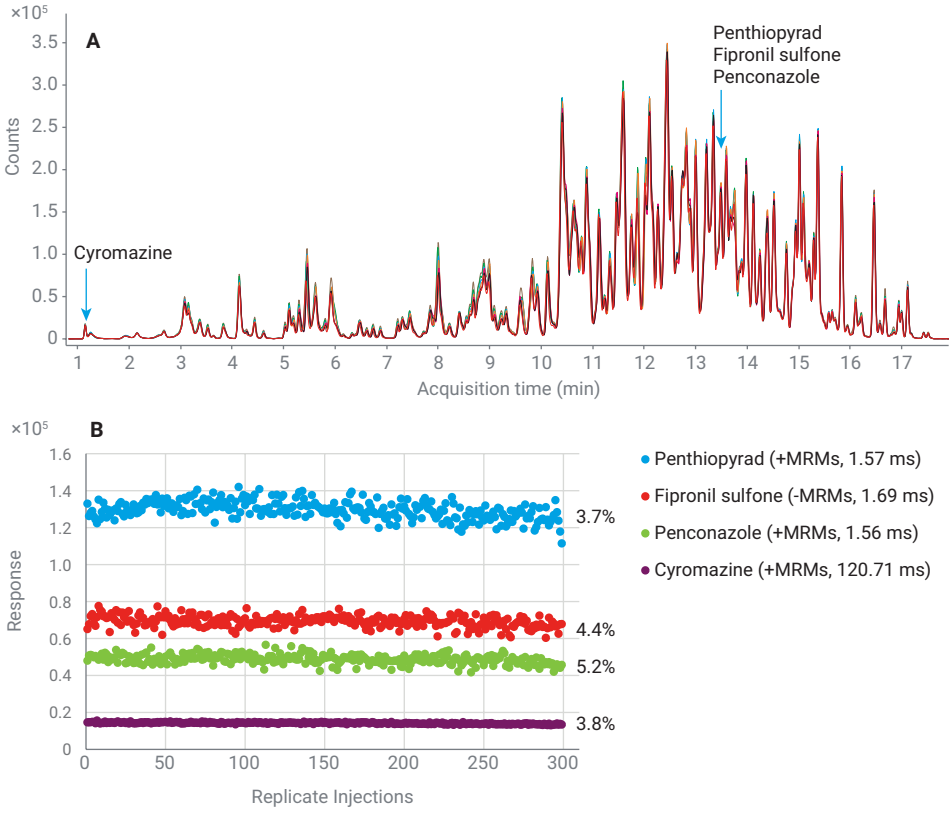

Figure 8. Reproducibility of replicate injections (n = 300) of black tea extract spiked at 10 µg/kg. (A) Overlaid TICs of every 50 injections of black tea extract. LC elution time for the four representative pesticides (cyromazine, penthiopyrad, fipronil sulfone, and penconazole) were indicated. (B) MS responses of the four selected pesticides from 300 replicate injections were plotted.

Conclusion

A comprehensive LC/MS/MS method for pesticide screening and quantitation analysis was directly transferred from a legacy Agilent LC/TQ system and further expanded on a newer system. The method was transferred to the Agilent 6475 triple quadrupole LC/MS system coupled with the Agilent 1290 Infinity II Bio LC system and the Agilent MassHunter 12.0 software. The new optimizer ensures automated workflow for improved compounds and ion source optimization. A final multiresidue pesticide method for the quantitation of more than 500 pesticides and pesticide metabolites was developed, and then evaluated in three food matrices including wheat, olive oil, and black tea.

The obtained results demonstrate:

- Easy method transfer from an Agilent legacy system to the 6475 LC/TQ system without the need of reoptimization
- Excellent chromatographic resolution of the Agilent 1290 Infinity II Bio LC system
- Outstanding quantitation performance in food matrices in terms of analytical sensitivity, precision, and accuracy
- Matrix effects are within an acceptable range for most pesticides
- Demonstrated system robustness for both LC chromatography and MS signals

References

1. SANTE/11312/2021 European Commission. https://food.ec.europa.eu/document/download/d4786faf-c574-4222-a5c6-45086b3920b8_en?filename=pesticides_mrl_guidelines_wrkdoc_2021-11312.pdf
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