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Development and Validation of a Quantitative Method for Multiresidue Pesticides in Food Matrices Using the Agilent 6475 LC/TQ System

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Introduction

Pesticide residues remaining in or on commodities raise concerns for food safety and environmental impact. Regulatory agencies have published policy statements to guide agricultural organizations on the proper use of pesticides and established Maximum Residue Limits (MRLs) of pesticides in various food matrices. Thus, there are widely demands for highly sensitive and robust platform for the quantification of pesticide residues analysis..

A new triple quadrupole LC/MS system, 6475 LC/TQ, which contains improvements on several aspects (Figure 1):

- Scheduled autotune and early maintenance feedback
- Enhanced software for method optimization
- iReflex (intelligent reflex) – new feature which enables reflexive reinjection logic
- Supporting Title 21 CFR Part 11 and Annex 11 compliance

An LC-MS/MS screening method for the quantification of 497 pesticides in food matrices was developed using the 1290 Infinity II Bio LC system coupled to 6475 LC/TQ. The LC/MS parameters were optimized with the new intelligent software to expand targeted pesticide list and optimize ion source. This method was validated in three food matrices, including wheat, olive oil and black tea. Results show excellent sensitivity, precision, accuracy and robustness on this new LC/TQ system.

Experimental

Standards and sample preparation

All Pesticide standards were purchased from Agilent or AccuStandard. Organic wheat, olive oil and black tea were purchased from local grocery store.

Food extracts were prepared following QuEChERS EN methods. A pesticide mix comprising 497 pesticide standards was prepared and spiked into solvent and food extracts to make matrix-matched standard curves with concentration ranging from 0.1 to 50 µg/L. After considering dilution factor during sample preparation, this concentration levels matches to 0.5 to 250 µg/kg for wheat and black tea, and 0.2 to 100 µg/kg for olive oil.

LC/MS analysis

All the samples were analyzed with a dynamic MRM (dMRM)-based LC-MS/MS method using MassHunter Workstation for LC/TQ 12.0 (Table 1 & 2).

Experimental

Instrumentation

- 1290 Bio High-Speed Pump (G7132A)
- 1290 Bio Multisampler with Cooler (G7137A)
- 1290 Multicolumn Thermostat (G7116B)
- 6475 Triple Quadrupole LC/MS (G6475A) with AJS source

1290 Infinity II Bio LC System		
Column	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 150 mm, 1.8 µm (p/n 959759-902)	
Sampler temp.	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	
Gradient program	Time	B (%)
	0.00	5
	3.00	30
	17	100
	20.00	100
Post time	3 minutes	

Table 1. 1290 Infinity II Bio LC Method

6475 Triple Quadrupole Mass Spectrometer	
Ion source	Agilent Jet Stream (AJS) source
Polarity	Positive and Negative
Gas temperature	200 °C
Drying gas	11 L/min
Nebulizer gas	35 psi
Sheath gas	350 °C
Sheath gas flow	12 L/min
Capillary voltage	+3500 V, -3000 V
Nozzle voltage	0 ±V
MS1/MS2 resolution	Unit/Unit
Cycle time	500 ms
Total MRMs	1003
Min/Max Dwell	1.12 ms/248.28 ms

Table 2. 6475 Triple Quadrupole LC/MS Method

Figure 1. 6475 Triple Quadrupole LC/MS with 1290 Infinity II Bio LC system

LC/MS Chromatograms

The targeted compound list in a multiresidue pesticide screening method developed for the previous Agilent LC/TQ instrument model (6470 LC/TQ) was imported and further expanded using the new intelligent optimizer in MassHunter Workstation for LC/TQ 12.0.

- MRMs library optimized on a previous Agilent LC/TQ models (e.g., 6470 LC/TQ) can be directly used on 6475 LC/TQ[1]
- New compounds were easily added using the new intelligent optimizer software[2,3]
- The final method resulting into monitoring 1003 MRMs within 20-minutes LC gradient (**Figure 2**), which shows **good chromatogram separation and greatly improves lab productivity**

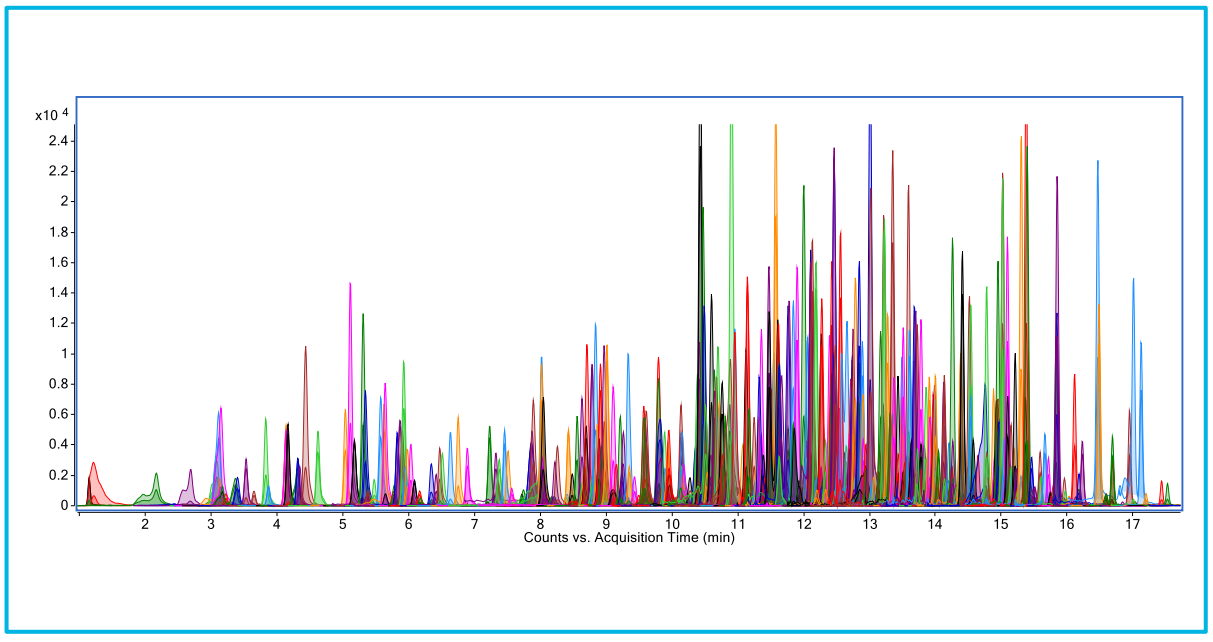


Figure 2. Overlaid MRM chromatograms of all the 497 pesticides post spiked at 1 µg/L in wheat matrix extract.

Quantification sensitivity with comprehensive dMRM method

Figure 3 shows the overview of the dMRM acquisition method. The retention time window from 12 to 14 minutes contains the most concurrent MRMs with <2 ms average dwell time per MRM.

Method performance for a total of 497 pesticides was evaluated in wheat, olive oil and black tea matrix extracts at 9 concentration levels (**Figure 4 & 5**). The results were summarized as below:

- **Excellent linearity** with 459 (92%) pesticides in wheat, 465 (94%) pesticides in olive oil and 458 (92%) pesticides in black tea showing $R^2 > 0.99$ based on matrix-matched calibration curves.
- **Excellent precision and accuracy** observed at all calibration levels.
- **Excellent sensitivity** - 465 (94%) pesticides in wheat, 486 (98%) pesticides in olive oil and 466 (94%) pesticides in black tea show lower limit of quantification (LLOQ) ≤ 10 µg/kg (**Figure 4**)

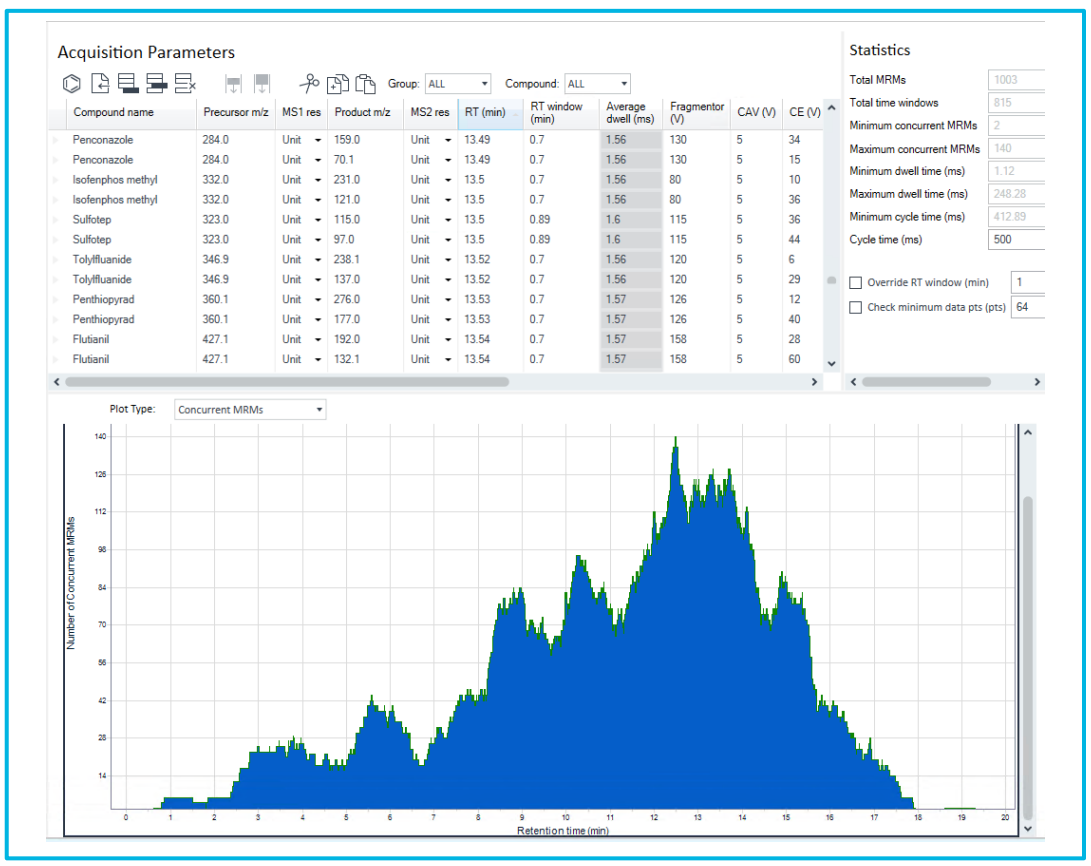


Figure 3. Overview of the comprehensive acquisition method showing user-friendly management of over 1000 MRMs.

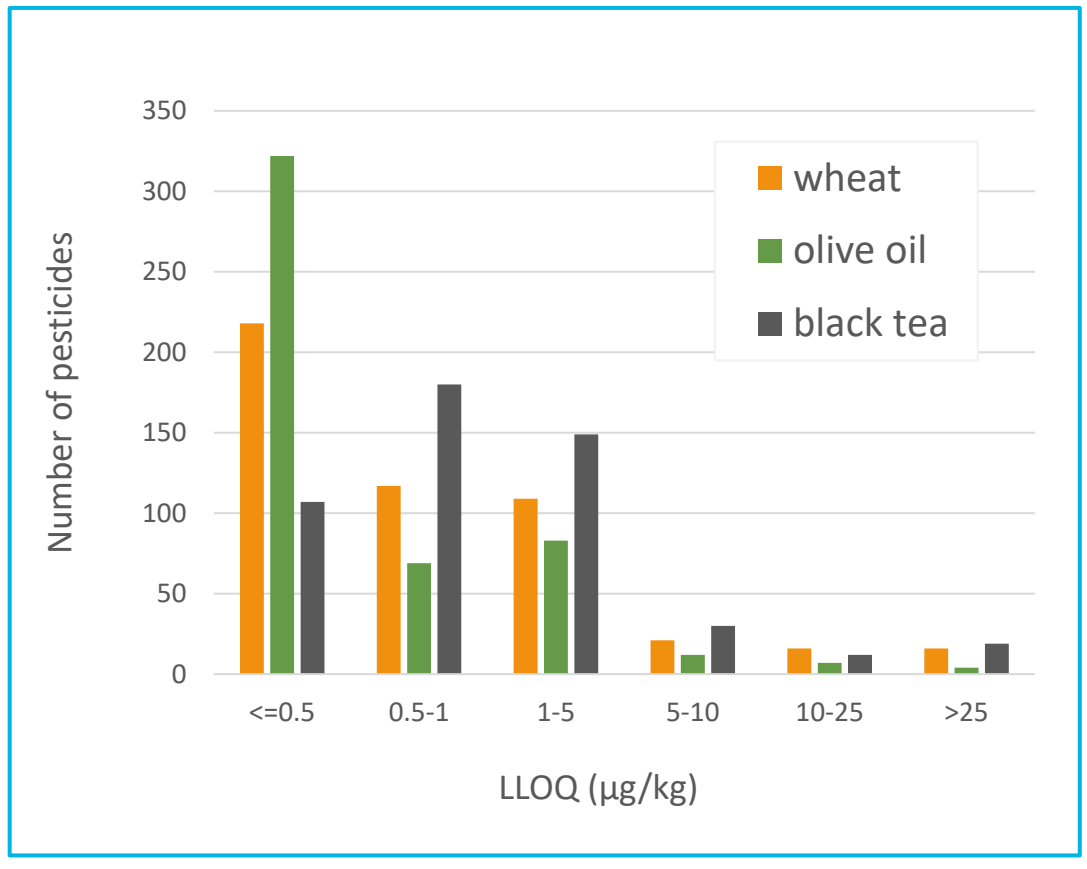


Figure 4. LLOQs for the 497 pesticides in wheat, olive oil and black tea extracts. Results were classified into six relevant concentration ranges after considering dilution factor during sample preparation.

Results and Discussion

Standard curves

Figure 5 shows representative standard curves of four selected pesticides in black tea matrix including one early eluted analyte (Cyromazine: RT=1.25 min) and three analytes (Penconazole, Sulfotep and Penthiopyrad) with dwell time <2.0 ms. Results show the 6475 LC/TQ delivers great linearity for all four analytes.

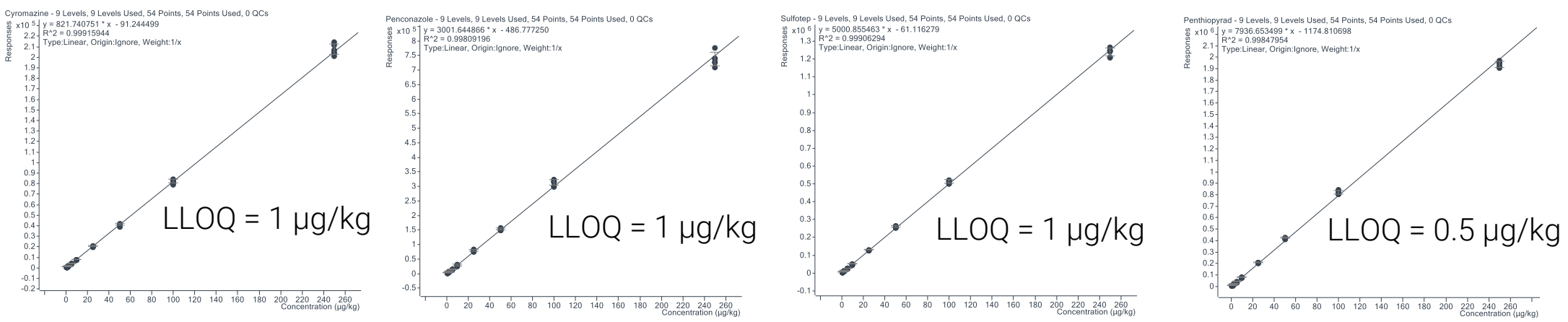
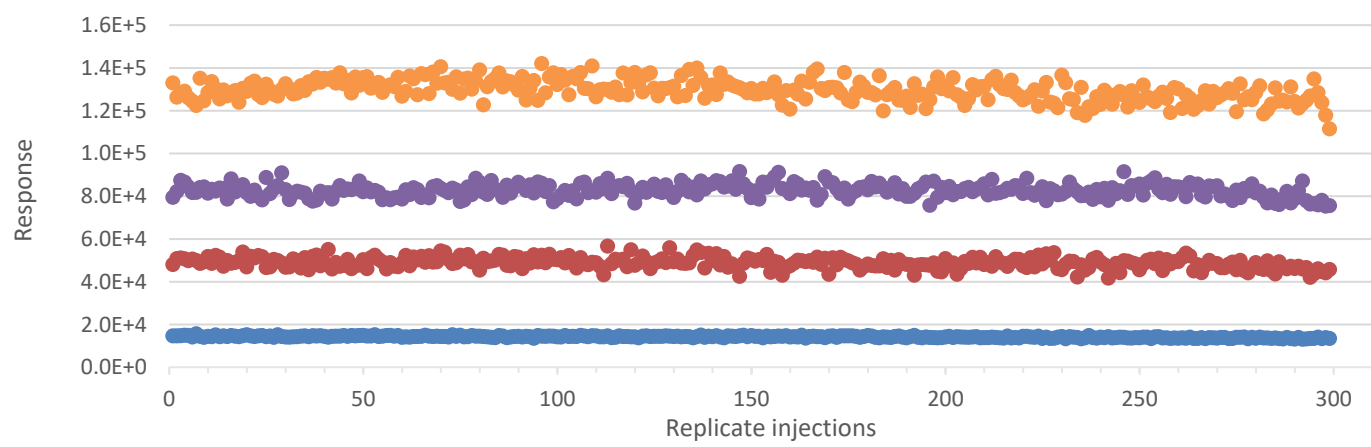


Figure 5. Standard curves of four selected pesticides in black tea matrix.

Robustness

To test robustness of the new LC/TQ system, replicate injections (n=300) of black tea matrix spiked with pesticide standards at 10 µg/kg were performed using the comprehensive dMRM method. **Outstanding reproducibility for both RT and MS signal was observed during 5-days continuous running, even for pesticides acquired with low dwell time (Figure 6).**



Compound name	dwell time (ms)	RT (min)	RT RSD (%) (n=300)	Response RSD (%) (n=300)
Cyromazine	120.71	1.25	0.41	3.8
Penconazole	1.56	13.48	0.05	5.2
Sulfotep	1.60	13.50	0.05	3.7
Penthiopyrad	1.57	13.51	0.05	3.7

Figure 6. Response of representative pesticides in black tea extract for 300 replicate injections.

Conclusions

The excellent quantification performance using a new LC/TQ platform, including the Agilent 1290 Infinity II Bio LC system coupled to the 6475 Triple Quadrupole LC/MS with the Jet Stream Technology Ion Source (AJS) has been demonstrated for large panel pesticide analysis in wheat, olive oil and black tea.

The platform robustness was assessed over 5 days' continuous injection using black tea extract spiked with pesticide standards, indicating the high ability of the system producing reliable result for day-to-day analysis.

https://explore.agilent.com/asms

DE80120981

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References

[1]MP 329 High fidelity legacy-to-modern method transfer on a novel triple quadrupole LC/MS platform for large output production labs.

[2]MP 327 An end-to-end software algorithm for LC/MS/MS method development, optimization, and QA/QC deployment.

[3]WP 493 Multi-residue Method Development Using Agilent 6475 LC/TQ System Implemented with Intelligent Optimization Software: Application to Forensic Drug Screening

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Trusted Answers