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Determination of Over 500 Pesticides in Spice by EMR–GPD Passthrough Cleanup and a Novel Triple Quadrupole LC/MS system

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Introduction

Pesticides are utilized in several agricultural applications to prevent, destroy, or control harmful organisms or diseases. They are also used to protect plants or plant products during production, storage and transport. Dry spices are complex matrices that present challenges to reliable pesticide analysis. Cinnamon powder is considered one of the most difficult matrices to analyze, with its high complexity and high pigment concentration. Rapid multi-residue LC/MS/MS analytical methods are needed to accurately and efficiently quantify large numbers of pesticides in this type of matrix.

Here we introduce a novel triple quadrupole LC/MS system, the 6495 LC/TQ, which contains improvements on several aspects (**Figure 1**):

- 4th generation iFunnel delivering robustness for high precision routine analysis
- Ion optics improvements for highest analytical sensitivity
- Artificial intelligence autotune (SWARM)

An LC-MS/MS screening method for screening 504 pesticides in spice matrix was developed using the 1290 Infinity II Bio LC system coupled to a 6495 LC/TQ (G6495D). New pesticides were easily added to an existing target list using new intelligent software that automatically optimizes method parameters. Results show excellent sensitivity, large linear range and robustness with this novel LC/TQ system.

Experimental

Standards and Sample Preparation

All Pesticide standards were purchased from Agilent or AccuStandard. Cinnamon was purchased from the local grocery store.

Cinnamon extract was prepared following QuEChERS EN methods followed by further cleanup using a Captiva EMR-GDP cartridge (p/n 5610-2091).[1] A pesticide mix comprising 504 pesticide standards was prepared and spiked into solvent and spice extracts to make matrix-matched standard curves with concentrations ranging from 0.01 to 50 µg/L corresponding to levels from 0.05 to 250 µg/kg in the sample after considering dilution factor during sample preparation.

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)
- 6495 Triple Quadrupole LC/MS (G6495D) with Agilent Jet Stream Source (AJS)

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

6495 Triple Quadrupole Mass Spectrometer	
Ion source	AJS
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	700 ms
Total MRMs	1001
Min/Max Dwell	0.72 ms/ 348.19 ms

Table 2. 6495 Triple Quadrupole LC/MS Method

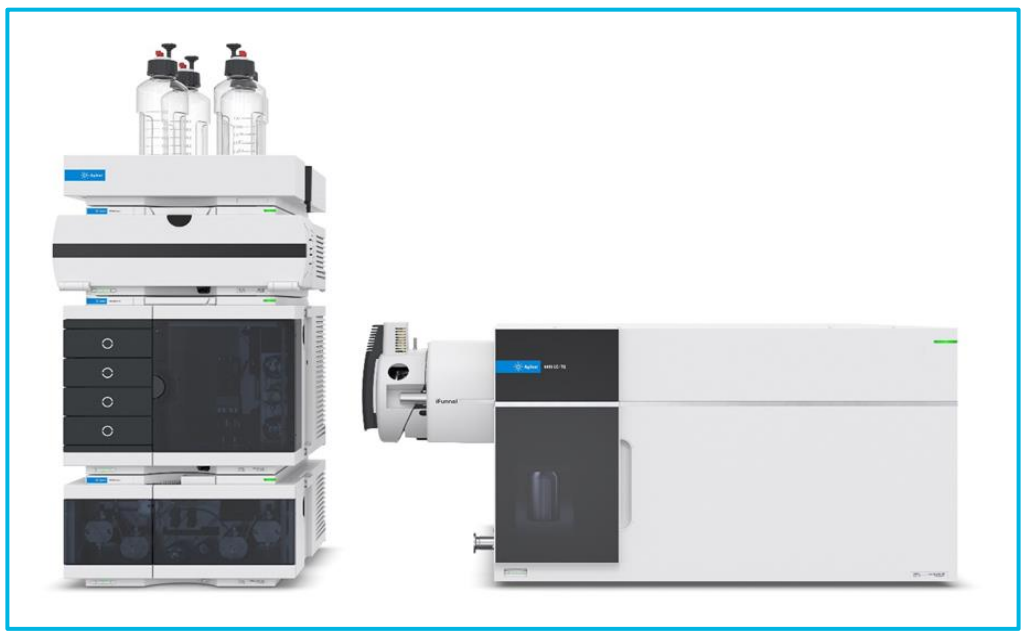


Figure 1. 6495 Triple Quadrupole LC/MS with 1290 Infinity II Bio LC system

LC/MS Chromatograms

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

- MRMs optimized on a previous Agilent LC/TQ models (e.g., 6475 LC/TQ) can be directly used on the 6495 LC/TQ.
- The final method resulting in monitoring 1001 MRMs within a 20 minutes LC gradient (Figure 2), shows **good chromatographic separation.**
- New compounds were easily added to the existing method using the new intelligent optimizer software.
- New individual iFunnel mode settings are available for each compound to optimize signal (Figure 3)

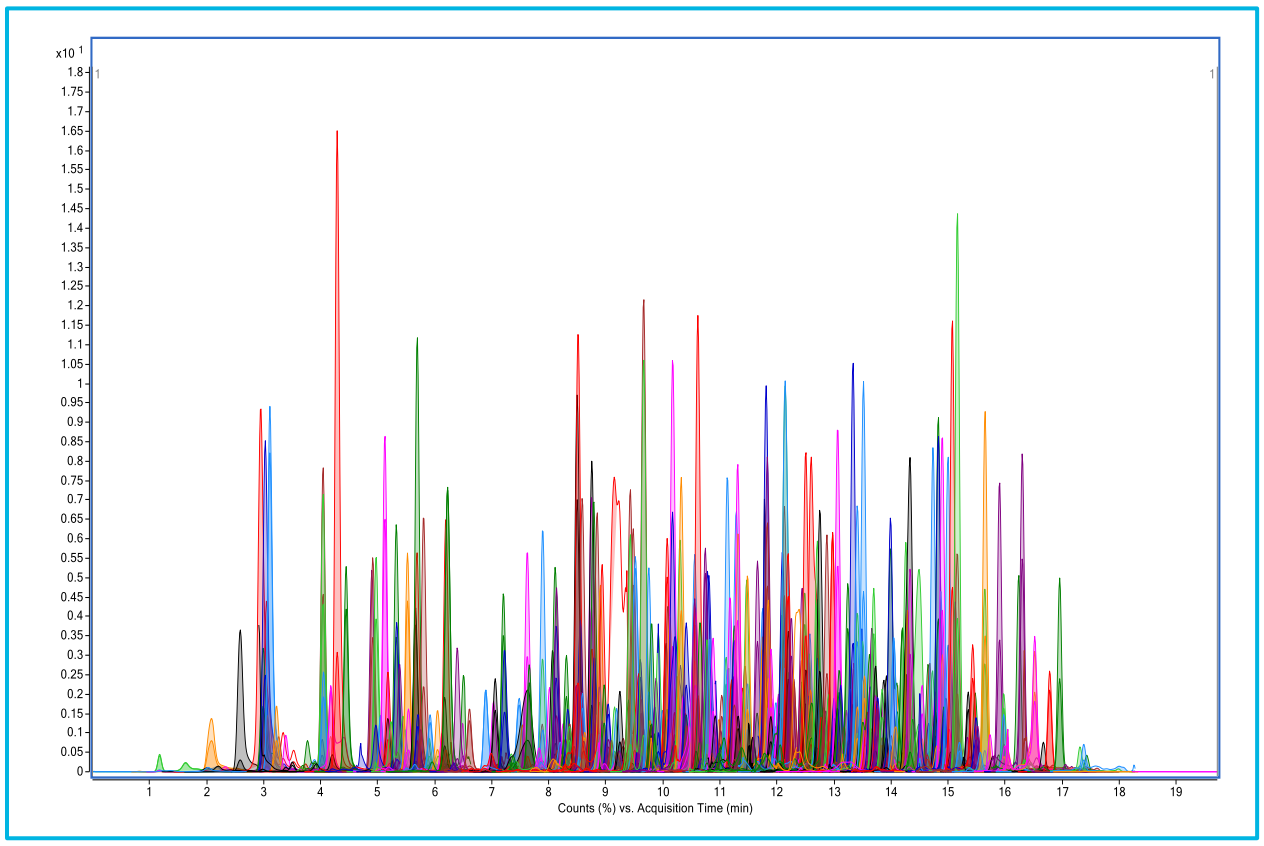


Figure 2. Overlaid MRM Chromatograms of all the 503 Pesticides Post Spiked at 1 µg/L in Matrix.

Quantification Sensitivity with Comprehensive dMRM Method

Figure 4 shows the overview of the dMRM acquisition method. The retention time window from 12 to 14 minutes contains the most concurrent MRMs with sub 1 ms average dwell time per MRM.

Method performance for a total of 503 pesticides was evaluated in cinnamon matrix extracts at 12 concentration levels (Figure 5). The results were summarized as below:

- **Excellent linearity** with 451 (90%) pesticides showing $R^2 > 0.99$ based on matrix-matched calibration curves.
- **Excellent precision and accuracy** observed at all calibration levels.

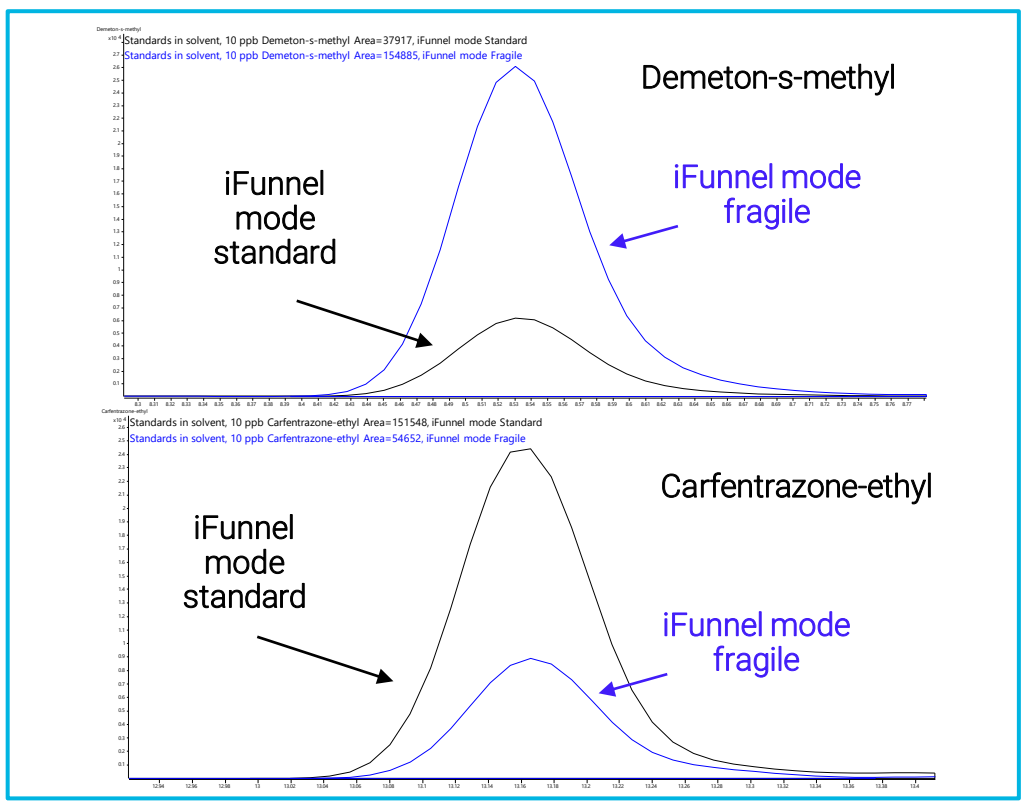


Figure 3. Influence of iFunnel Mode on Peak Intensity for 2 Selected Compounds

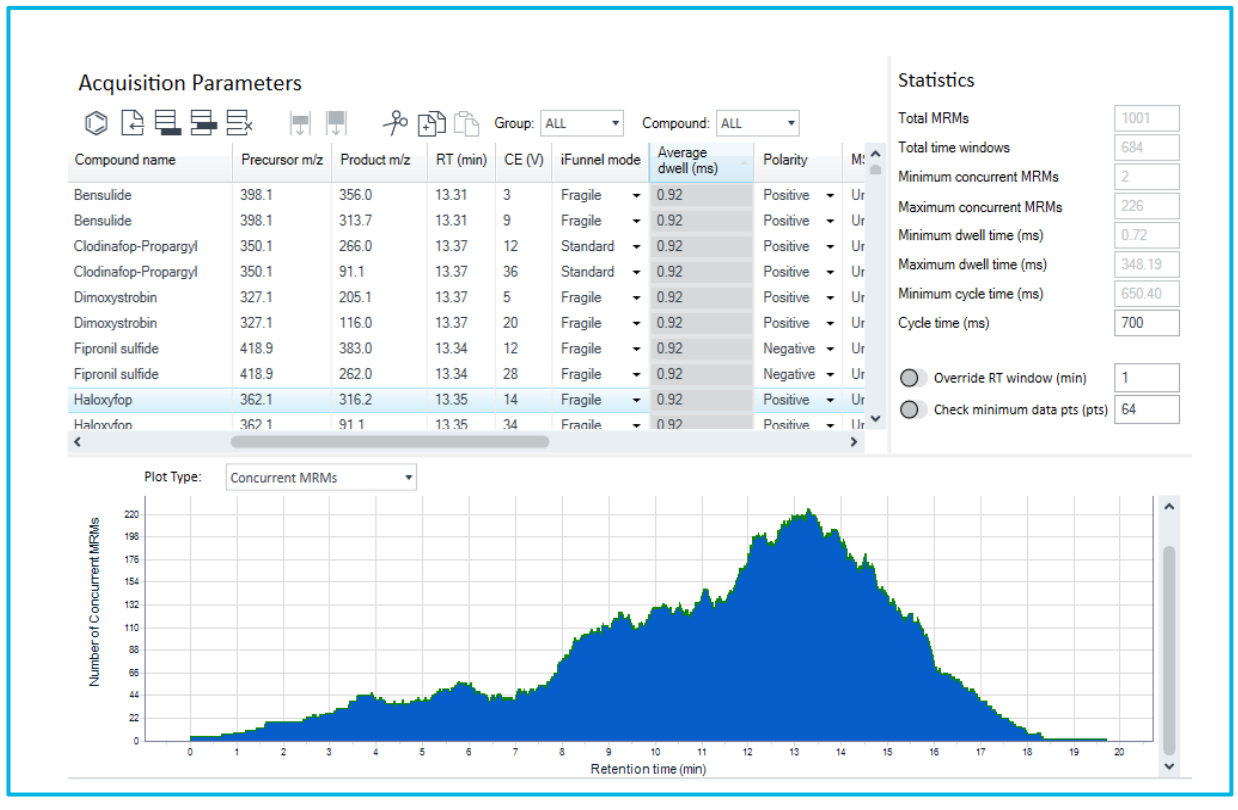


Figure 4. Overview of the Comprehensive Acquisition Method Showing User-Friendly Management of Over 1000 MRMs.

Results and Discussion

Standard Curves

Figure 5 shows representative standard curves of four selected pesticides in cinnamon matrix including one early eluted analyte (Nicotine: RT=1.17 min) and three analytes (Fipronil Desulfinyl, Carfentrazone-ethyl and Bensulide) with sub 1ms dwell time in negative or positive ionization mode. Results show the 6495 LC/TQ delivers great linearity for all four analytes as well as an excellent linear range.

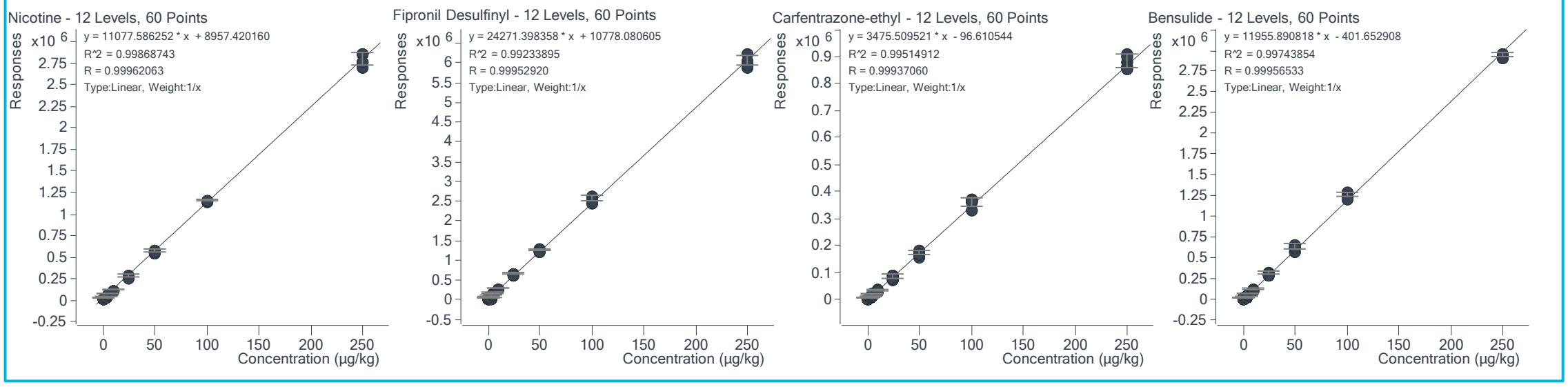
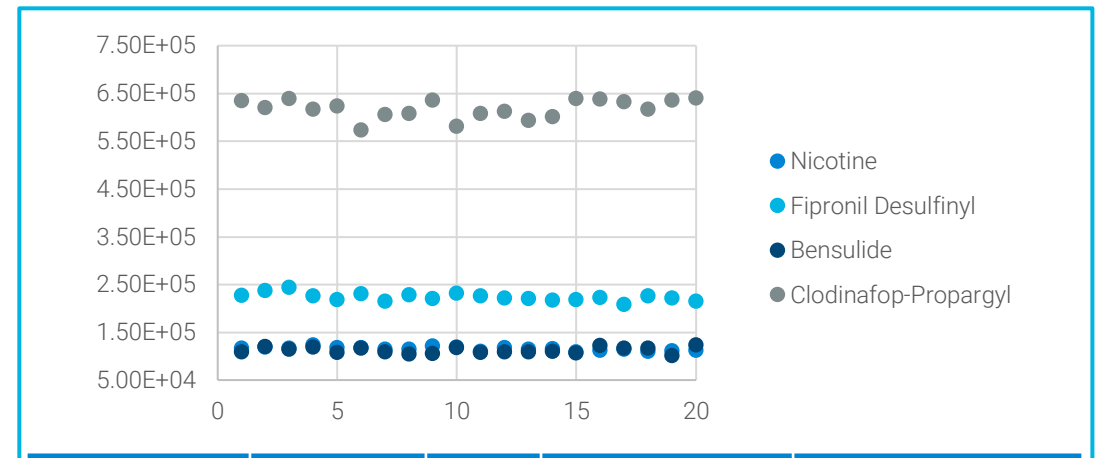


Figure 5. Standard Curves of Four Selected Pesticides in Cinnamon Matrix.

Robustness and Matrix Effect

To test robustness of the new LC/TQ system, replicate injections (n=20) of cinnamon matrix spiked with pesticide standards at 10 µg/kg were performed using the comprehensive dMRM method. **Good reproducibility for MS signal was observed during these replicate injections, even for pesticides acquired with low dwell time (Figure 6).** Furthermore, efficient sample preparation showed limited matrix effect for most of the pesticides (**Figure 7**).



Compound name	dwell time (ms)	RT (min)	Response RSD (%) (n=20)	Polarity and iFunnel settings
Nicotine	152.66	1.15	3.3	Positive, Fragile
Fipronil Desulfinyl	0.97	12.88	3.6	Negative, Fragile
Clodinafop-Propargyl	0.92	13.37	3.2	Positive, Standard
Bensulide	0.92	13.31	5.6	Positive, Fragile

Figure 6. Response of Representative Pesticides. Cinnamon Extract for 20 Replicate Injections.

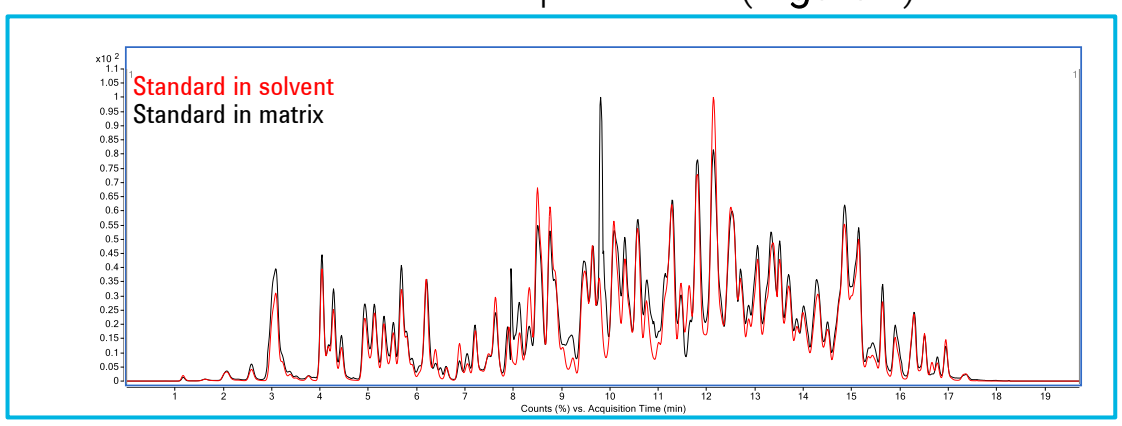


Figure 7. Overlaid MRM chromatograms spiked at 10 µg/kg in solvent and in matrix .

Conclusions

The excellent quantification performance using a novel LC/TQ platform, including the Agilent 1290 Infinity II Bio LC system coupled to the 6495 Triple Quadrupole LC/MS with the Jet Stream Technology Ion Source (AJS) has been demonstrated for large panel pesticide screening analysis in a known difficult matrix such as cinnamon.

https://www.agilent.com/en/promotions/asms

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References

[1]Zhao, L.; Andrianova, A. A.; Determination of Over 300 Pesticides in Cinnamon Using Captiva EMR–GPD passthrough cleanup and LC/MS/MS and GC/MS/MS detection. Agilent Technologies application note, 5994-5671EN, 2023

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