Application Note

Food Safety Testing

Analysis of 764 Pesticides in Tomato Using an Agilent 6495D Triple Quadrupole LC/MS System

Pesticide residue workflow for determination of 764 pesticides in tomato QuEChERS extract

Authors

Peter Kornas
Agilent Technologies, Inc.
Waldbronn, Germany

Linfeng Wu
Agilent Technologies, Inc.
Santa Clara, CA, USA

Hui Zhao
Agilent Technologies, Inc.
Wilmington, DE, USA

Abstract

A comprehensive LC/MS/MS method for 764 pesticides in QuEChERS tomato raw extract was established using an Agilent 6495D triple quadrupole LC/MS system. With 4th-generation iFunnel technology and novel inlet design, the 6495D LC/TQ system delivers ultimate sensitivity with high precision at sub-millisecond dwell times, without compromising robustness, for the most difficult analytes in complex food matrices. The iFunnel can uniquely be tuned on a compound-by-compound basis for optimal transmission of all analytes in an assay for wide-scope multiresidue pesticide analysis that includes fragile compounds. Speed of acquisition and duty cycle are enhanced with the latest onboard computing, enabling 150% more concurrent dynamic multiple reaction monitoring (dMRM) for extended large-panel assays as well as shortened run times. The 6495D also provides excellent linearity and allows for easy method transfer from previous LC/TQ models. These improvements make it a robust and reliable option for performing routine trace analysis in the most demanding production environments. Overall, the 6495D LC/TQ is a significant upgrade in terms of performance and usability for pesticide analysis.

Introduction

Pesticides are crucial in agriculture and in the food industry for enhancing crop and food production. However, residues of these chemicals on food such as fruits, vegetables, and cereals can pose health risks and environmental issues. Therefore, maximum residue levels (MRLs) were established, often at very low parts per billion (ppb), by regulatory bodies such as the European Commission.[1] This creates significant challenges, especially when hundreds of compounds need to be screened simultaneously and quantified in complex food matrices. The large number of pesticides makes the analysis complex, often requiring multiple analytical methods and intensive laboratory workflows, leading to high operating costs and slow turnaround times.

An MRM-based LC/MS/MS method was previously developed for 764 pesticides in tomato matrix using an Agilent 1290 Infinity II LC system coupled to an Agilent 6470 LC/TQ.[2,3] This method was directly transferred to the 6495D LC/TQ system due to the enhancement in acquisition cycle time and capability to handle up to 500 concurrent dMRMs, a 150% improvement upon the predecessor. With the new iFunnel settings, which are now a compound-dependent parameter, analytical sensitivity was further improved.

Experimental

Chemicals and reagents

Agilent LC/MS-grade acetonitrile (ACN), methanol (MeOH), water, and ammonium formate were used for the study. LC/MS-grade formic acid was purchased from VWR. All other solvents used were HPLC grade from Sigma-Aldrich.

Standards and solutions

The following ready-to-use and custom premixed pesticide standards were acquired:

- Agilent LC/MS Pesticide Comprehensive Test Mix (part number 5190-0551)
- Agilent Custom Pesticide Test Mix (part number CUS-00000635 – CUS-00000643)
- Agilent Custom Org Standard (part number CUS-00004663)
- AccuStandard Custom Pesticide Standard (part number S-96086-01 – S-96086-10)

Additional single standards, either as standard solution or as powders were purchased from AccuStandard and LGC Standards GmbH.

When single standards were purchased as powders, stock solutions with a concentration of 1,000 mg/L were prepared in acetone and stored at –20 °C.

Two intermediate standard mixes (Mix 1 and Mix 2) at concentrations of 1,000 µg/L were prepared in ACN from stock standard solutions and used for the rest of the experiments.

Sample preparation

Pesticide-free and organically labeled tomatoes were obtained from local grocery stores. The tomato was homogenized using a domestic blender and kept at 4 °C for short-term storage.

The following products and equipment were used for sample preparation:

- Agilent QuEChERS EN extraction kits (part number 5982-5650CH)
- Vortex mixer (VWR International GmbH, Darmstadt, Germany)
- Centrifuge Universal 320 R (Andreas Hettich GmbH, Tuttlingen, Germany)

A 10 ± 0.1 g sample of homogenized tomato was weighed into a 50 mL tube. A QuEChERS extraction was then performed by adding 10 mL of acetonitrile and mixing the samples vigorously for two minutes using a mechanical shaker. Then, QuEChERS extraction salts were added, and after mixing and centrifuging, the acetonitrile extract was directly used for LC/MS/MS analysis. The procedure is illustrated in Figure 1.

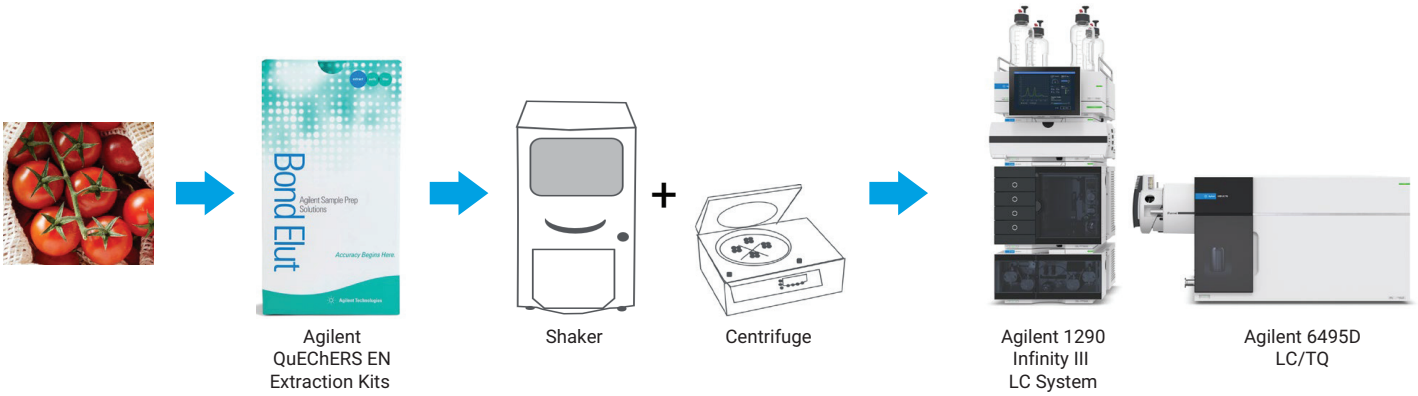

Figure 1. Overview of the sample preparation and analysis procedure.

Preparation of matrix-matched calibration standards

Matrix-matched calibration standards were prepared for both standard mixes in tomato matrix. Serial dilutions were performed from Mix 1 and Mix 2 to prepare 10 calibration concentration levels of 0.1, 0.2, 0.5, 1, 2, 5, 10, 25, 50, and 100 µg/L. Calibration standards were prepared fresh and stored at 4 °C if not used immediately.

Instrumentation

Chromatographic separation was performed using an Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 150 mm, 1.8 µm column (part number 959759-902) installed on an Agilent 1290 Infinity III LC system.

The individual modules of the 1290 Infinity III LC system included:

- Agilent 1290 Infinity III high-speed pump (G7120A)
- Agilent 1290 Infinity III autosampler (G7167B)
- Agilent 1290 Infinity III thermostatted column compartment (G7116B)

A 6495D triple quadrupole LC/MS system with an Agilent Jet Stream (AJS) electrospray ion source was operated in dynamic MRM (dMRM) mode. The LC/TQ autotune was performed in Unit and Wide modes. All data acquisition and processing were performed using Agilent MassHunter software (version 12.1). The LC/MS/MS parameters are shown in Table 1.

Table 1. Agilent 6495D LC/TQ parameters.

Parameter	Value
Agilent 1290 Infinity III LC System	
Column	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 150 mm, 1.8 µm (part number 959759-902)
Column Temperature	40 °C
Injection Volume	2 µL
Autosampler Temperature	6 °C
Mobile Phase A	5 mM Ammonium formate in water with 0.1% formic acid
Mobile Phase B	5 mM Ammonium formate in methanol with 0.1% formic acid
Flow Rate	0.4 mL/min
Gradient	Time (min) A (%) B (%) 0 95 5 3 70 30 17 0 100 20 0 100
Post-Run Time	3 min
Needle Wash	Multi wash
Agilent 6495D Triple Quadrupole Mass Spectrometer	
Ionization Mode	Simultaneous positive/negative ESI with Agilent Jet Stream (AJS)
Scan Type	Dynamic MRM (dMRM)
Gas Temperature	200 °C
Gas Flow	12 L/min
Nebulizer	35 psi
Sheath Gas Temperature	400 °C
Sheath Gas Flow	12 L/min
Capillary Voltage	2,500 V (+)/3,000 V (–)
Nozzle Voltage	0 V
Total MRMs	1,590
Min/Max Dwell Time	0.9 ms/247.6 ms

Results and discussion

For the analysis of 764 pesticides using the 6495D LC/TQ, an acquisition method developed on a previous Agilent LC/TQ instrument (G6470BA) was easily imported to the new LC/TQ. Adjustments were made for drying gas temperature, as higher drying gas flows were needed. All other ion source and MRM parameters could be used directly on the 6495D.

For the analysis, 1,590 transitions were monitored. Depending on the fragmentation behavior of the individual compound, two or three target-specific MRM transitions were selected per pesticide (except for procymidone, where only one transition was stable enough to be monitored). This was done to satisfy the SANTE regulatory requirements for identification and confirmation by LC/MS/MS.[4] The two most abundant fragments were defined as primary transitions that were acquired within the retention time window and subsequently used as the quantifier and qualifier ion. Figure 2 shows the overview of the acquisition method for the analysis of the target compounds.

With the ZORBAX RRHD Eclipse Plus C18 column and the chromatographic method, good separation and distribution were achieved for the 764 pesticides within a run time of 20 minutes (Figure 3).

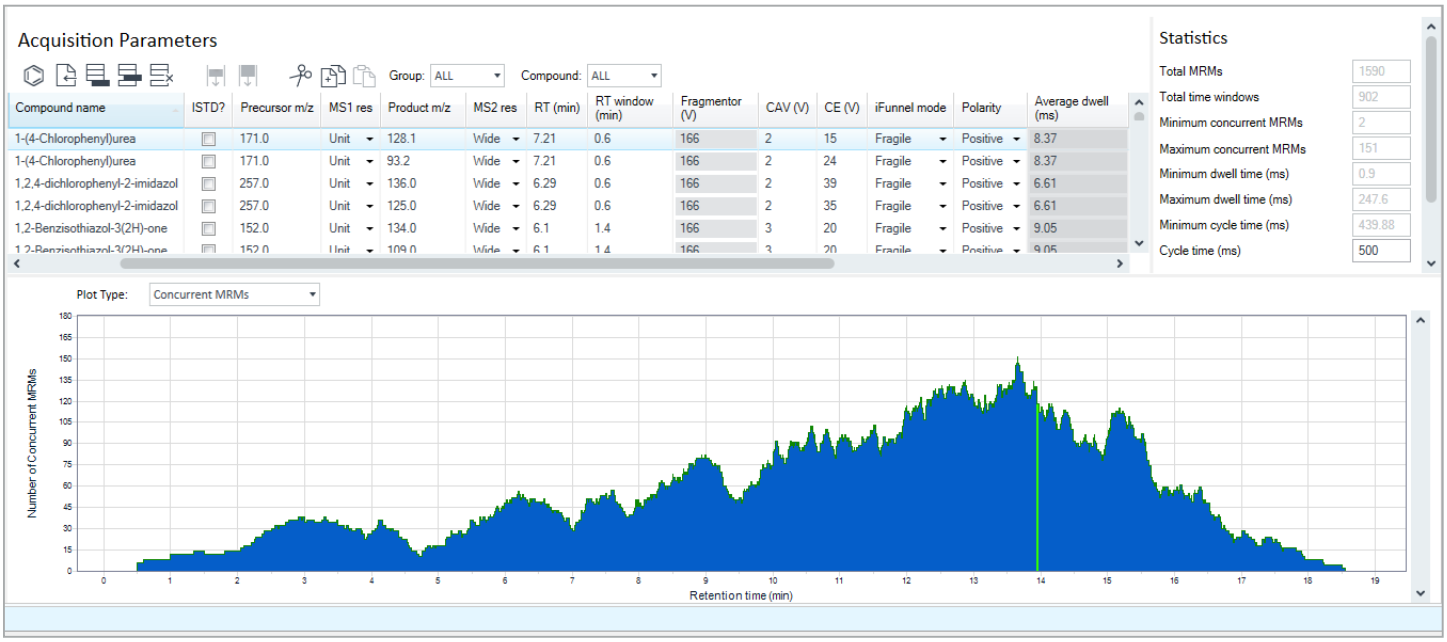

Figure 2. Overview of the acquisition method showing user-friendly management of more than 1,500 MRMs.

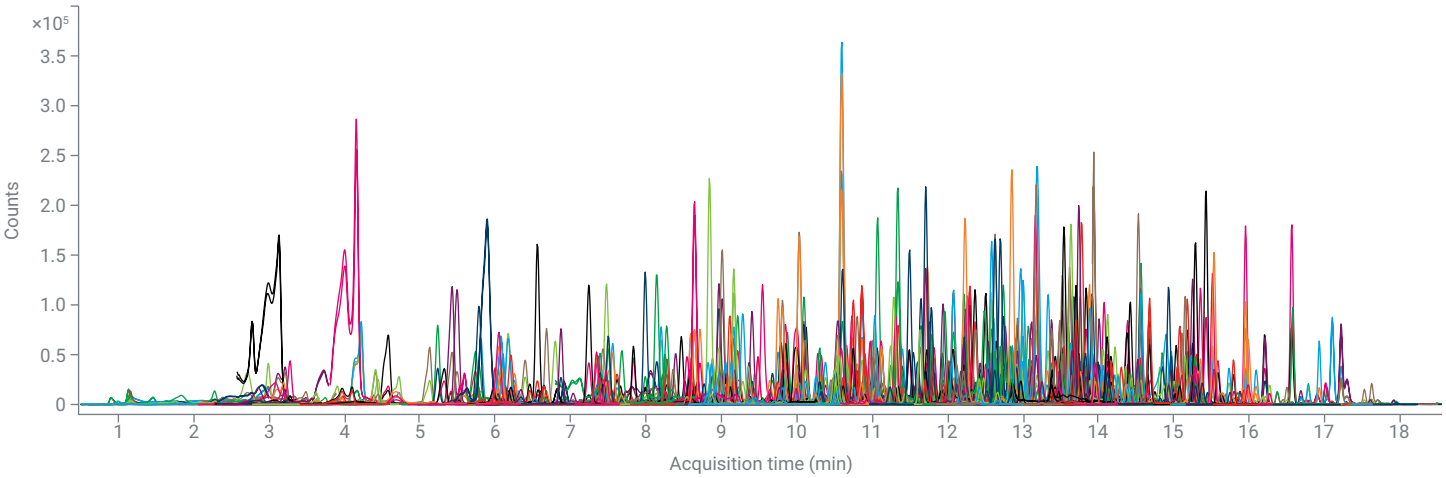

Figure 3. Overlaid MRM chromatograms of all 764 pesticides in tomato at 1 µg/L.

With the high acquisition speed of the 6495D LC/TQ, a minimum dwell time of 0.9 ms was achieved for a maximum of 151 concurrent dMRMs when using a cycle time of 500 ms, which shows a significant improvement compared to the predecessor 6495 TQ system. Typical chromatographic peak widths were between 8 and 12 seconds. The selected cycle time of 500 ms ensured that sufficient data points were collected across the chromatographic peaks for good reproducibility. An example is given in Figure 4, where 17 data points were collected across the chromatographic peak for both transitions of carfentrazone-ethyl, the quantifier and qualifier transition. This compound is a good representative of the obtained peaks, as it has a peak width of 9 seconds, and it elutes in the range of the chromatogram with the highest number of concurrent MRMs, resulting in the lowest dwell time of 0.9 ms.

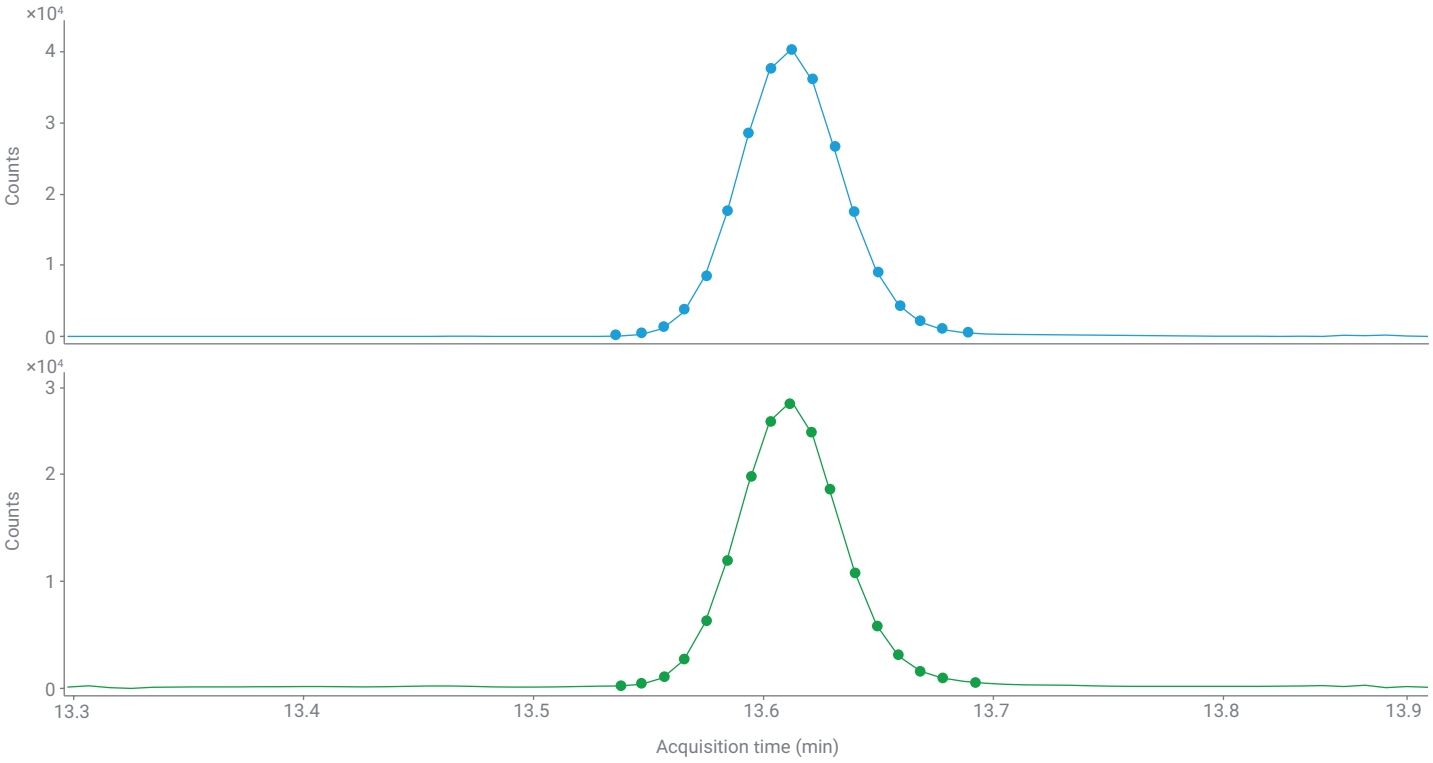

Figure 4. Data points per peak for the quantifier and qualifier transition for carfentrazone-ethyl.

With the new iFunnel mode settings on the 6495D LC/TQ, it is now possible to select different iFunnel settings per compound transition. Three different iFunnel settings are available: Standard, Fragile, and Large Molecule. In the predecessor 6495C LC/TQ, the same iFunnel settings are applied for all target analytes. This feature gives users the possibility to further improve analytical sensitivity for some compounds of interest. Several examples are shown in Figure 5. For ethifencarb-sulfone and demeton-S-methyl, better analytical sensitivity was achieved using the Fragile iFunnel mode, whereas for sulcotrione and carfentrazone-ethyl, the Standard iFunnel mode gave better results.

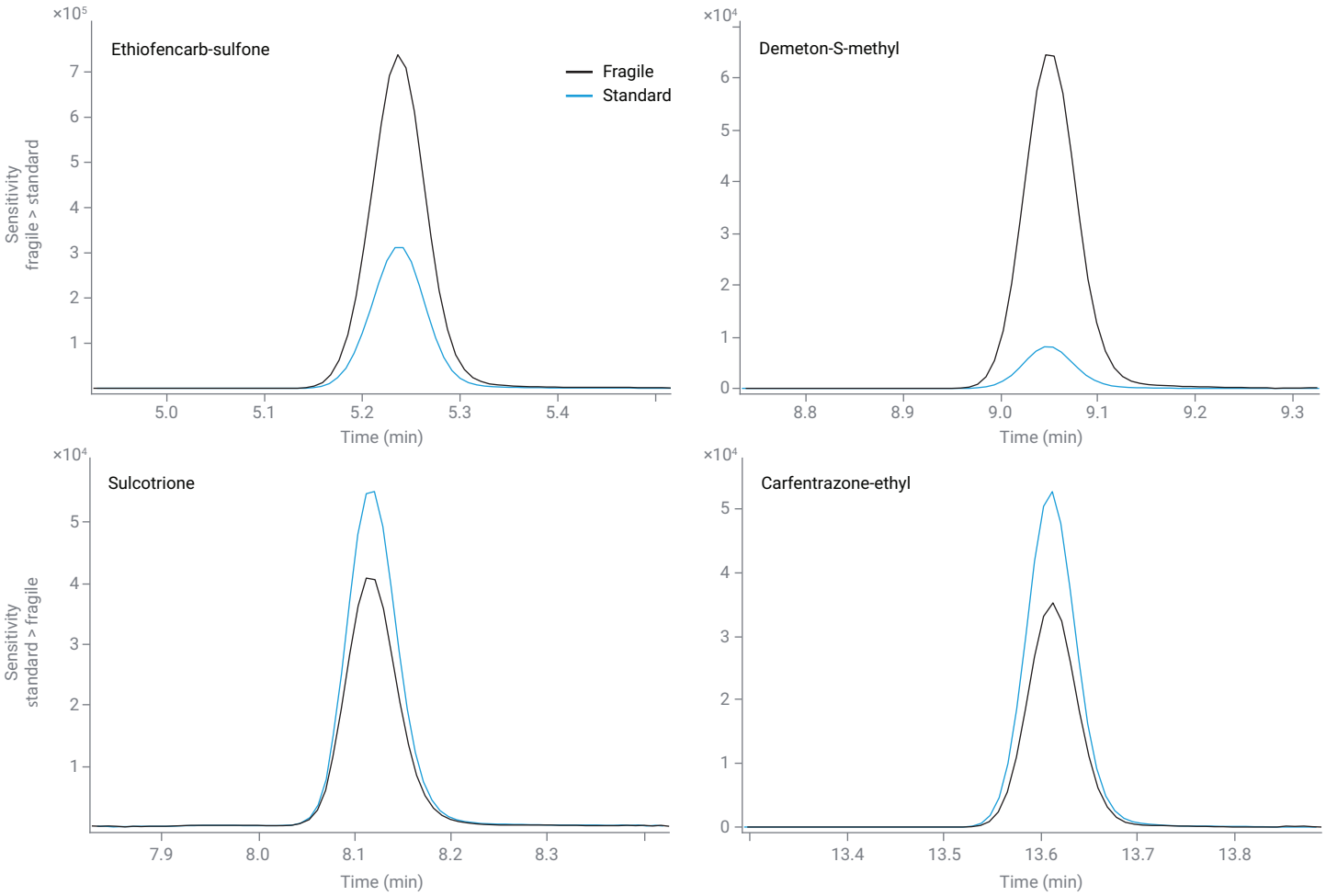

Figure 5. Peak Intensity influenced by the iFunnel mode settings (black: fragile, blue: standard).

With the 6495D LC/TQ, excellent linearity was achieved for the 764 target analytes, showing $R^2 \geq 0.99$ based on matrix-matched calibration curves. Calibration curves were generated for both STD mixes (Mix 1 and Mix 2) using matrix-matched standards ranging from 0.1 to 100 µg/L (corresponding to 0.1 to 100 µg/kg) using 10 calibration points. For the evaluation of linearity, a linear regression was used with a weight of 1/x, and the origin was ignored.

Figure 6 shows representative calibration curves of six selected compounds in tomato matrix. All six compounds eluted in the part of the chromatogram with the highest number of concurrent MRMs with sub 1 milliseconds dwell time and were analyzed in positive (carfentrazone-ethyl, cyazofamid, and penconazole) or negative (fipronil, fludioxonil, and thifluzamide) ionization mode.

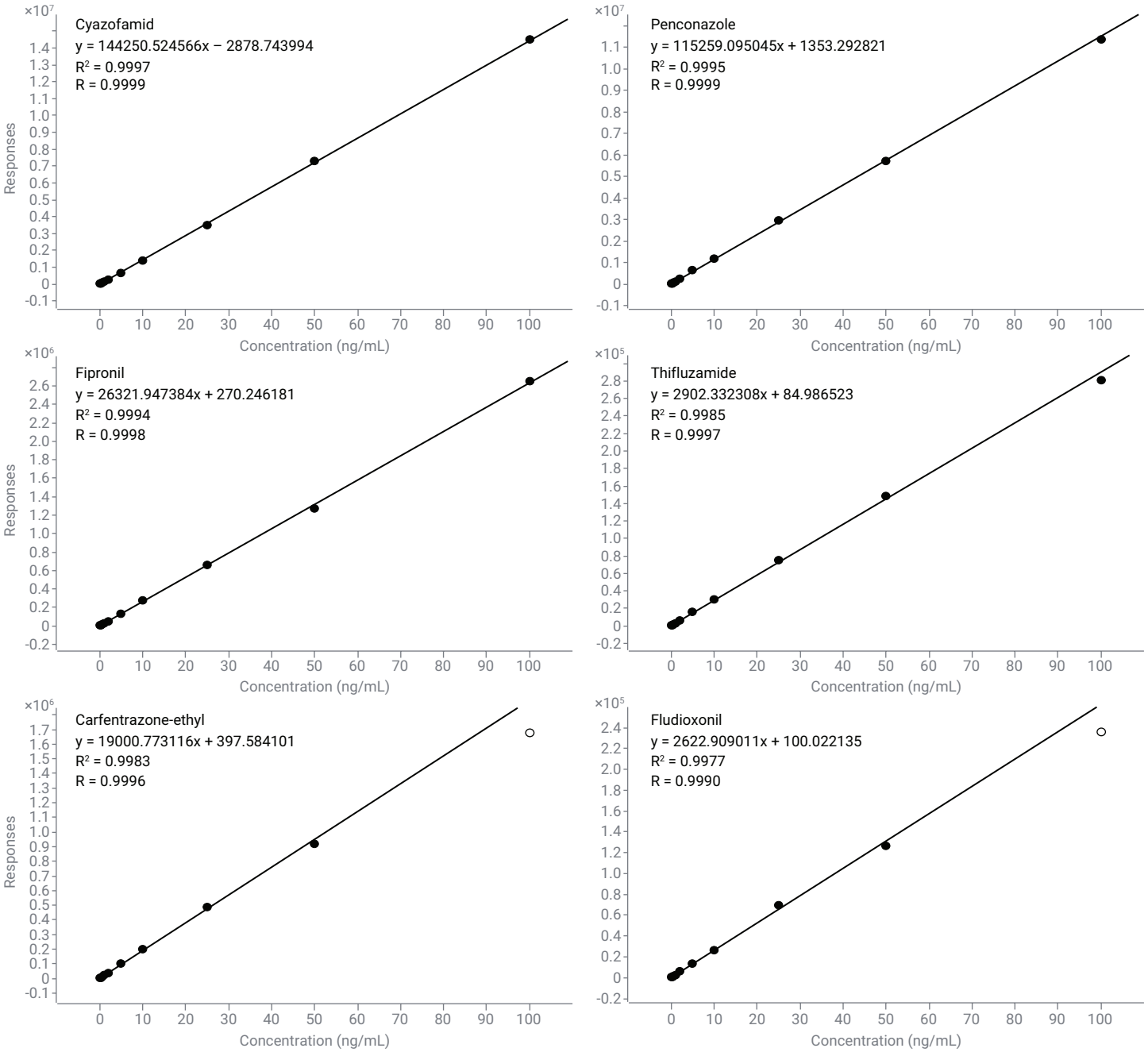

Figure 6. Calibration curves of six selected compounds in tomato matrix.

Some compounds (e.g., carfentrazone-ethyl and fludioxonil) showed saturation at the highest calibration level of 100 µg/L; therefore, this calibration level was omitted.

The 6495D triple quadrupole LC/MS system showed excellent sensitivity for the target analytes and, compared to the Agilent 6475 triple quadrupole LC/MS system without iFunnel, an MS signal increase of a factor of 10 was observed. Some examples are given in Figure 7, where this high-sensitivity increase is shown for the spiked tomato QuEChERS extracts.

The compounds displayed in Figure 7 show that at a level of 0.1 ng/mL in tomato matrix, signal-to-noise (S/N) ratios of ≥ 30 can be achieved for the selected compounds when using the 6495D LC/TQ, whereas the 6475 LC/TQ showed S/N ratios below 10.

Depending on each lab's requirements for detection limit, the 6495D and 6475/6470 are aligned to provide excellent analytical performance for the same number of analytes in one run for complex food matrices.

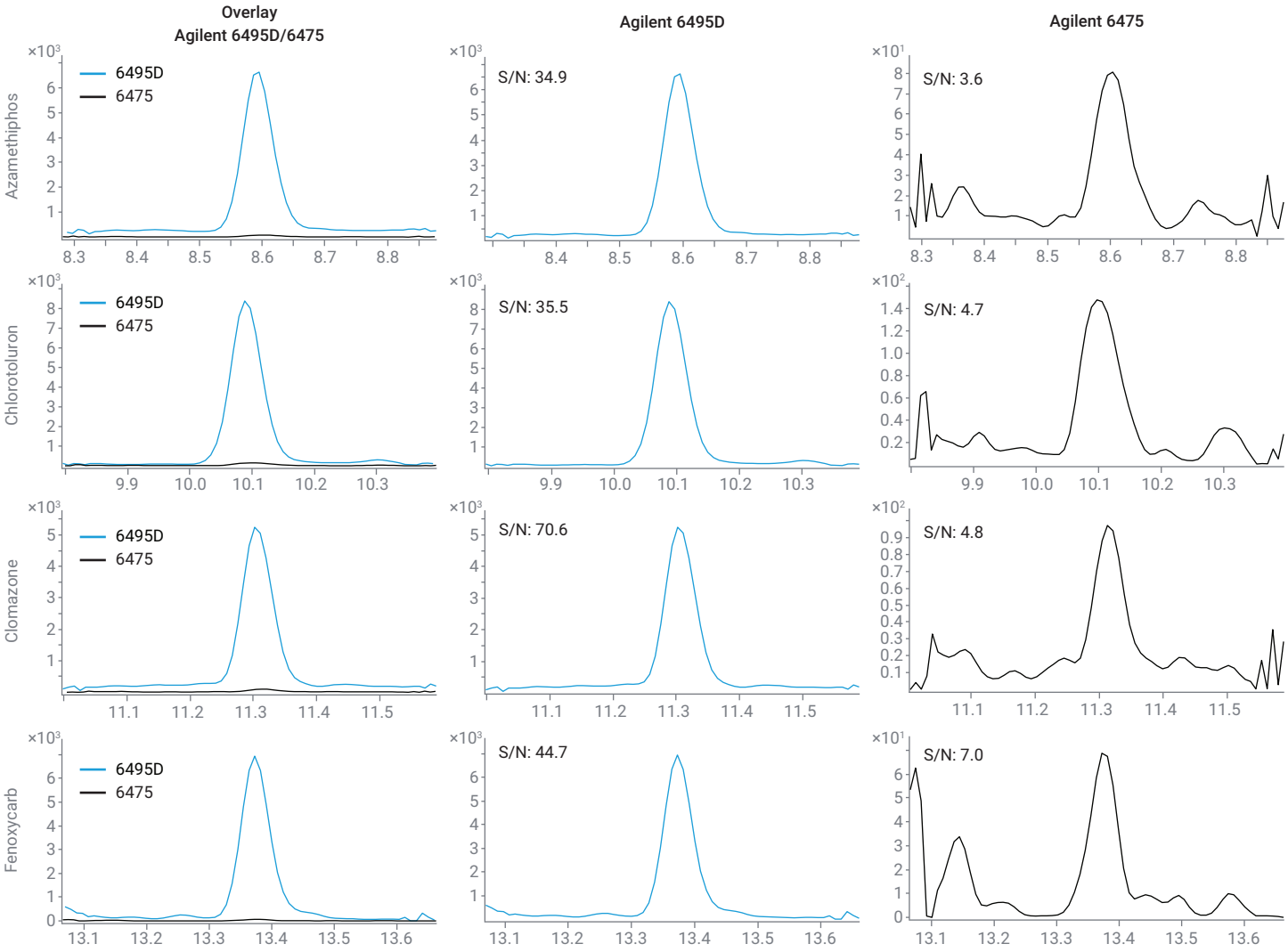

Figure 7. Observed analytical sensitivity increase on an Agilent 6495D LC/TQ compared to an Agilent 6475 LC/TQ for selected compounds (0.1 ng/mL in tomato extract).

Conclusions

This study demonstrates the performance of an Agilent 6495D triple quadrupole LC/MS system with an Agilent 1290 Infinity III LC for the analysis of 764 pesticides in tomato QuEChERS raw extract. Enhanced onboard computing enables increased acquisition speed and up to 150% more concurrent dMRMs for extended large-panel assays and shortened run times. With 4th-generation iFunnel technology and tunable settings, users can now select the best iFunnel setting for each compound transition to achieve optimal sensitivity. Good linearity was observed for all 764 target analytes, with $R^2 \geq 0.99$ for the calibration range 0.1 to 100 µg/L.

References

1. Regulation (EC) 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 and Amending Council Directive 91/414/EEC.
2. Kornas, P.; *et al.* Quantitation of 764 Pesticide Residues in Tomato by LC/MS according to SANTE 11312/2021 Guidelines. *Agilent Technologies application note*, publication number 5994-5847EN, **2023**.
3. Quantitative Analysis of Multi-Residue Pesticides in Food Matrices Using Agilent 6470 Triple Quadrupole LC/MS System – Method Protocol, **2022**.
4. SANTE 11312/2021: Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed.

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