Application Note

Food Testing & Agriculture

Pesticide Analysis in Unique Commodities using the Agilent 6470 Triple Quadruopole LC/MS System

Analysis of 250+ pesticide residues in coffee, ginger, turmeric, and pepper

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Abstract

This application note describes an LC/MS/MS method for the quantitation of pesticide residues in complex matrices such as coffee beans and various spices. Quantitation of pesticides in these types of matrices is extremely challenging because of the high amounts of irrelevant endogenous components, such as natural pigments and other phytochemicals. Matrix components in these commodities are known to cause matrix effects (ionization suppression or enhancement), resulting in inaccurate quantitation. A dilution of the final extract during sample preparation was found to be an effective strategy for reducing matrix effects.

The spice matrices tested in this application note were coffee, dried ginger, dried turmeric, and black pepper. Data acquisition was carried out using dynamic multiple reaction monitoring (dMRM) mode, an MRM technique that includes automated scheduling of MRM time segments, maximizing sensitivity while keeping enough data points across a chromatographic peak. This application note also demonstrates the features of Agilent MassHunter quantitative analysis software for a fast and efficient data review process, with features such as automated calculation of MRM ion ratio to avoid false-positive results.

Introduction

SANTE/12682/2019 has classified spices under difficult or unique commodity groups. Roasted coffee and dried spices fall within this group and are considered one of the difficult-to-analyze matrices, because of the dehydrated state in which they are received. QuEChERS has been a widely accepted method to extract pesticide residues from various food commodities. However, because of the large number of pesticides requiring monitoring and the constant reduction of maximum residue limits (MRL), sample preparation and analysis can become challenging.

In this application note, a highly selective dMRM-based LC/MS/MS method was developed using an Agilent 6470 triple quadrupole LC/MS (LC/TQ). The sensitivity of the 6470 LC/TQ provides enough flexibility to dilute the final extract while still enabling easy detection of compounds at their required MRLs. Features in the MassHunter quantitative analysis software, such as automatic MRM ion ratio calculation, batch at-a-glance view, and compounds at-a-glance view help the analyst efficiently review and confidently report results.

Sample matrices

Whole unroasted coffee beans (*Coffea arabica*), whole dried ginger (*Zingiber officinale*), whole dried turmeric (*Curcuma longa*), and whole black pepper (*Piper nigrum*) were the commodities selected for this study. These were collected from Wayanad district of Kerala state, southern India.

These samples were initially crushed with a mortar and pestle. Crushed samples were ground to fine powder with the help of a mixer grinder without much heating of the sample.

Experimental

Chemicals and reagents

The Agilent LC/MS pesticide comprehensive test mix (part number 5190-0551) was used for analysis. Agilent Bond Elut QuEChERS extraction kit (part number 5982-5755) and Agilent Bond Elut Dispersive SPE kit (part number 5982-5421) were used for sample preparation. Ammonium fluoride and ammonium formate were purchased from Sigma-Aldrich (now Merck). LC/MS-grade solvents such as methanol and water were purchased from Honeywell (Charlotte, NC, USA). MS-grade formic acid was purchased from Fluka (now of Honeywell).

Instrument configuration

- Agilent 1290 Infinity II high-speed pump (G7120A)
- Agilent 1290 Infinity II multisampler (G7167B)
- Agilent 1290 Infinity II multicolumn thermostat (G7116B)
- Agilent 6470 triple quadrupole LC/MS (G6470A)

Table 1. Chromatography conditions.

Parameter	Value
Column	Agilent ZORBAX RRHD Eclipse Plus C18, 150 mm × 3.0 mm, 1.8 µm (p/n 959759-302)
Mobile Phase A	0.5 mM ammonium fluoride and 4.5 mM ammonium formate + 0.1% formic acid in water
Mobile Phase B	0.5 mM ammonium fluoride and 4.5 mM ammonium formate + 0.1% formic acid in water: methanol (5:95, V/V)
Flow Rate	0.45 mL/min
Injection Volume	2 µL
Column Temperature	45 °C
Sample Diluent	Acetonitrile: water (60:40)
Needle Wash	MeOH: acetonitrile: water (25:50:25)
Gradient	**Time (min) / %A / %B** 0 / 95 / 5 0.5 / 95 / 5 3.5 / 50 / 50 17.0 / 0 / 100 20.0 / 0 / 100 20.1 / 95 / 5 22.0 / 95 / 5

Table 2. MS source parameters.

Parameter	Value
Ionization Source	AJS ESI
Ionization Mode	ESI Positive
Gas Temperature	250 °C
Gas Flow	16 L/min
Nebulizer	40 psi
Sheath Gas	350 °C
Sheath Gas Flow	12 L/min
Capillary Voltage	3,500 V
Nozzle Voltage	300 V

Sample preparation

2.0 g of pulverized sample was accurately weighed and transferred to a 50 mL centrifuge tube. 10 mL of water was added to the sample and allowed to soak for at least 30 minutes. 10 mL acetonitrile was added as an extraction solvent. After adding contents from the Bond Elut QuEChERS extraction kit, the entire mixture was vortexed for one minute at 2,500 rpm. Supernatant was collected after centrifugation at 6,000 rpm for six minutes. 1 mL of the supernatant was transferred to the Bond Elut dispersive SPE kit and vortexed for one minute. The mixture was centrifuged at 10,000 rpm for five minutes, then the supernatant was filtered through a 0.2 µm syringe filter. This final extract was diluted five times using acetonitrile: water (60:40).

A comprehensive pesticide test mix containing 253 LC/MS-amenable pesticides was diluted appropriately and used for generating the calibration curves. Nine concentration levels (0.05, 0.1, 0.2, 0.5, 1, 5, 10, 20, and 50 ng/mL) were prepared and analyzed.

Table 3. Dilution chart.

Working Standard Concentration (ng/mL)	Volume Taken (µL)	Diluent Volume (µL)	Obtained Concentration (ng/mL)
1,000	100	900	100
100	500	500	50
50	400	600	20
20	500	500	10
10	500	500	5
5	400	600	2
2	500	500	1
1	500	500	0.5
0.5	400	600	0.2
0.2	500	500	0.1

Data acquisition and data analysis

The pesticide analysis method development was aided using the Agilent pesticide tMRM database (G1733-G5862).

All samples were acquired using the Agilent MassHunter data acquisition software version 10.1.

Chromatograms were viewed through Agilent MassHunter qualitative analysis software version 10.0. Quantitation of each batch was carried out using MassHunter quantitative analysis software version 10.1.

Results and discussion

Organically grown coffee and spice samples were collected from farmers and the same preparation procedure was used for preparation of blank matrix. Extracted coffee blank and spice blank samples were spiked with different concentration levels of pesticides levels to prepare matrix-matched calibration curves. Calibration curves were made between concentration levels 0.1 to 50 ng/mL, with a linear regression of 1/x weighing. Regression coefficients were found to be more than 0.9950 for most compounds in this study. These mixtures were then used as standards for the determination of limits of quantification (LOQ), limits of detection (LOD), and analyte recovery experiments. LOD and LOQ for most of the compounds were determined to be 0.1 and 0.2 ng/mL, respectively.

Validation parameters such as calibration curve linearity, reproducibility, recovery, specificity, and sensitivity in terms of LOQ and LOD were characterized to ensure good method performance. Accuracies of each calibration point were within ±20%. No manual integration was needed.

Spiking procedure for recovery calculation

Spiking at the 10 ppb level was performed in coffee, turmeric, ginger, and pepper.

A 20 µL amount of 1 ppm (µg/mL) pesticide standard mix was spiked to 2 g of sample powder. The absolute quantity of pesticides present in 20 µL of 1 ppm pesticide mix is 20.0 ng. Therefore, when 20 ng is spiked to 2 g coffee powder, the spike level concentration becomes 20 ng/2 g = 10 ppb. During QuEChERS extraction, 10 mL of acetonitrile is added as extraction solvent. Therefore, the concentration becomes 20 ng/10 mL = 2 ng/mL. After the cleanup process, a further 1 mL is added to the 4 mL of diluent. The resultant concentration of the pesticide in the final extract becomes (2 ng/mL)/5= 0.4 ng/mL.

In other words, the overall dilution factor (per gram of sample) was 25. The effective final concentration injected to the system would become 10 ppb/25 = 0.4 ppb.

Recoveries of the representative pesticides in coffee matrix are given in the form of a RADAR plot. Recovery % varies from 54.5% (febuconazole) to 111% (avermectin B1A).

Sample analysis result

Coffee beans, turmeric whole, ginger whole, and black pepper whole were analyzed in triplicates. Overlaid chromatograms of three replicate injections of four commodities are shown in Figure 7, demonstrating the consistency in the results.

Chlorpyrifos was detected in the three spice samples: ginger, turmeric, and pepper. Carbendazim was present in turmeric and pepper. Metalaxyl was found in ginger and pepper. The ion ratios of the detected compounds in sample and in standard were within +30% as per SANTE/12682/2019.

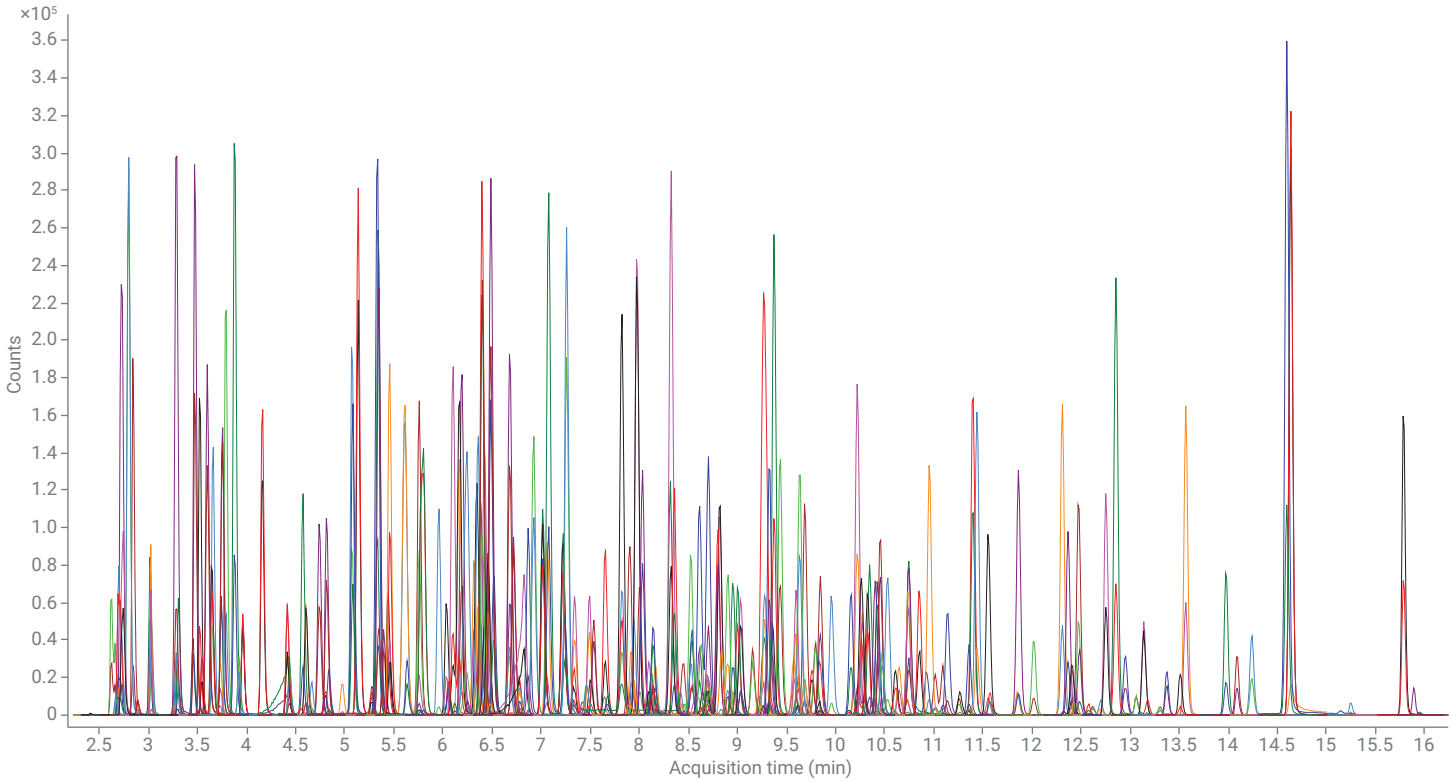

Figure 1. Extracted ion chromatogram (EIC) of matrix-matched standards at 10 ng/mL in coffee matrix.

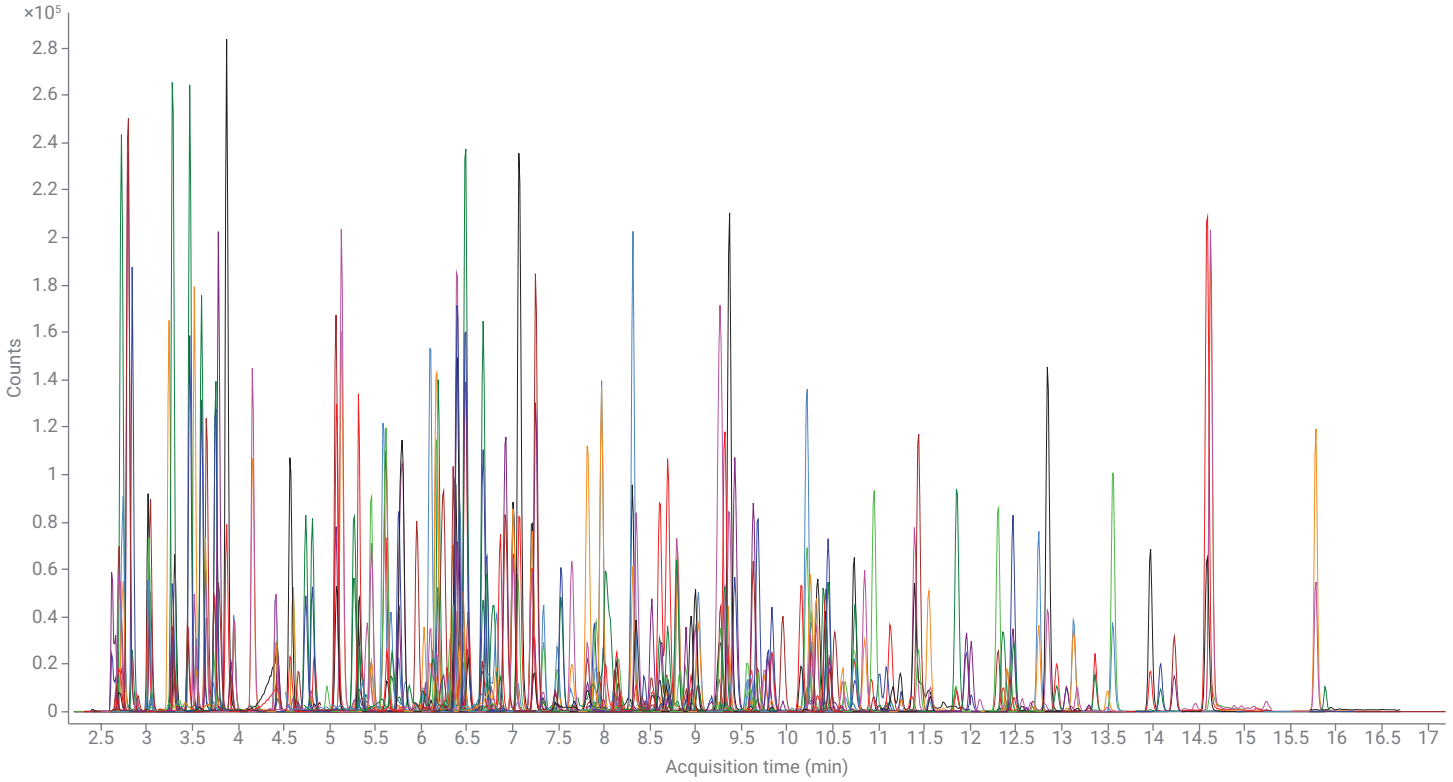

Figure 2. Extracted ion chromatogram (EIC) of matrix-matched standards at 10 ng/mL in turmeric matrix.

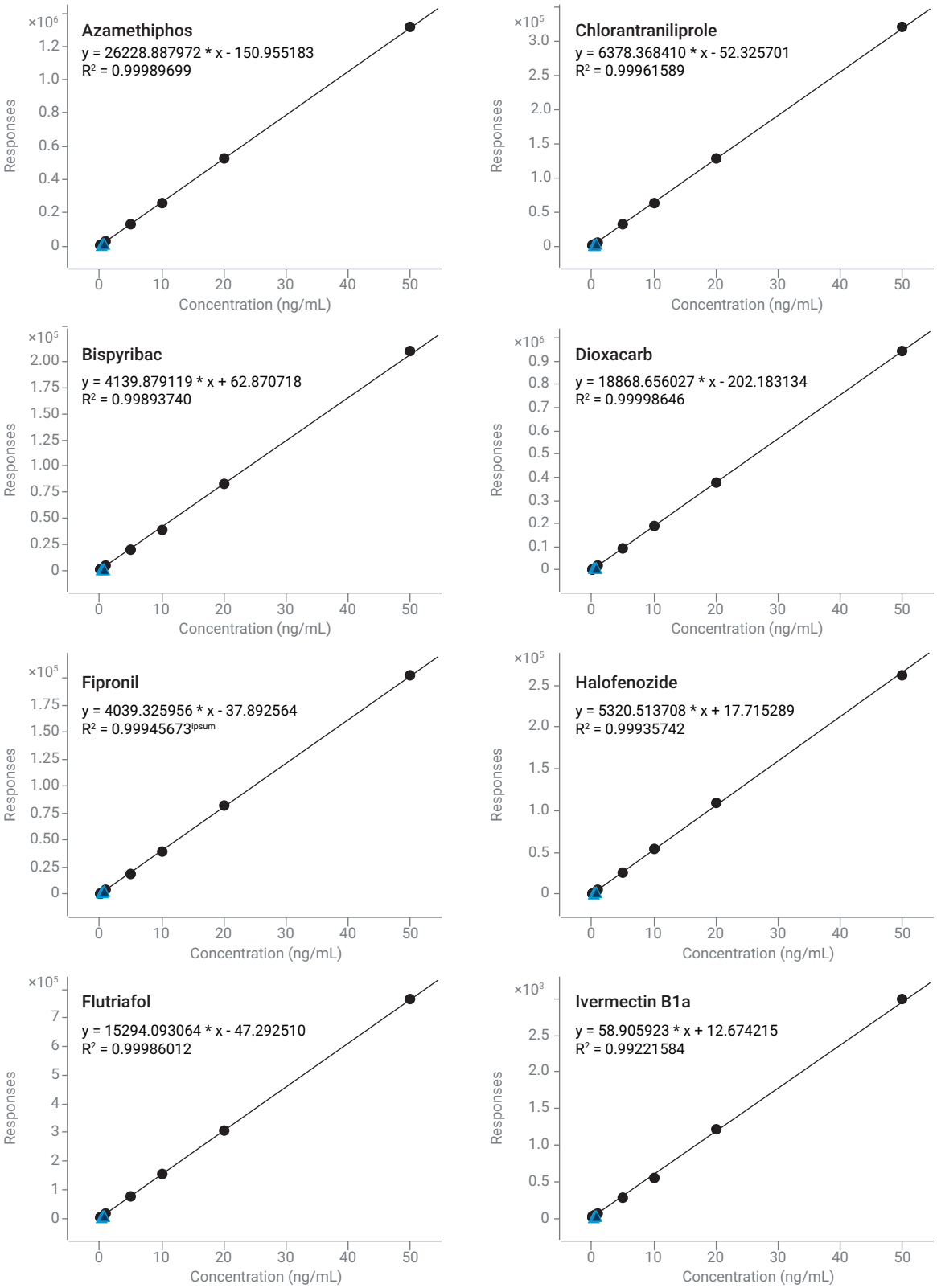

Figure 3. Representative matrix-matched calibration curves in ginger matrix.

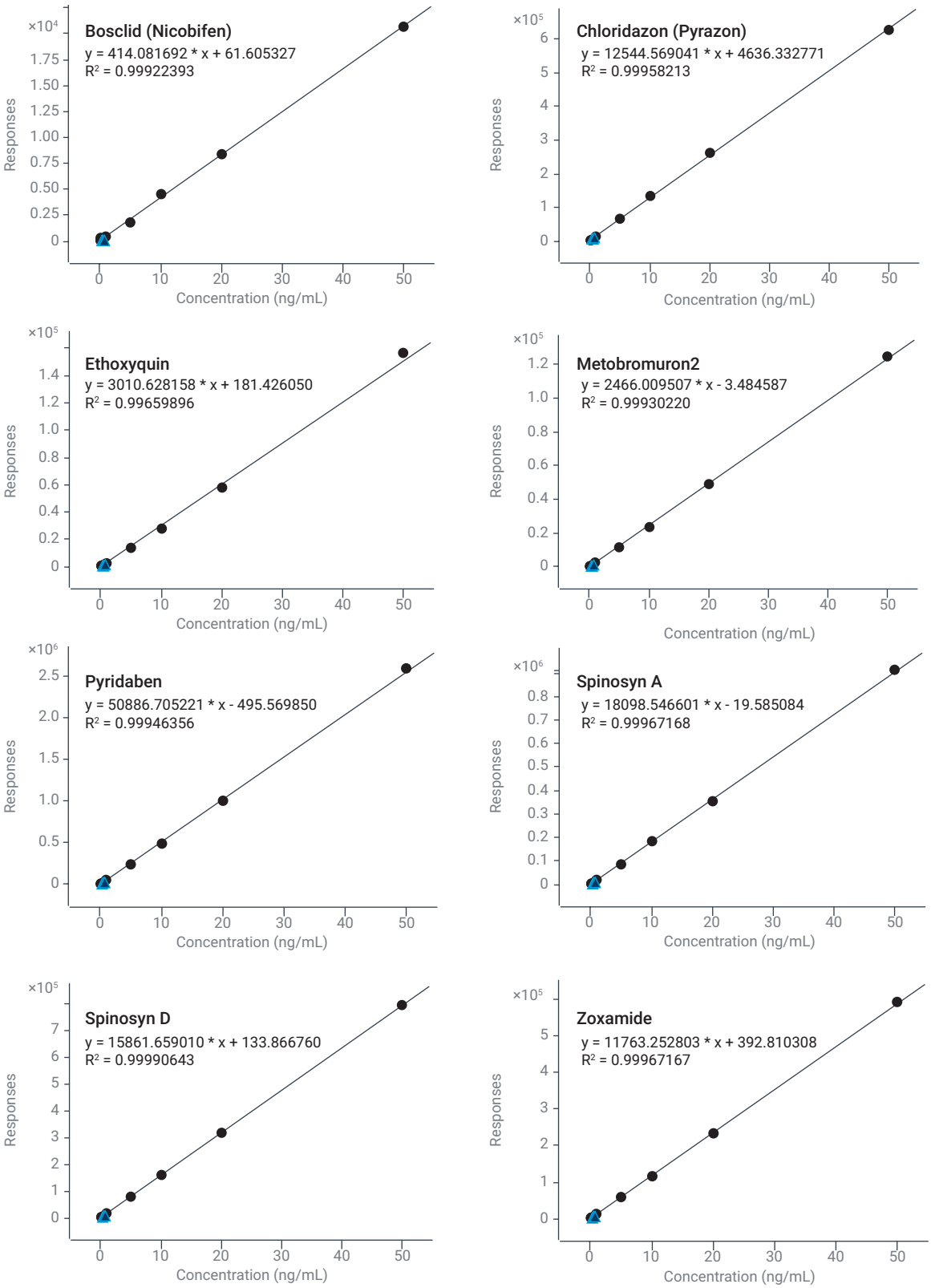

Figure 4. Representative matrix-matched calibration curves in pepper matrix.

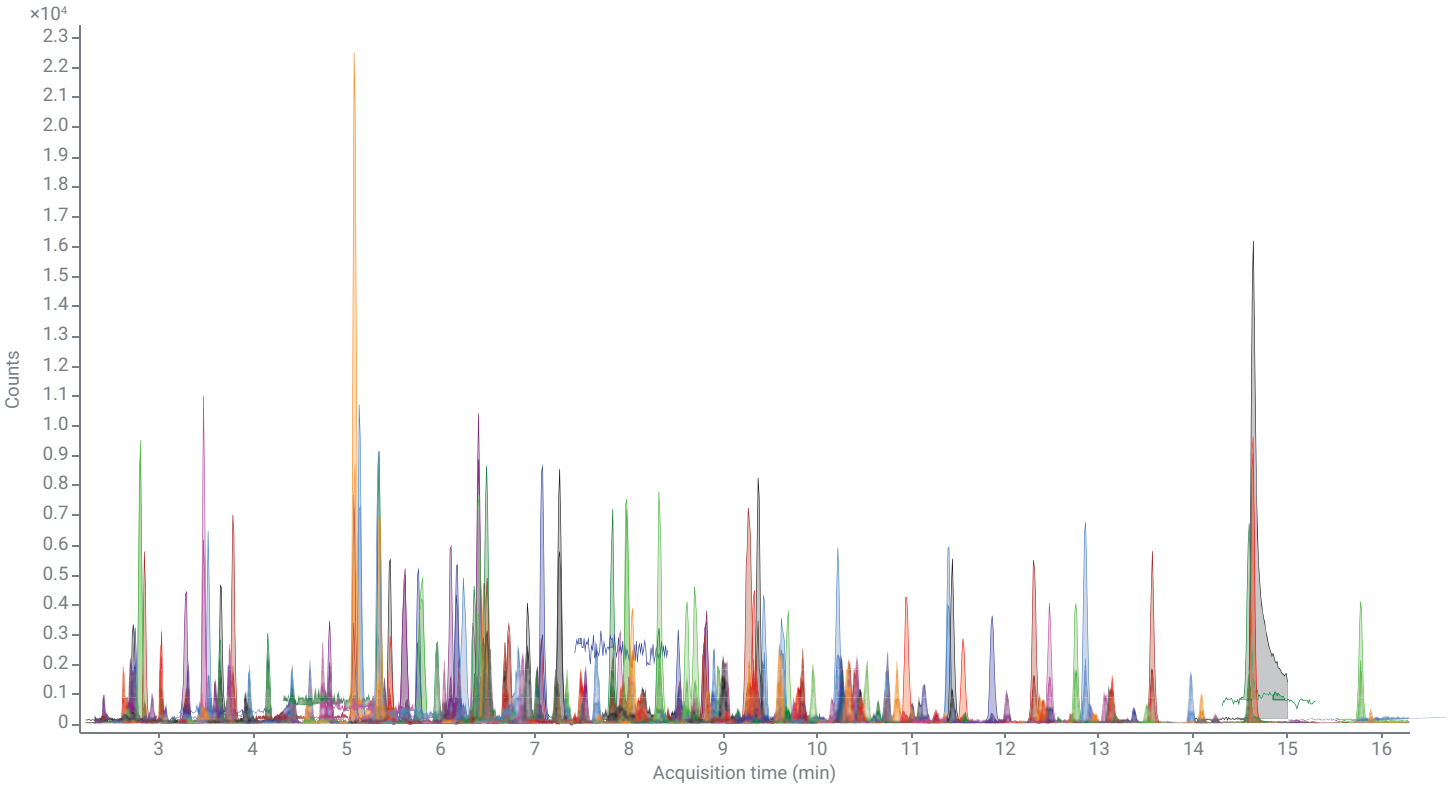

Figure 5. Extracted ion chromatogram (EIC) of pre-spike level at 10 ng in coffee matrix. Effective concentration is 0.4 ng/g.

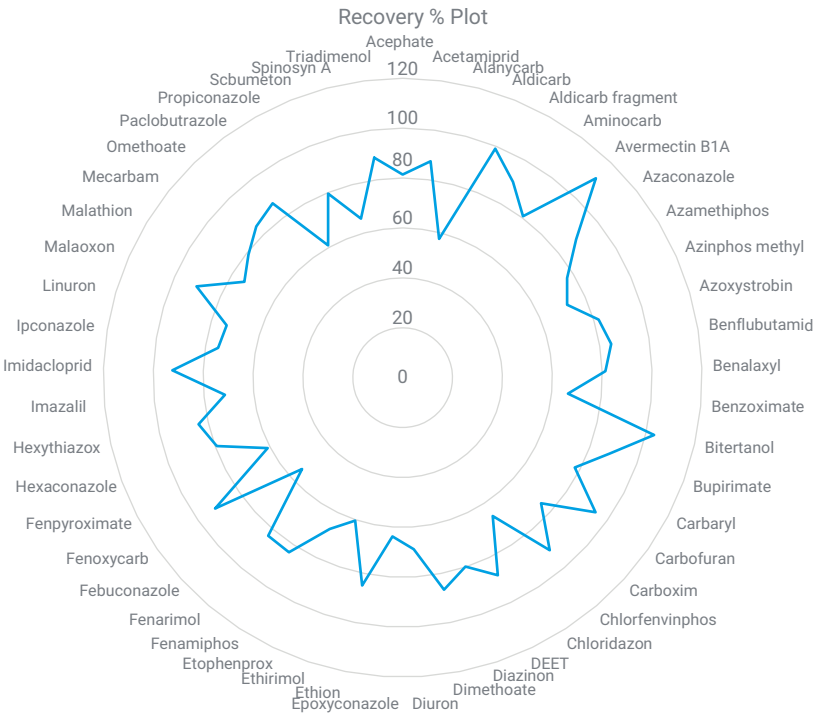

Figure 6. RADAR plot of recovery % for representative pesticides in coffee matrix.

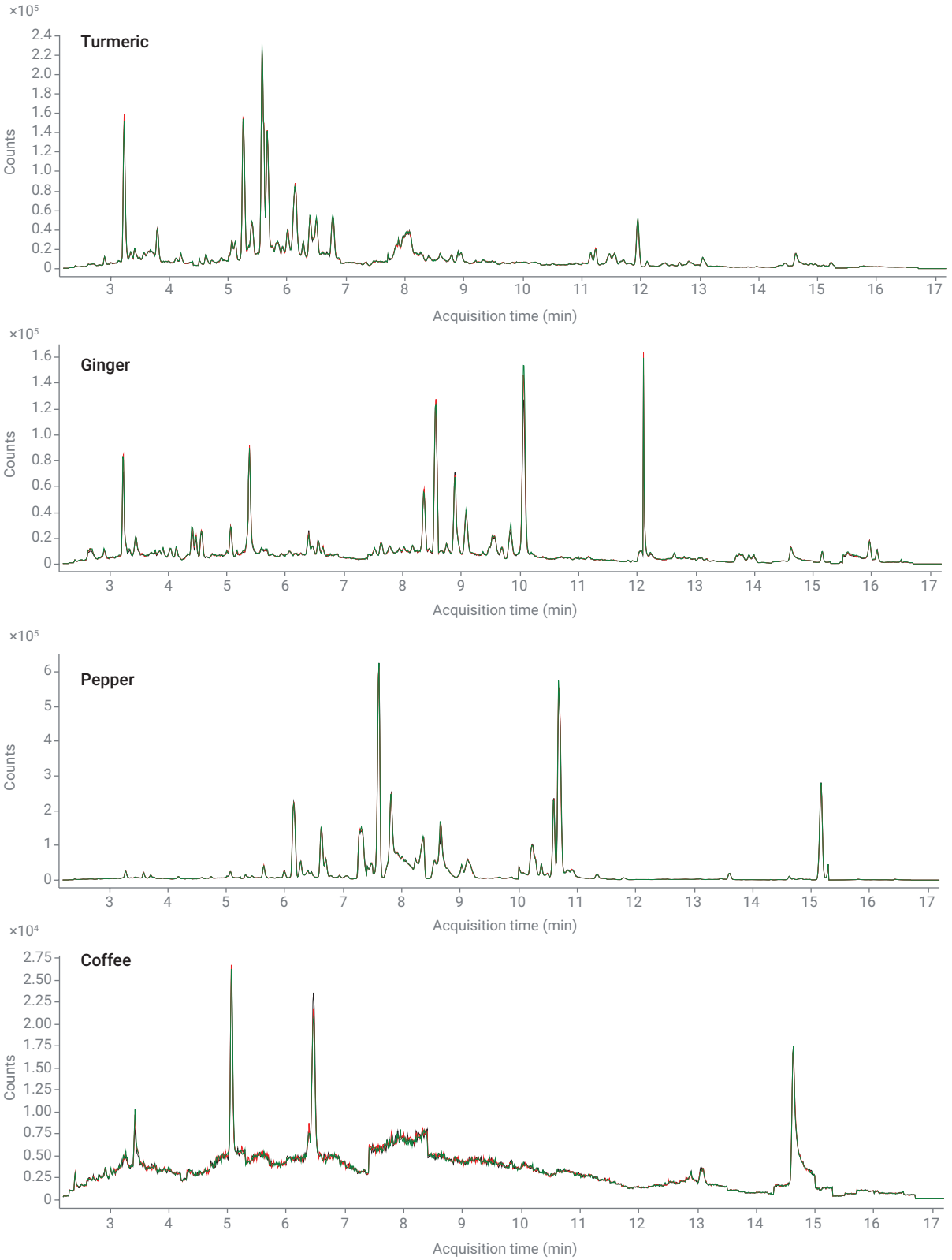

Figure 7. Overlaid chromatograms of triplicate injections of turmeric, ginger, pepper, and coffee samples.

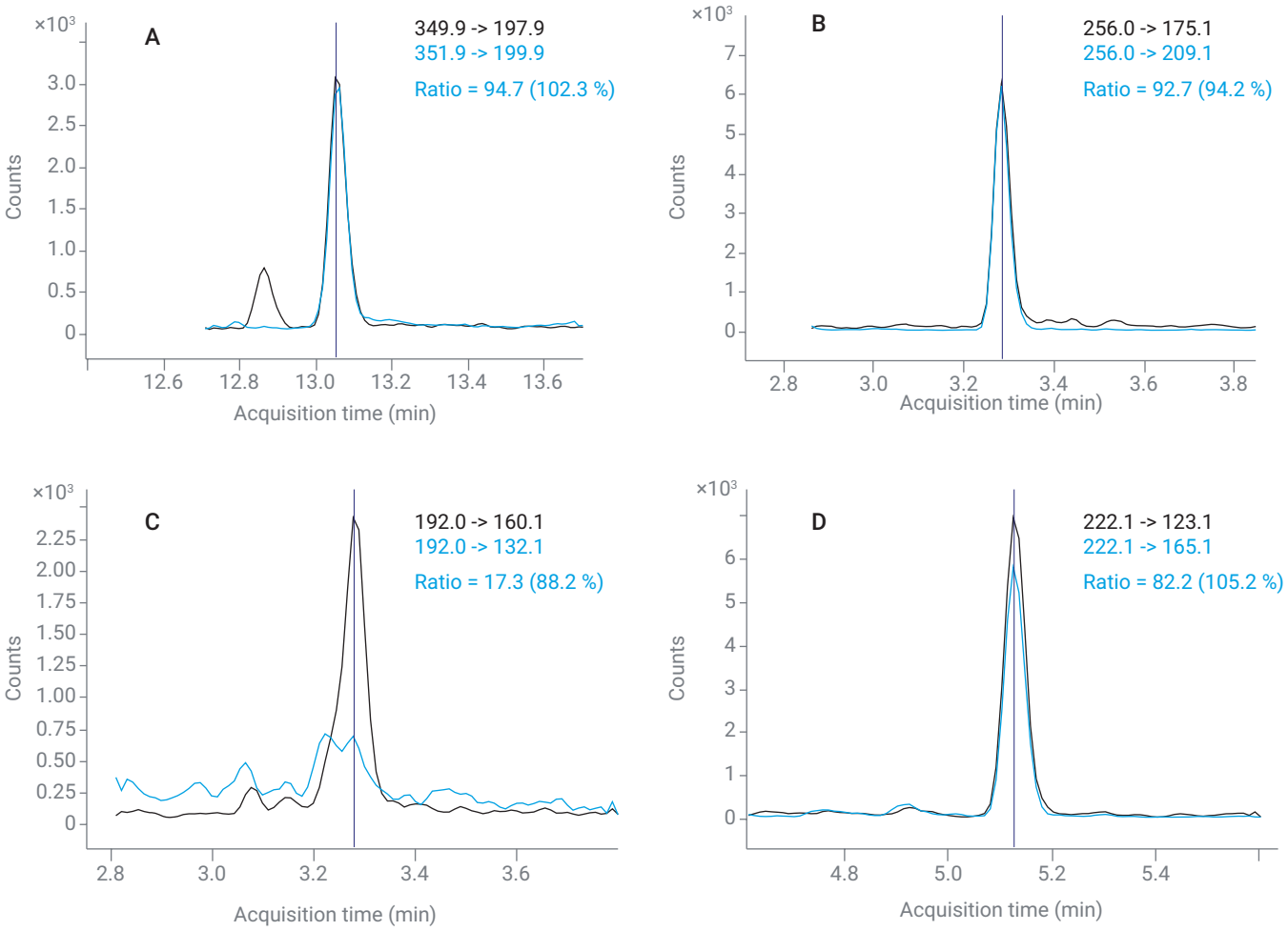

Figure 8. Ion ratio confirmation for (A) chlorpyrifos in turmeric; (B) imidacloprid in pepper; (C) carbendazim in turmeric; and (D) carbofuran in turmeric.

Figure 9. Compounds at-a-glance feature allows the user to review all compound chromatograms in a batch by compound name or by sample.

Table 4. Pesticide concentrations in food.

Commodity	Pesticide Found	Concentration of Pesticide (ng/g)
Coffee	Buprofezin	1.13
	Prosulfocarb	1.05
Pepper	Carbendazim	5.06
	Imidacloprid	63.85
	Metalaxyl	9.91
	Chlorpyrifos	4.01
Turmeric	Carbendazim	22.28
	Carbofuran	17.12
	Azoxystrobin	0.67
	Chlorpyrifos	24.5
Ginger	Metalaxyl	23.22
	Chlorpyrifos	12.0

Conclusion

A highly sensitive and robust dMRM method was developed to quantify pesticide residues in complex and unique matrices such as coffee and spice matrices—ginger, pepper, and turmeric. The dMRM-based method provided enough data points across the chromatographic peak, which maintains ideal Gaussian peak symmetry.

The developed method was partially validated to evaluate the method performance in terms of LOD, LOQ, specificity, linearity, reproducibility, and recovery. Samples were analyzed using the developed method and the detected pesticides were reported. This method will be useful for routine pesticide quantitation in complex matrices.

References

1. Sheshadri, A.; Glauner, T.; Faye, T. Multiclass Determination of Organic Contaminants in Red Chili and Turmeric Powders. *Agilent Technologies application note*, publication number 5991-8243EN, **2017**.
2. Stone, P. J. W. *et al.* Determination of Pesticides and Mycotoxins in Cannabis Flower as Defined by Legalized U.S. State Recreational Cannabis Regulations. *Agilent Technologies application note*, publication number 5994-1734EN, **2020**.
3. Zou, A. *et al.* Comprehensive LC/MS/MS Workflow of Pesticide Residues in Food Using the Agilent 6470 Triple Quadrupole LC/MS System. *Agilent Technologies application note*, publication number 5994-2370EN, **2020**.

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