Application Note

Food & Beverage Testing

Determination of Over 300 Pesticides in Cumin Powder

Using Captiva EMR–LPD passthrough cleanup and LC/MS/MS and GC/MS/MS detection

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Abstract

This application note presents the development and optimization of a method for the analysis of multiresidue pesticides in cumin powder. The method involves sample extraction with the Agilent Bond Elut QuEChERS EN extraction kit, followed by passthrough cleanup with the Agilent Captiva Enhanced Matrix Removal–Low Pigment Dry (EMR–LPD) cartridge, then LC/MS/MS and GC/MS/MS analysis. The newly developed method provided efficient matrix removal, acceptable target quantitation results, and a low failure rate for analysis of a large panel of pesticides in the challenging cumin matrix. Excellent method quantitation results were achieved for both LC-amenable pesticides (126) and GC-amenable pesticides (201), with 70 to 120% average recovery achieved for > 95% of targets, and < 20% average RSD for > 97% targets in cumin. The matrix removal assessment by dried residue weight indicated that ~ 60% of cumin co-extractives were removed. The passthrough cleanup was also demonstrated to be a simplified method that saves time and effort for analysts.

Introduction

Dry spices are consumed worldwide for both edible and medicinal purposes. However, the cultivation, storage, and production of spices usually involves the application of pesticides for control of pests, bacteria, and fungi. The wide use of pesticides has raised concerns about their impact on the environment and human health. Consequently, the use of pesticides needs to comply with existing national and/or regulatory agencies worldwide such as the European Union (EU) and Codex Alimentarius Commission (CAC).[1] Analysis of pesticides in spices therefore represents a critical practice for the safety and regulation of spices.

Dry spices are classified as difficult or unique commodities that significantly challenge reliable pesticide analysis[2,3], especially in sample preparation for simultaneous extraction of pesticides and matrix removal. The most common methods use QuEChERS or modified QuEChERS extraction followed by dispersive solid phase extraction (dSPE), plus other cleanup methods.[3,4]

Agilent Captiva EMR with Carbon S cartridges apply passthrough cleanup methodology for fast and efficient sample matrix removal. The Captiva EMR–General Pigmented Dry (EMR-GPD) and EMR–Low Pigmented Dry (EMR–LPD) cartridges are designed for cleanup of complex, dry matrices. Both cartridges contain the Agilent proprietary sorbents Carbon S and Captiva EMR–Lipid, blended with primary secondary amine (PSA) and C18 into an optimized formula for sample cleanup.

- Captiva EMR–Lipid provides highly selective and efficient lipid removal.
- PSA provides efficient fatty acid removal.
- Carbon S provides efficient pigment removal.
- EC-C18 provides further hydrophobic matrix cleanup.

The blended formula was carefully developed and optimized to deliver the best balance between matrix removal and target recovery for complex dry matrices with different levels of pigment components. For general pigmented dry matrix, Captiva EMR–GPD is usually recommended, while for low pigmented dry matrix, Captiva EMR–LPD is recommended.

QuEChERS extraction followed by Captiva EMR–GPD passthrough cleanup has been shown to be successful for pesticide analysis in both cayenne pepper[5] and cinnamon[6], since these two spices contain more pigment interferences. Cumin is not a heavily pigmented spice, thus Captiva EMR–LPD passthrough cleanup is better suited to this spice matrix. In this study, QuEChERS extraction followed by EMR–LPD passthrough cleanup was used for the analysis of over 300 common pesticides by LC/MS/MS and GC/MS/MS.

Experimental

Chemicals and reagents

Pesticide standards and internal standards (IS) were either obtained as the standard mix stock solutions from Agilent Technologies (part number 5190-0551) and Restek (Bellefonte, PA, U.S.), or as individual standard stock solutions or powder from Sigma-Aldrich (St. Louis, MO, U.S.). HPLC-grade acetonitrile (ACN) was from Honeywell (Muskegon, MI, U.S.). Reagent-grade acetic acid (AA), ammonium acetate, and ammonium fluoride were also from Sigma-Aldrich.

Solutions and standards

A combined LC- and GC-standard spiking solution, and an IS spiking solution were prepared at 10 µg/mL in 1:1 ACN:water or ACN only and stored at –20 °C in a freezer. The standard spiking solutions were warmed up thoroughly to room temperature, sonicated before use, and returned after use.

The ACN with 1% AA extraction solvent was prepared by adding 10 mL glacial AA into 990 mL ACN and storing at room temperature.

Equipment and material

The LC/MS/MS study was performed using an Agilent 1290 Infinity LC system coupled to an Agilent 6490 triple quadrupole LC/MS (G6490). The 1290 Infinity LC system consisted of an Agilent 1290 Infinity binary pump (G4220A), an Agilent 1290 Infinity autosampler (G4226A), and an Agilent 1290 Infinity thermostatted column compartment (G1316C). The 6490 LC/TQ was equipped with an Agilent Jet Stream Electrospray ion source. Agilent MassHunter Workstation software was used for data acquisition and analysis.

The GC/MS/MS study was performed using the Agilent 8890 GC coupled with an Agilent 7000E triple quadrupole GC/MS. The GC was configured with the Agilent 7693A automatic liquid sampler (ALS) and 150-position tray. The system used a multimode inlet (MMI). The mid-column backflush configuration was set up using two identical 15 m columns connected by Agilent purged ultimate union (PUU) and controlled by the 8890 GC pneumatic switching device (PSD) module. See the application note by Andrianova[7] for the 7000E GC/TQ configuration. Data were acquired in dynamic MRM (dMRM) mode. The acquisition method was retention time locked to match the retention times in the Agilent MassHunter pesticides and environmental pollutants MRM database (P&EP), version 4, which was used to seamlessly create the MS method. Agilent MassHunter Workstation software was used for data acquisition and analysis.

Other equipment used for sample preparation included: a Centra CL3R centrifuge (Thermo IEC, MA, U.S.), a Geno/Grinder (SPEX, NJ, U.S.), a Multi Reax test tube shaker (Heidolph, Schwabach, Germany), pipettes and a repeater (Eppendorf, NY, U.S.), an Agilent positive pressure manifold 48 processor (PPM-48; part number 5191-4101), the Agilent Bond Elut QuEChERS EN extraction kit (part number 5982-5650), and the Agilent Captiva EMR–LPD cartridge, 6 mL (part number 5610-2092).

Instrument conditions

Table 1 lists the LC/MS/MS conditions. Table 2 lists the GC/MS/MS conditions. For dMRM parameters, refer to the application note by Zhao[8] for LC/MS/MS conditions and the P&EP, version 4 (part number G9250AA) for GC/MS/MS conditions.

Table 1. Agilent 1290 Infinity LC and Agilent 6490 triple quadrupole LC/MS method conditions.

LC Conditions	
Columns	Agilent ZORBAX Eclipse Plus C18 column, 2.1 × 100 mm, 1.8 µm (p/n 959758-902) Agilent ZORBAX Eclipse Plus C18 column, UHPLC guard, 2.1 × 5 mm, 1.8 µm (p/n 821725-901)
Flow Rate	0.3 mL/min
Column Temperature	40 °C
Injection Volume	2 µL
Mobile Phase	A) 10 mM Ammonium formate, 0.5 mM ammonium fluoride in water, 0.125% FA B) 10 mM Ammonium formate, 0.5 mM ammonium fluoride in 95:5 ACN:water, 0.125% FA
Needle Wash	1:1:1:1 ACN:MeOH:IPA:water, 0.2% formic acid
Gradient	Time (min) %B Flow (mL/min) 0.0 15 0.3 6.0 95 0.3 8.01 100 0.3
Stop Time	10 min
Post Time	2.3 min
MS Conditions	
Ionization Mode	Electrospray ionization (ESI)
Gas Temperature	120 °C
Gas Flow	20 L/min
Nebulizer	40 psi
Sheath Gas Heater	225 °C
Sheath Gas Flow	11 L/min
Capillary Voltage	4,500 V (positive and negative)
Nozzle Voltage	0 V (both positive and negative)
iFunnel Parameters	High-pressure RF: 150 V (+), 90 V (–)
	Low-pressure RF: 60 V (+), 60 V (–)
Polarity	Positive and negative, see Table 4 from reference 1.

Table 2. Agilent 8890 GC and 7000E triple quadrupole GC/MS method conditions.

Parameter	Value
Columns	Two Agilent HP-5ms UI, 15 m × 0.25 mm, 0.25 µm film thickness (p/n 19091S-431UI-KEY)
Carrier Gas	Helium
Column 1 Flow	1.016 mL/min
Column 2 Flow	1.216 mL/min
Injection Volume	1 µL cold splitless
Inlet Liner	Agilent Ultra Inert dimpled liner, 2 mm (p/n 5190-2297)
MMI Temperature Program	60 °C for 0.1 min, 600 °C/min to 280 °C and hold
Oven Temperature Program	60 °C for 1 min, 40 °C/min to 170 °C, then 10 °C/min to 310 °C and hold for 2.25 min
Run Time	20 min
Backflush Conditions	1.5 min postrun 310 °C oven temperature Postrun total flow 25 mL/min
Transfer Line Temperature	280 °C
Source	Inert extractor source with a 3 mm lens, 280 °C
Vacuum Pump	Performance turbo
Quadrupole Temperature	150 °C
Data Monitoring	dMRM
EM Voltage Gain Factor	10
Solvent Delay	3 min

Sample preparation

The organic cumin powder was purchased from a local grocery store. The 2 g of cumin powder was extracted with QuEChERS EN extraction, followed with matrix passthrough cleanup on the Captiva EMR–LPD 6 mL. The appropriate post-treatment was applied to prepare the sample eluent for LC/MS/MS and GC/MS/MS detection. The detailed sample preparation procedure is shown in Figure 1. The entire sample preparation procedure resulted in a 5x dilution factor.

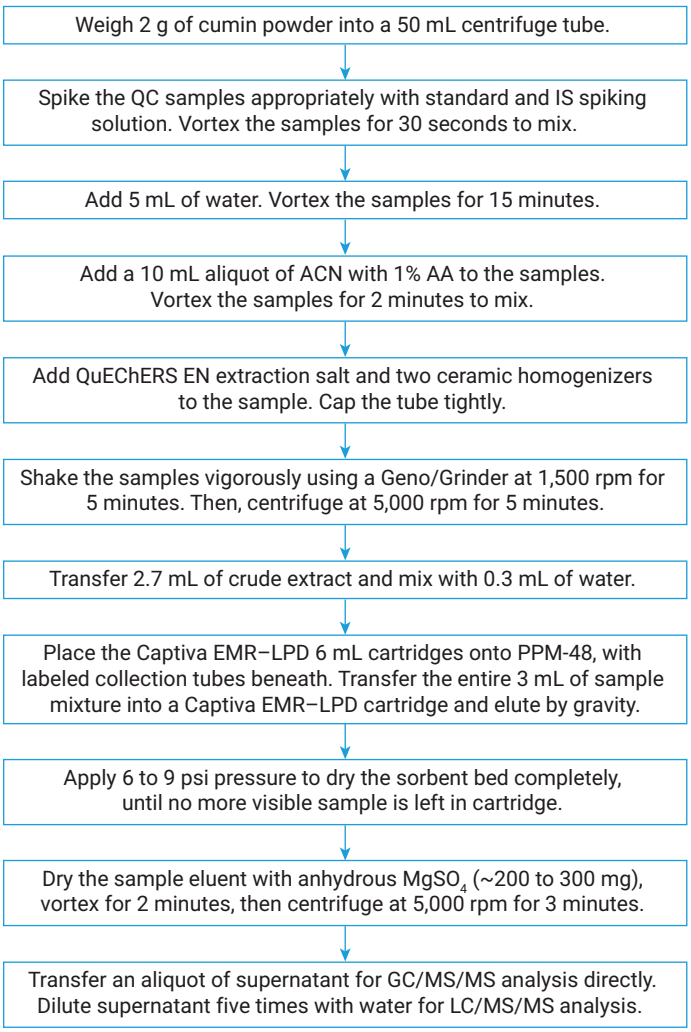

Figure 1. Sample preparation procedure for cumin powder samples by Agilent Bond Elut QuEChERS EN extraction followed by Agilent Captiva EMR–LPD passthrough cleanup.

Method performance evaluation

The developed sample preparation method was evaluated in terms of matrix removal, target recovery, reproducibility, matrix effect, and matrix-matched calibration curve linearity and limits of quantitation (LOQs) in cumin. To evaluate recovery, reproducibility, and matrix effect, prespiked quality control (PR-QC) samples were prepared at 10 and 100 ng/g in cumin in replicates of six, corresponding to 2 and 20 ng/mL in crude sample extract after extraction. The spiked samples and matrix blank samples were then prepared using the developed method. Postspiked QCs (PO-QC) were prepared in matrix blank extract before water dilution, corresponding to 2 and 20 ng/mL. Neat QCs were directly spiked at 2 and 20 ng/mL in reagent blank (ACN with 1% AA), using LC-standard spiking solution only, then diluted appropriately with water. Six replicates of each type of QC were prepared. The peak area ratios of corresponding targets in PR-QCs versus PO-QCs were used to calculate target recovery. The peak areas in PR-QCs were used for the sample preparation method reproducibility RSD calculation. The peak area ratios of corresponding targets in PO-QCs versus neat QCs were used for target matrix effect calculation. Matrix-matched calibration curve linearity and LOQs were evaluated by postspiking at the levels of 0.5, 1, 2, 5, 10, 50, 100, 250, 400, and 500 ng/mL in cumin matrix blank extract, corresponding to 2.5 to 2,500 ng/g in cumin. Analyte identification, confirmation, and quantitation were determined from retention times and MRM transitions.

Results and discussion

Captiva EMR–LPD passthrough versus dSPE cleanup

The Captiva EMR–LPD passthrough cleanup was shown to be an excellent alternative matrix cleanup method after traditional QuEChERS extraction to replace a typical dSPE cleanup. The passthrough workflow provides an easy, multimode chemical filtration, where the unwanted matrix co-extractives are removed efficiently and selectively, but the targeted pesticides are passed through for analysis. Compared to a traditional dSPE cleanup procedure, the Captiva EMR–LPD passthrough cleanup is a simplified method. Captiva EMR–LPD saves time and effort by obsoleting many of the steps needed in the dSPE procedure, such as multiple sample transfers, centrifuging, and capping and uncapping of dSPE tubes. It also significantly improves the sample volume recovery from ~50% on dSPE cleanup to > 90% on EMR passthrough cleanup.

Cumin is a relatively less pigmented spice matrix than other spices such as cayenne pepper and cinnamon. Therefore, Captiva EMR–LPD was used for this spice matrix cleanup.

The cleanup method performance was compared based on target recovery, reproducibility (RSDs), and matrix effect. The two dSPE kits being compared have similar sorbent components, with variations in sorbent amount, where dSPE 1 contains more PSA, GCB, and C18 than dSPE 2. Figure 2 shows the evaluation results for matrix cleanup evaluation. The cumin extract after QuEChERS was yellow, then turned very light yellow and transparent after cleanup. The color of sample extract after EMR–LPD and dSPE 2 were similar, but the color of sample extract after dSPE 1 was slightly lighter, indicating more pigment removal through this cleanup. The dried residue weight study was also conducted. Per 1 mL of sample extract, the Captiva EMR–LPD passthrough cleanup removed 60% of total matrix co-extractives, while dSPEs 1 and 2 removed 55% and 37% respectively. Figure 2C shows sample extract chromatograms collected through the GC/MS full scan, where the lowest chromatographic background was obtained by sample preparation with Captiva EMR–LPD cleanup (shown in blue).

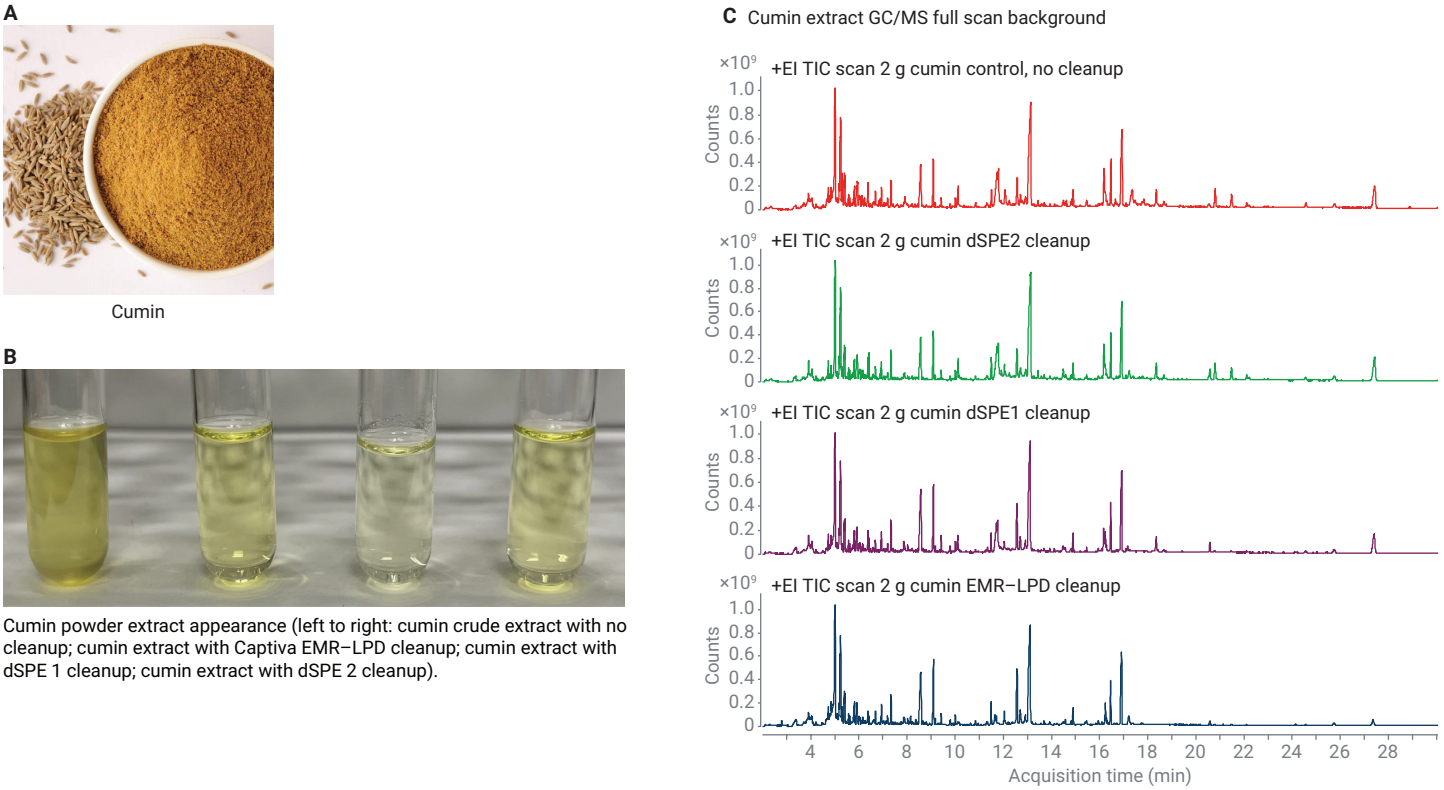

Figure 2. Preliminary study on cumin matrix. (A) Appearance of typical cumin seeds and powder. (B) Crude extract after QuEChERS extraction. (C) GC/MS full scan chromatographic background.

Figure 3 shows the recovery of sensitive pesticides during sample cleanup. Captiva EMR–LPD cleanup showed significant improvement on the recovery of sensitive pesticides, especially for labile pesticides that are acidic or basic. Compared to dSPE cleanups 1 and 2, the recoveries of acidic pesticides such as 2,4-D, 2,4,5-T, MCPA, dichlorprop, and mecoprop were at least doubled using Captiva EMR–LPD. Recovery of other sensitive pesticides such as pymetrozine, nicosulfuron, bentazon was also significantly increased. The dSPE 1 showed slightly higher pigment removal in cumin matrix but caused significant loss of sensitive pesticides. The dSPE 2 compromised significantly on matrix removal by using less sorbents, but the heavier matrix co-extractives, especially for more hydrophobic interferences, caused many false positives for later-eluted pesticides, resulting in an unacceptably high recovery of > 120%.

Matrix effect using LC/MS/MS is closely related to sample extract cleanliness, and poor matrix removal efficiency usually can cause significant matrix effect on the targets. Figure 4 shows the partial targets' matrix effect on LC/MS/MS for cumin sample extracts spiked with pesticides at 20 ng/mL. The results indicate that cumin samples that were cleaned up with Captiva EMR–LPD provided the best matrix effect results on targets. However, samples cleaned with dSPE 2 resulted in many significant matrix suppressions on targets, especially for later-eluted targets. Samples cleaned with dSPE 1 delivered better results on matrix effect than samples cleaned with dSPE 2, but it caused more loss of sensitive pesticides.

Method quantitation performance assessment

The method quantitation performance was evaluated by target recovery, reproducibility, and matrix effect on LC/MS/MS, as well as matrix-matched calibration linearity and limits of quantitation (LOQs). The targeted pesticides included 126 LC-amenable pesticides using LC/MS/MS detection and 201 GC-amenable pesticides using GC/MS/MS detection.

Target recovery, reproducibility, and matrix effect: These parameters are directly related to method quantitation accuracy and data quality. Therefore, it is important to use these parameters to demonstrate quantitation method performance. The SANTE/11312/2021 guideline was referred to for method performance assessment.[1] Figure 5 shows the individual target results at 10 and 100 ng/g in cumin for pesticide recovery, reproducibility (RSD), and matrix effect (LC/TQ only) with detection using LC/MS/MS and GC/MS/MS. Results were calculated based on the average of 10 and 100 ng/g spiking levels, with six replicates at

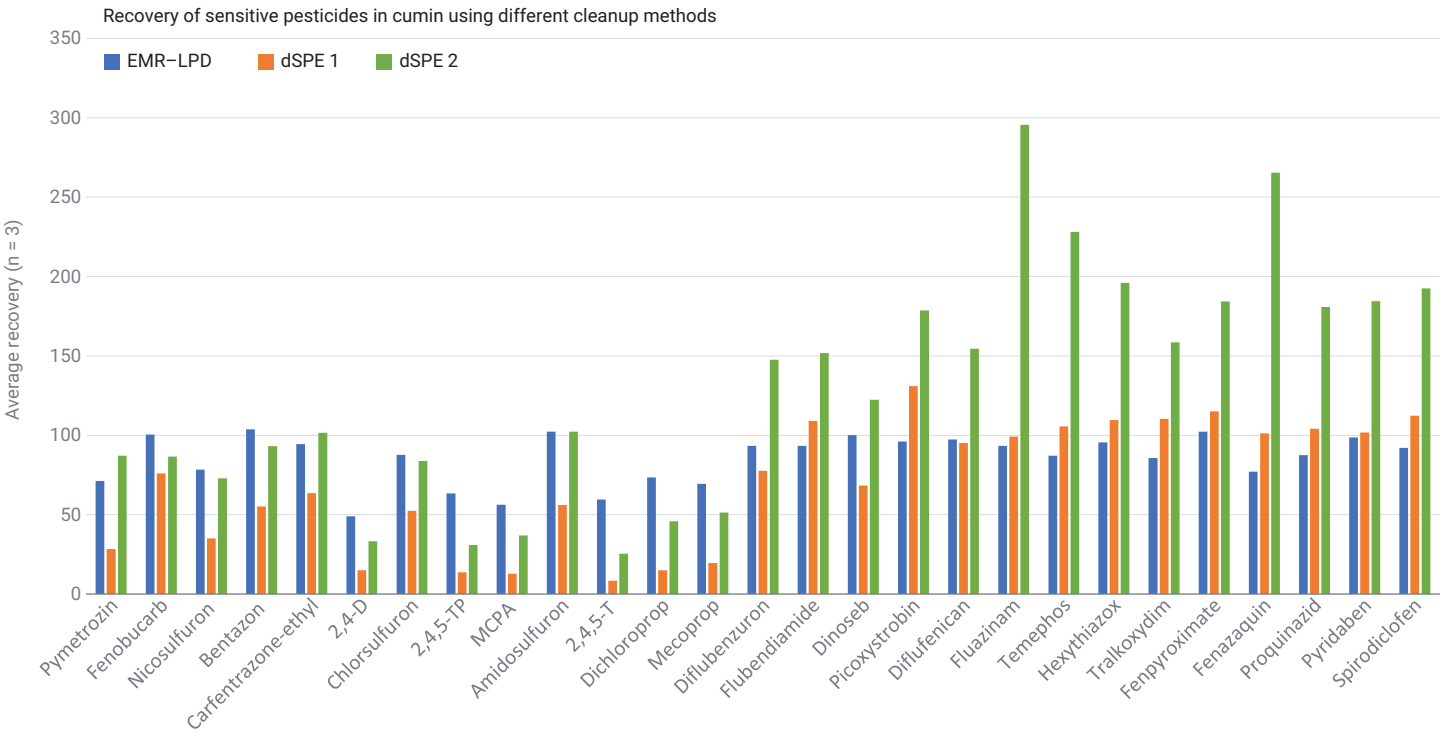

Figure 3. Recovery comparison for sensitive pesticides in spiked cumin sample crude extract after extraction using different matrix cleanup methods. Pesticide standard was spiked at 20 ng/mL in cumin crude extract.

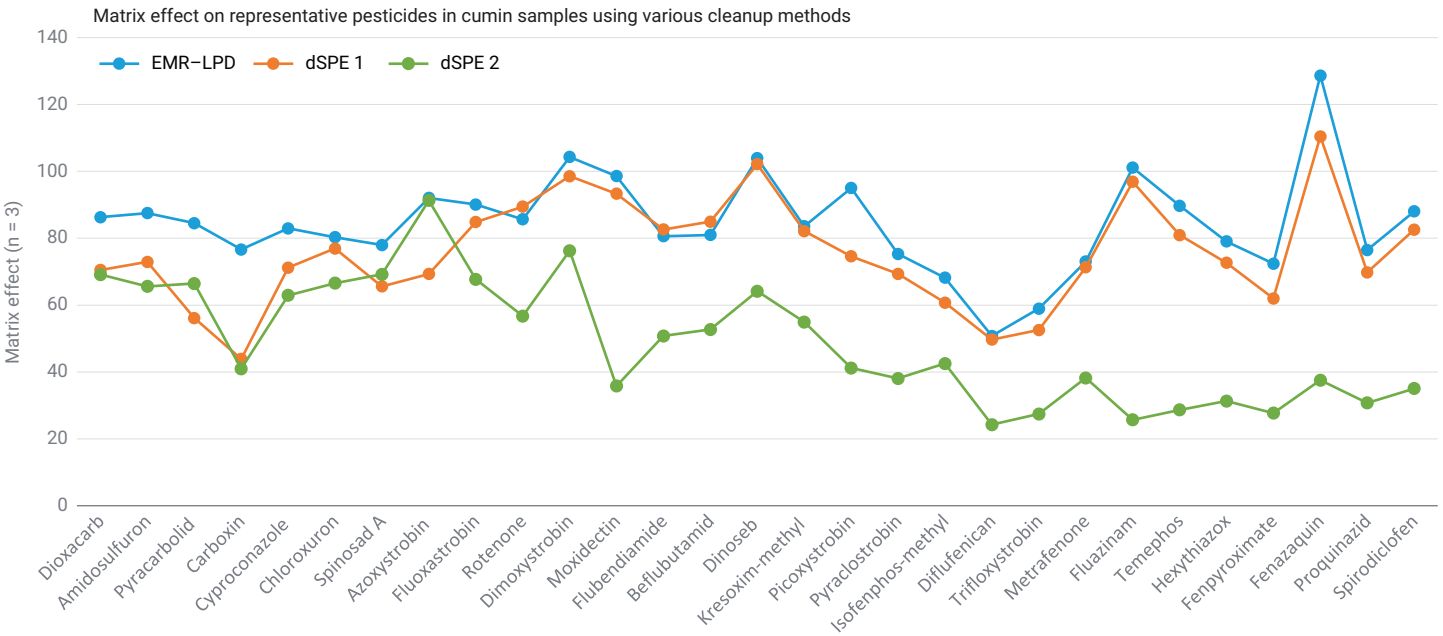

Figure 4. Matrix effect comparison for representative pesticides spiked in cumin sample extract prepared with different matrix cleanup methods. Pesticide standard was postspiked at 20 ng/mL in cumin extract after matrix cleanup.

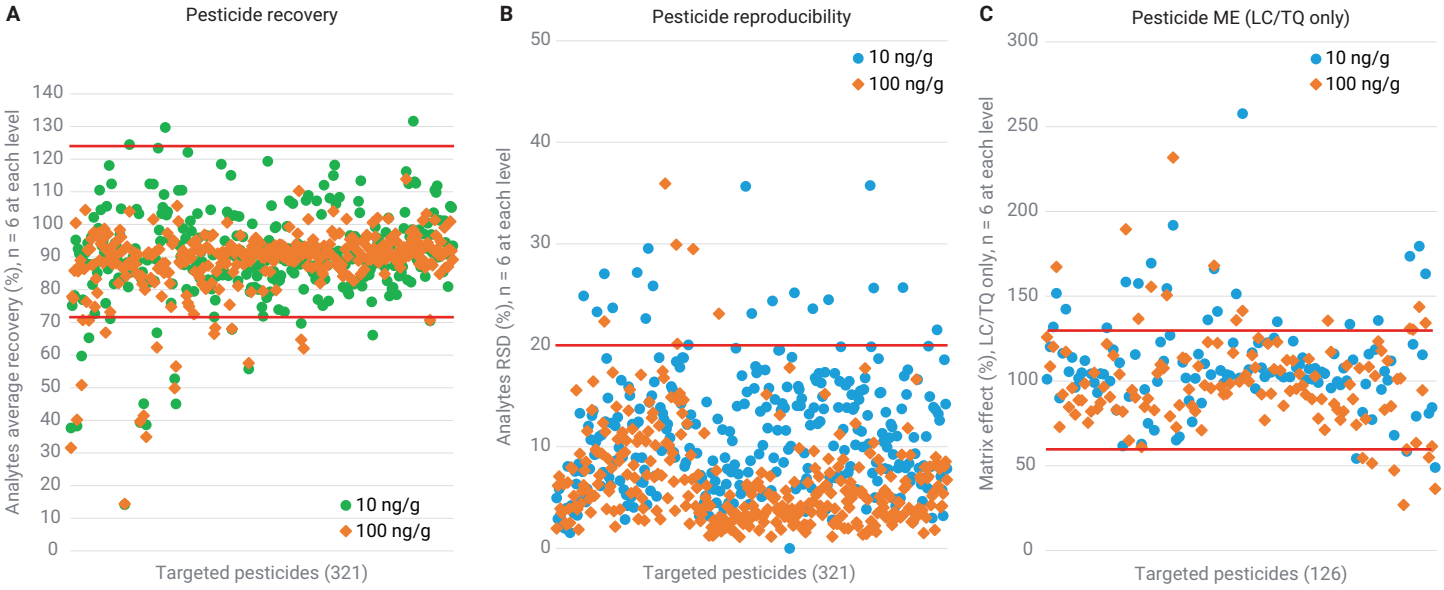

Figure 5. Method quantitation of targets' results at 10 and 100 ng/g in cumin for (A) pesticide recovery, (B) pesticide reproducibility, and (C) pesticide matrix effect (LC/TQ only).

each level. For 126 LC-amenable pesticides, the statistical data analysis shows that 119 targets demonstrated 70 to 120% recovery, and 121 targets demonstrated 40 to 120% recovery. Furthermore, 121 targets gave < 20% RSD and 102 pesticides gave a matrix effect between 60 and 130%. For 201 GC-amenable pesticides, 194 targets delivered 70 to 120% recovery, with 200 targets delivering < 20% RSD. Of the total of 327 pesticides, 11 targets were not detectable at the 10 ng/g level, due to either matrix interferences, matrix effect, or target stability, which caused difficult detection at the 10 ng/g level.

Matrix-matched calibration and LOQ: Matrix-matched calibration standards were made by postspiking the standards into a final sample extract at the range of 0.5 to 500 ng/mL. Considering the 5x dilution factor introduced during sample extraction, this corresponded to 2.5 to 2,500 ng/g in cumin. Linear regression and $1/x^2$ weight were used for calibration curve generation, with quadratic regression or 1/x weight being used for some exceptions. The calibration dynamic range of individual targets was determined based on the specific target's sensitivity and selectivity at a low and high concentration level in alignment with the calibration curve. Figure 6 shows the summary for the results of targeted pesticides' matrix-matched calibration curves in cumin. Results show that for the total of 321 pesticides, a full dynamic calibration range (0.5 to 500 ng/mL in cumin extract) with $R^2 > 0.99$ was achieved for 90.5% of targets, either with linear or quadratic regression. A portion (7.6%) of targets showed a modified dynamic range with $R^2 > 0.99$, due to either the lack of sensitivity or selectivity at the low end, or matrix positive contribution. The remaining 1.8% of targets showed compromised calibration curves with $R^2 < 0.99$.

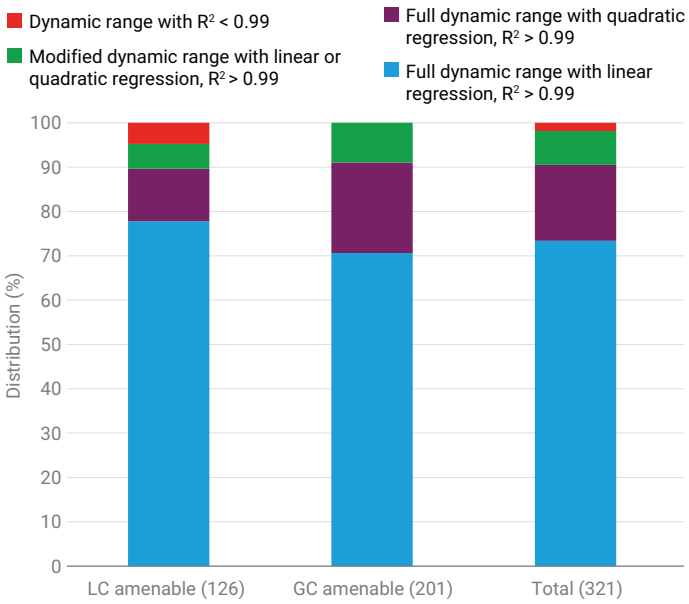

Figure 6. Results for targeted pesticides' matrix-matched calibration curves in cumin by LC/MS/MS and GC/MS/MS detection. The full dynamic range was 0.5 to 500 ng/g in cumin extract.

Conclusion

A simple, rapid, and reliable method using Agilent Bond Elut QuEChERS EN extraction followed by Agilent Captiva EMR–LPD cartridge passthrough cleanup was developed and verified for over 300 pesticides in cumin powder by LC/MS/MS and GC/MS/MS. The novel Captiva EMR–LPD cleanup method provides convenient and simplified sample passthrough cleanup; selective and efficient matrix removal for cumin powder; and acceptable pesticide recovery, reproducibility, and matrix effect.

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