Application Note

Food & Beverage Testing

Analysis of Pesticides in Celery

Using Captiva EMR–GPF passthrough cleanup application

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Abstract

This application note describes the implementation of Agilent Captiva Enhanced Matrix Removal–General Pigmented Fresh (EMR–GPF) passthrough cleanup for the analysis of multiresidue pesticides in celery by LC/MS/MS. Compared to dispersive SPE (dSPE) cleanup, Captiva EMR–GPF demonstrates a faster workflow, equivalent pigment-removal efficiency, and improved recoveries and reproducibility, especially for sensitive pesticides.

Introduction

The newly designed Agilent Captiva EMR–GPF cartridge is optimized to deliver a convenient passthrough cleanup for general pigmented fresh nonleafy vegetable and fruit matrices such as berries, peppers, grapes, celery, and so on. An advanced synthetic hybrid carbon sorbent, Carbon S, which is used in Captiva EMR–GPF cartridges, provides efficient and selective matrix pigment removal and significantly reduces unwanted interactions with targets, especially for sensitive compounds. Compared to cleanup using the traditional Agilent Bond Elut QuEChERS Universal dispersive SPE kit with graphite carbon black (GCB), Captiva EMR–GPF passthrough cleanup demonstrates an easier, more efficient and selective sample cleanup method, while delivering excellent recoveries on targets overall, especially for sensitive pesticides. The results show that the newly developed method is a more reliable matrix cleanup strategy for multiclass, multiresidue pesticide analysis. The method can easily be adopted with the common Agilent Bond Elut QuEChERS AOAC extraction kit, and improves overall target recovery pass rate for reliable quantitation in fresh general pigmented produce matrices.

Experimental

Equipment and consumables

- Eppendorf Centrifuge 5810R (Hamburg, Germany)
- SPEX SamplePrep 2010 Geno/Grinder (Metuchen, NJ, USA)
- Agilent Bond Elut QuEChERS AOAC extraction kit with ceramic homogenizers (part number 5982-5755CH)
- Agilent Bond Elut QuEChERS Universal dispersive SPE kit with GCB, 15 mL (part number 5982-0029)
- Agilent Captiva EMR–GPF cartridge, 3 mL (part number 5610-2090)
- Agilent positive pressure manifold 48 processor (PPM-48) (part number 5191-4101)

Instrument conditions

LC/MS/MS detection was performed on an Agilent 1290 Infinity II LC system, including the Agilent 1290 Infinity II high-speed pump (G7120A), the Agilent 1290 Infinity II multisampler (G7167B), and the Agilent 1290 Infinity II multicolumn thermostat (G7116B), coupled with an Agilent triple quadrupole LC/MS (G6470A) with an Agilent Jet Stream Electrospray ion source. Agilent MassHunter Workstation software was used for data acquisition and analysis. Table 1 lists the LC/MS/MS method conditions.

Sample preparation

The sample preparation included sample extraction with buffered QuEChERS protocol, using the Agilent Bond Elut QuEChERS AOAC extraction kit, and dispersive SPE (dSPE) cleanup using the Agilent Bond Elut QuEChERS Universal dSPE with GCB. Homogenized celery, 15 g, was weighed into a 50 mL polypropylene tube and extracted using 15 mL of acetonitrile (ACN) with 1% acetate acid. After addition of AOAC extraction salts, the samples were shaken vigorously for 5 minutes by Geno/Grinder at 1,000 rpm. Tubes were then centrifuged at 4,000 rpm for 5 minutes at 10 °C.

Table 1. LC/MS/MS method conditions.

LC/MS/MS Parameter	Setting		
Column	Agilent Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm column (p/n 695775-902)		
Column Temperature	40 °C		
Autosampler Temperature	10 °C		
Injection Volume	2 µL		
Mobile Phase	A) Water, containing 4.5 mM ammonium formate, 0.5 mM ammonium fluoride, 0.1% formic acid B) Methanol, containing 4.5 mM ammonium formate, 0.5 mM ammonium fluoride, 0.1% formic acid		
Gradient	Time (min) %A Flow rate (mL/min) 0 98 0.4 0.5 98 3 80 16 0 18 0 18.1 98 20 98		
Stop Time	20 min		
Source Parameters			
Gas Temperature	250 °C		
Gas Flow	10 L/min		
Nebulizer	40 psi		
Sheath Gas Temperature	350 °C		
Sheath Gas Flow	11 L/min		
Capillary Voltage	+3,500		
Nozzle Voltage	+300		
Time Segments Agilent 1290 Infinity II LC system			
Start Time (min)	Scan type	Diverter valve	Delta EMV (+)
0	DMRM	To waste	0
1.2	DMRM	To MS	400
19	DMRM	To waste	0

The following procedure is demonstrated in Figure 1: For Universal dSPE cleanup, an aliquot of 8 mL of upper ACN crude extract was transferred to a dSPE tube. Sample tubes were capped tightly and shaken for 3 minutes, followed by centrifugation for 5 minutes at 4,000 rpm. For Captiva EMR–GPF passthrough cleanup, an aliquot of 3 mL of crude extract was loaded into the 3 mL cartridge, and eluted by gravity until no visible liquid was left in the cartridge. The cartridge was dried by positive pressure (6 to 9 psi) on the PPM-48. An aliquot of 200 µL from the supernatant in the dSPE tube, or the eluent from the passthrough cleanup, was transferred and mixed with 800 µL of water for LC/MS/MS analysis.

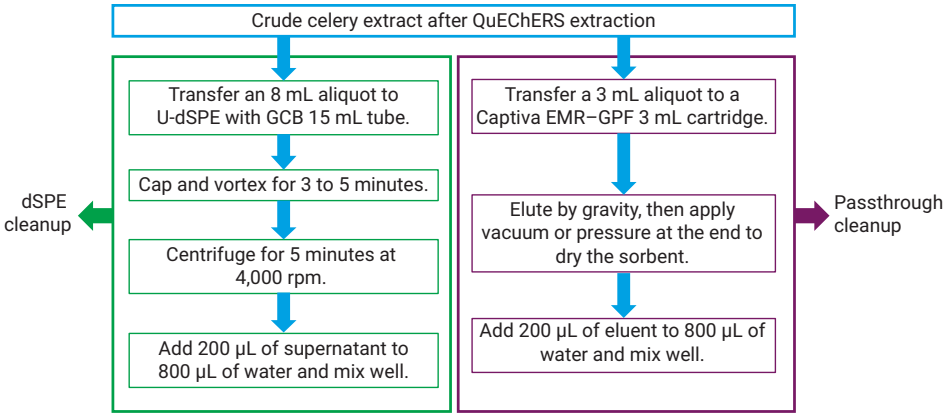

Figure 1. Procedures following extraction with the Agilent Bond Elut QuEChERS AOAC extraction kit: (left) dSPE cleanup using the Agilent Bond Elut QuEChERS Universal dispersive SPE kit with GCB (U-dSPE with GCB); (right) passthrough cleanup using the Agilent Captiva EMR–GPF cartridge.

Results and discussion

Sample preparation procedure

For traditional Universal dSPE with GCB cleanup, after QuEChERS extraction, the processing of supernatant for cleanup with dSPE took time on multiple steps, such as uncapping and capping the dSPE tubes, mixing, and centrifugation. For Captiva EMR–GPF cleanup, these procedures were simplified by replacement with gravity elution, which requires less effort and can save time by 30 to 40%.

Recovery and reproducibility with Captiva EMR–GPF

The 52 pesticides, including seven acidic targets and seven planar targets, were validated on their recovery and reproducibility in celery with Captiva EMR–GPF at two quality control (QC) levels (Figure 2). The results demonstrated that more than 90% of the pesticides achieved good recoveries (70 to 120%), except for several sensitive pesticides with acidic or planar characteristics. All RSDs were below 20%.

Additionally, sample loading volume on the Captiva EMR–GPF cartridge is a critical step that may affect analyte recovery, especially for planar pesticides. For the Captiva EMR–GPF cartridge, both 2 mL and 3 mL sample loading volumes were investigated and compared based on planar pesticide recovery. As shown in Figure 3, significant improvement in planar compound recovery was achieved with 3 mL rather than 2 mL sample loading volume. This is likely due to matrix competition for sorbent interactions. When sample loading volume is low, matrix interactions with the sorbent is not enough to cover the

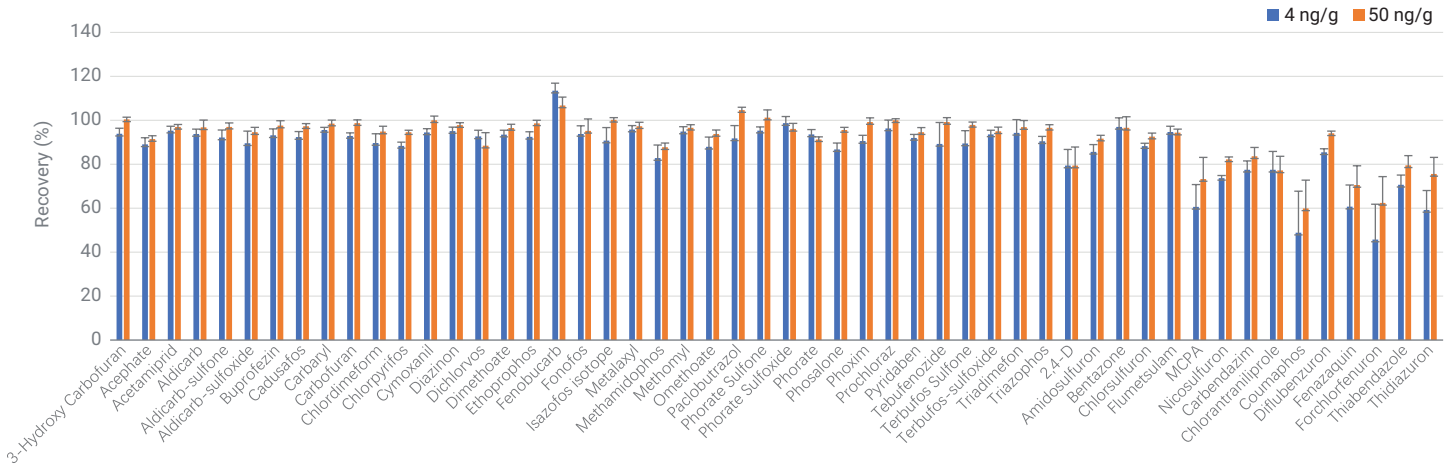

Figure 2. Average recoveries and RSDs for pesticides in celery under two QC levels (n = 5) after cleanup using the Agilent Captiva EMR–GPF cartridge.

active sites, and unwanted retention for targets can happen. As a result, it is very important to load the appropriate sample volume as recommended, to avoid the loss of planar targets.

Captiva EMR–GPF passthrough cleanup versus Universal dSPE with GCB

The recoveries and RSDs using Captiva EMR–GPF passthrough cleanup was compared to the results using Universal dSPE with GCB, with two levels of prespiked QCs, 4 ng/g and 50 ng/g, in celery, in replicates of five. Figure 4 shows the statistical results of the comparison. Overall, Captiva EMR–GPF passthrough cleanup delivered slightly better results than Universal dSPE with GCB, with over 80% of average recovery and ≤10% of average RSD for all 52 pesticides.

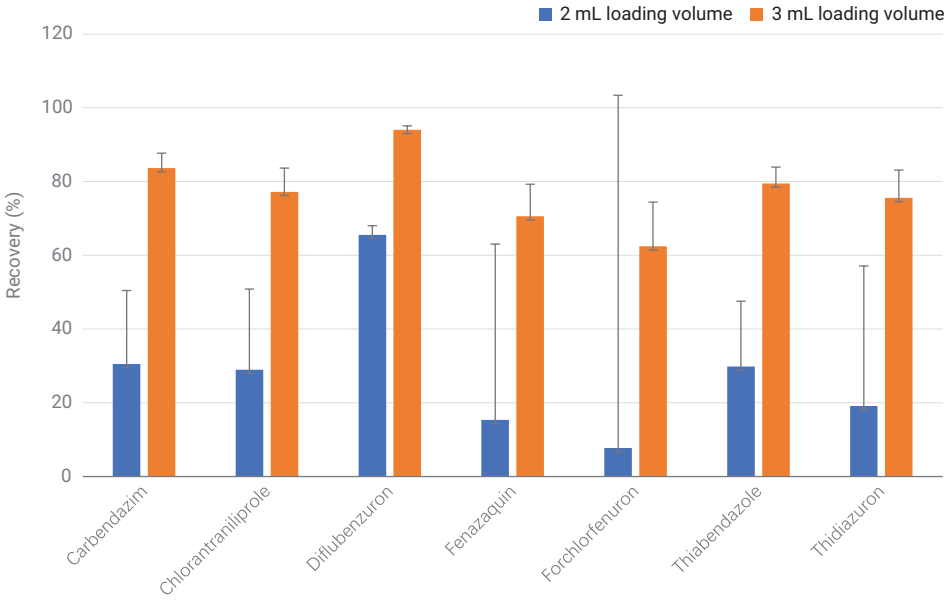

Figure 3. Comparison of the effects of sample loading volume on Agilent Captiva EMR–GPF cartridges for planar pesticide recovery at 50 ng/g spiking in celery (n = 5).

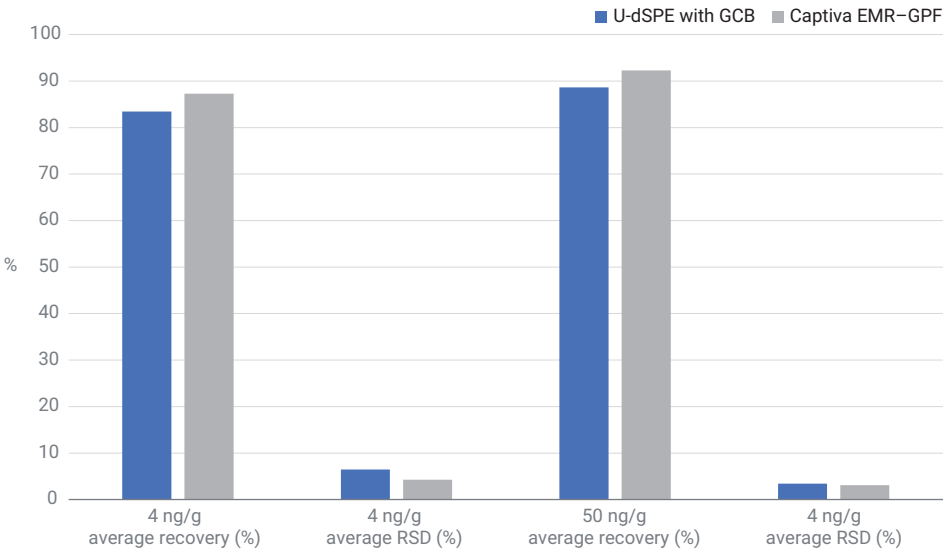

Figure 4. Comparison of Agilent Captiva EMR–GPF passthrough cleanup with traditional Agilent Bond Elut QuEChERS Universal dSPE with GCB (U-dSPE with GCB) cleanup on average recoveries and RSDs for pesticides in celery (n = 5).

The sensitive pesticides such as planar pesticides and acidic pesticides were studied specifically for comparison. As shown in Figure 5, for seven planar pesticides, Captiva EMR–GPF passthrough cleanup delivered equivalent recoveries to the Universal dSPE with GCB cleanup.

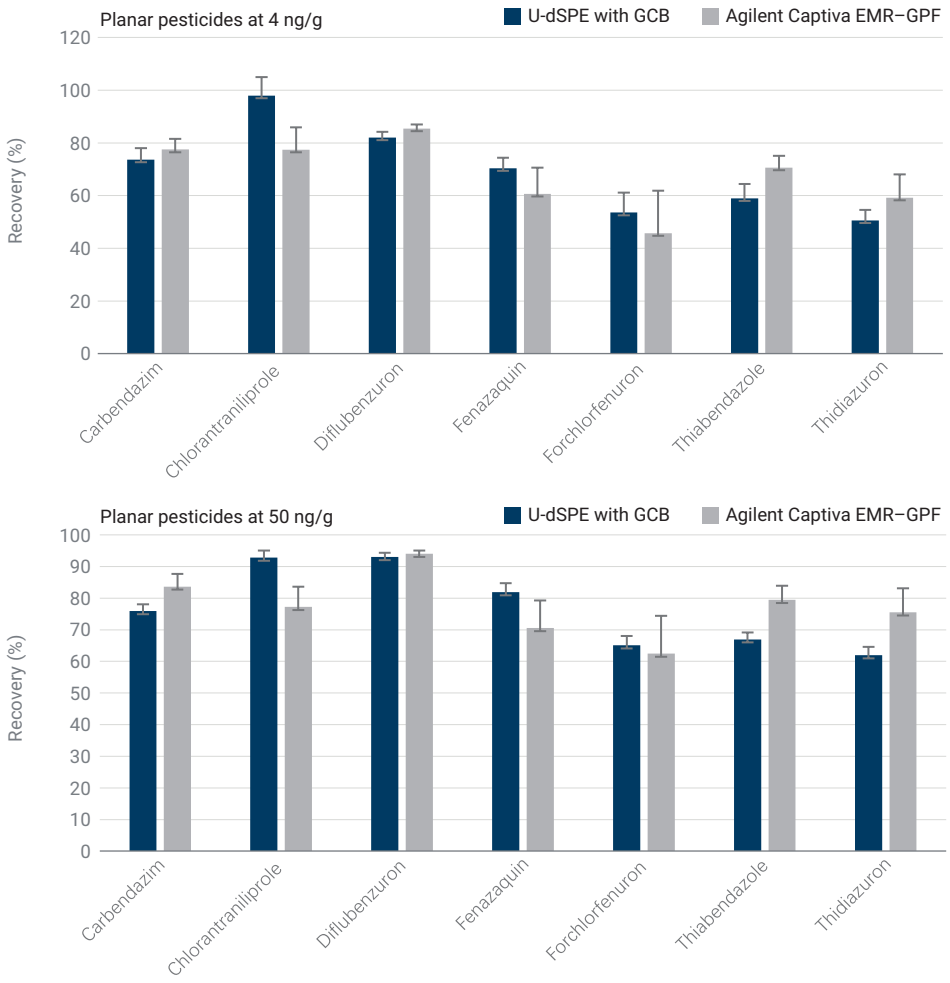

Figure 5. Comparison of Agilent Captiva EMR–GPF passthrough cleanup and the Agilent Bond Elut QuEChERS Universal dSPE with GCB (U-dSPE with GCB) cleanup on planar pesticide recoveries in celery (n = 5).

More importantly, as shown in Figure 6, there was significant loss of acidic and other sensitive pesticides using Universal dSPE with GCB, demonstrated by low recoveries and poor reproducibility. However, the Captiva EMR–GPF passthrough cleanup demonstrated significant improvement. These results are in alignment with the results of other Captiva EMR–GPF applications.[1,2] The improvement can be attributed to the following two factors: (A) Carbon S sorbent is an advanced carbon hybrid material with optimized carbon content and pore structure. It makes the interactions between sorbent and other compounds more controlled, thus significantly improves the interaction selectivity and reduces the unwanted loss between sorbent and target molecules. (B) The passthrough cleanup without simultaneous water removal by $MgSO_4$ provides better buffering protection to the sensitive compounds and thus prevents their loss during cleanup. The broader improved recovery on other sensitive pesticides, as well as overall method performance improvement with reduced failure rate, makes the Captiva EMR–GPF passthrough cleanup a more suitable sample cleanup method for multiclass, multiresidue large-panel pesticides in food. This has been demonstrated in other applications.[2,3]

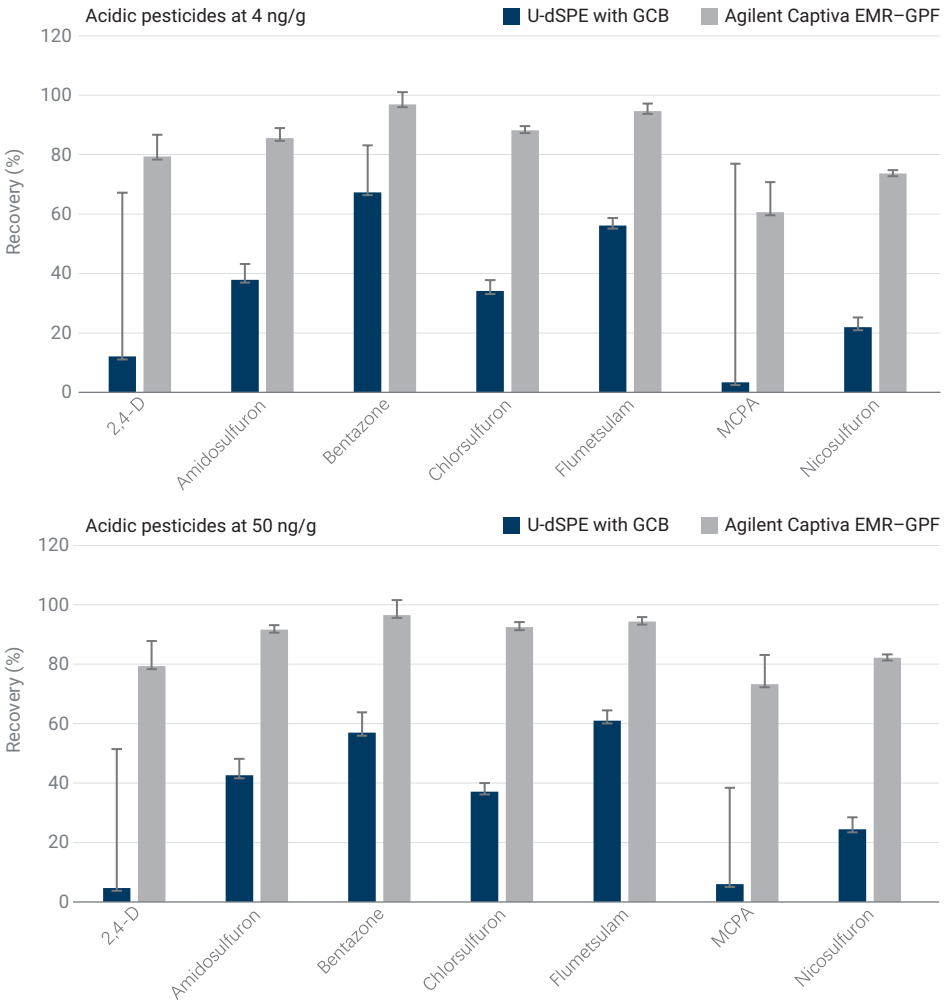

Figure 6. Comparison of Agilent Captiva EMR–GPF passthrough cleanup with Agilent Bond Elut QuEChERS Universal dSPE with GCB (U-dSPE with GCB) cleanup on acidic pesticides in celery (n = 5).

Matrix pigment removal

Figure 7 shows the visual appearance of sample supernatants before and after cleanup by Universal dSPE with GCB and Captiva EMR–GPF passthrough cleanup. Compared to the sample without cleanup after QuEChERS extraction (left), both the samples after Universal dSPE with GCB (middle) and Captiva EMR–GPF (right) cleanup appear to be colorless and transparent, indicating equivalent performance in pigment-removal efficiency. Although celery is a green vegetable, it is considered to have a general pigmented fresh matrix, not a high chlorophyll leafy matrix. Therefore, Captiva EMR–GPF cartridges are recommended, rather than Agilent Captiva Enhanced Matrix Removal–High Chlorophyll Fresh (EMR–HCF) cartridges; otherwise, significant analyte loss might be experienced.

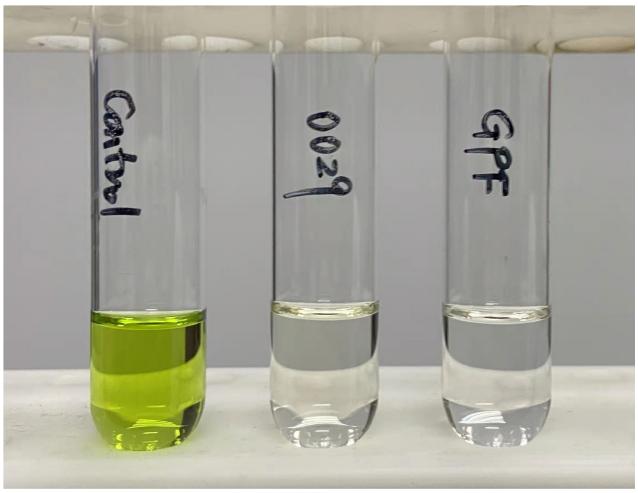

Figure 7. Supernatant of celery samples from Agilent Bond Elut QuEChERS AOAC extraction (left), followed by cleanup using the Agilent Bond Elut QuEChERS Universal dSPE kit with GCB (middle) and the Agilent Captiva EMR–GPF cartridge (right).

Conclusion

The Agilent Captiva EMR–GPF passthrough cleanup demonstrates exceptional performance for pesticide analysis in celery. Compared to traditional cleanup using the Agilent Bond Elut QuEChERS Universal dSPE kit with GCB, it provides a fast yet simplified workflow, equivalent pigment removal efficiency, and improved recoveries and reproducibility for sensitive pesticides. Captiva EMR–GPF passthrough cleanup is confirmed to be a beneficial replacement to Universal dSPE with GCB cleanup for general pigmented fresh matrices such as celery.

References

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2. Zhao L.; Determination of Multiclass, Multiresidue Pesticides in Bell Peppers using Captiva EMR–GPF Passthrough Cleanup by LC/MS/MS and GC/MS/MS, *Agilent Technologies application note*, publication number 5994-4767EN, **2022**.
3. Zou, A. *et al.* Analysis of 510 Multiple Pesticides Residues in Black Pepper using Captiva EMR Passthrough Cleanup by LC/MS/MS, *Agilent Technologies application note*, publication number 5994-4768EN, **2022**.

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