Application Note

Food & Beverage Testing

Agilent

Trusted Answers

Determination of Multiclass, Multiresidue Pesticides in Bell Peppers

Using Captiva EMR–GPF passthrough cleanup by LC/MS/MS and GC/MS/MS

Author

Limian Zhao

Agilent Technologies, Inc.

Abstract

This application note presents the development and validation of a multiresidue method for the analysis of pesticide residues in a mixed bell pepper matrix composed of different color bell peppers. The method uses extraction with the Agilent Bond Elut QuEChERS AOAC extraction kit, followed by Agilent Captiva Enhanced Matrix Removal–General Pigmented Fresh (EMR–GPF) passthrough cleanup, and then LC/MS/MS and GC/MS/MS detection, separately. The novel sample preparation workflow provides efficient and selective matrix cleanup, delivered a fast, simplified, and convenient one sample preparation for both LC/MS/MS and GC/MS/MS analysis. Compared to traditional dispersive SPE (dSPE) cleanup, the Captiva EMR–GPF passthrough cleanup delivers highly efficient and selective matrix/pigment removal, improves target recovery and reproducibility, and reduces the matrix effect and interferences. For analysis of a large panel of pesticides (240 pesticides), on both LC/MS/MS and GC/MS/MS, the workflow showed that 98% target were within the acceptance recovery window (60 to 120%), 99% target were within the RSD acceptance window (≤20%), and 94% targets gave out good calibration linearity (R^2 >0.99 in the calibration range).

Introduction

Natural pigments in fresh fruits and vegetables can be highly abundant, such as chlorophyll and lutein from green vegetables; anthocyanidins and anthocyanins from red, blue, purple, and black fruits; and carotenoids and xanthophylls from orange and yellow fruits and vegetables. These pigments can easily be extracted through an extraction procedure using an organic solvent. Without the further removal of pigment co-extractives, the direct injection of a highly pigmented sample extract on detection instrumentation, such as LC/MS/MS or GC/MS/MS, could result in multiple matrix effects, including matrix ion suppression on LC/MS/MS; matrix interferences on GC/MS/MS; and accumulated matrix deposition on the detection flow path and MS source, and so on. Therefore, it is important to apply enhanced cleanup to remove pigment co-extractives before instrument analysis.

Graphite carbon black (GCB) has widely been used in sample preparation for efficient pigment removal.[1,2] Especially for the commonly used QuEChERS preparation method in food analysis, GCB has been used in dSPE kits for pigment removal. Although GCB has shown to be efficient at pigment removal, it can also cause unwanted analyte loss, especially for compounds with planar structure, such as hexachlorobenzene, thiabendazole, cyprodinil, and so on. Other types of synthetic carbon material or polymer-based sorbent have also been used for matrix pigment removal in competition products, but there is still a compromise to analyte recovery or matrix/pigment removal. It has been challenging to achieve a balance between effective matrix/pigment removal and analyte recovery, especially for sensitive compounds.

Agilent Carbon S sorbent is an advanced hybrid carbon material with optimized carbon content and pore structure. Compared to GCB sorbent, Carbon S sorbent provides equivalent or better pigment removal from plant-origin sample matrices, and significantly improves sensitive analyte recoveries. As a result, Carbon S sorbent achieves a better balance between analyte recovery and matrix pigment removal efficiency than traditional GCB sorbent. This advanced sorbent has thus been used for the Captiva EMR products expansion, where the convenient passthrough cleanup is adopted for efficient and selective matrix removal. Compared to traditional dSPE cleanup, passthrough cleanup provides simplified workflow steps, such as the elimination of uncapping and capping the dSPE tubes, vortexing, and centrifugation.

This application note evaluated sample preparation using Captiva EMR–GPF passthrough cleanup for the analysis of 230 pesticides in a bell pepper mix by both LC/MS/MS (129 LC-amenable pesticides) and by GC/MS/MS (101 GC-amenable pesticides). The bell pepper mix included red, green, orange, and yellow peppers. This matrix was selected to represent various general pigmented vegetables.

Experimental

Chemicals and reagents

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

Solutions and standards

Standard spiking solution A (129 LC-amenable pesticides) was prepared as 10 µg/mL in 1:1 ACN/water. Standard spiking solution B (101 GC-amenable pesticides) was prepared as 10 µg/mL in ACN. Combined IS spiking solution A (two IS compounds for LC) was prepared at 10 µg/mL in 1:1 ACN/water. Combined IS spiking solution B (three IS compounds for GC) was prepared at 10 µg/mL in ACN. All four spiking solutions were stored at –20 °C in a freezer. The standard spiking solutions were warmed up thoroughly at room temperature, sonicated before use, and returned after use.

The ACN with 1% acetic acid extraction solvent was prepared by adding 10 mL of glacial acetic acid into 990 mL of ACN and stored at room temperature.

Equipment and material

The LC/MS/MS detection was performed using an Agilent 1290 Infinity LC system consisting of an Agilent 1290 Infinity binary pump (G4220A), an Agilent 1290 Infinity high performance autosampler (G4226A), and an Agilent 1290 Infinity thermostatted column compartment (G1316C). The LC system was coupled to an Agilent triple quadrupole LC/MS (G6490) equipped with an Agilent Jet Stream iFunnel electrospray ion source. Agilent MassHunter workstation software was used for data acquisition and analysis.

The GC/MS/MS was performed using an Agilent 8890 GC system coupled with an Agilent 7000D triple quadrupole GC/MS. The GC system was equipped with electronic pneumatic control (EPC), a multimode inlet (MMI) with air cooling, a G4513A automatic injector, and a

backflushing system based on a purged ultimate union, controlled by an auxiliary electronic pressure control (AUX EPC) module. Agilent MassHunter workstation software was used for data acquisition and analysis.

Other equipment used for sample preparation includes: Centra CL3R centrifuge (Thermo IEC, MA, USA); Geno/Grinder (SPEX, NJ, USA); Multi Reax test tube shaker (Heidolph, Schwabach, Germany); pipettes and repeater (Eppendorf, NY, USA); Agilent positive pressure manifold 48 processor (PPM-48) (part number 5191-4101); Agilent Bond Elut QuEChERS AOAC extraction kit (part number 5982-5755); Agilent Captiva EMR–GPF cartridge, 3 mL (part number 5610-2090); Agilent ceramic homogenizers, 50 mL tubes, 100/pk (part number 5982-9313).

Instrument conditions

Instrument methods were followed according to previously used methods.[3,4] Table 1 lists the LC/MS/MS conditions. Table 2 lists the GC/MS/MS conditions. Table 3 lists the dynamic MRM (dMRM) parameters for all of targets. Figure 1 shows a typical MRM chromatogram of targeted pesticides using the above (A) LC/MS/MS conditions and (B) GC/MS/MS conditions, for a fortified bell pepper sample at the level of 100 ng/g. The sample was prepared using QuEChERS AOAC extraction followed by Captiva EMR–GPF passthrough cleanup.

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 (positive), 90 V (negative) Low-pressure RF: 60 V (positive), 60 V (negative)
Polarity	Positive and negative, refer to Table 2

Table 2. Agilent 8890 GC and Agilent 7000D GC/MS/MS conditions.

Parameter	Value
Columns	Agilent J&W HP-5ms Ultra Inert GC column, 15 m × 0.25 mm, 0.25 µm film thickness (two) (p/n 19091S-431UI)
Carrier Gas	Helium
Column 1 Flow	1.0 mL/min
Column 2 Flow	1.4 mL/min
Injection Volume	1 µL cold splitless
Inlet Liner	Agilent inlet liner, Ultra Inert, splitless, single taper, glass wool, 4 mm id (p/n 5190-2293)
MMI Temperature Program	75 °C for 0.02 min, 750 °C/min to 350 °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 3 min
Run Time	20.75 min
Backflush Conditions	3 min post run 310 °C oven temperature 50 psi AUX EPC pressure, and 2 psi inlet pressure
Transfer Line Temperature	280 °C
Source Temperature	EI source, 300 °C
Quadrupole Temperature	150 °C
Data Monitoring	Dynamic MRM mode (dMRM)
Gain Factor	10
Solvent Delay	3 min

Table 3. Targeted pesticides dMRM conditions on (A) LC/MS/MS and (B) GC/MS/MS.

A	dMRM Conditions on LC/MS/MS						
Target Name	RT (min)	First MRM Transition (*m/z*)	CE (V)	Second MRM Transition (*m/z*)	CE (V)	Delta RT (min)	Polarity
Methamidophos	1.156	142 → 124.9	13	142 → 94.1	9	1	POS
Pymetrozine	1.238	218.1 → 105	25	218.1 → 51.2	73	1	POS
Acephate	1.253	184 → 143	9	184 → 95	25	1	POS
Omethoate	1.391	214 → 183	9	214 → 124.9	17	1	POS
Aminocarb	1.609	209.1 → 152.2	9	209.1 → 137	21	1	POS
Propamocarb	1.775	189.2 → 102	17	189.2 → 74	25	1	POS
Dinotefuran	1.994	203.1 → 129	5	203.1 → 43	61	1	POS
Carbendazim	2.750	192.1 → 160	17	192.1 → 65.1	61	1	POS
Monocrotophos	2.930	224.1 → 127	13	224.1 → 58	29	1	POS
Nitenpyram	2.950	271.1 → 125.9	25	271.1 → 56.1	49	1	POS
Thiabendazole	3.001	202.1 → 175.1	25	201.1 → 131	37	1	POS
Fuberidazole	2.259	185.1 → 157.1	25	185.1 → 156.1	33	1	POS
Thiamethoxam	3.512	292 → 211	9	292 → 131.9	17	1	POS
Cymoxanil	3.680	199.1 → 157.2	21	199.1 → 156.1	29	1	POS
Mexacarbate	3.750	223.2 → 151.1	25	223.2 → 136.1	45	1	POS
Ethirimol	3.786	210.2 → 140.1	17	210.2 → 43	61	1	POS
Metamitron	3.852	203.1 → 104	21	203.1 → 41.9	49	1	POS
Fenuron	3.951	165.1 → 72.1	21	165.1 → 46	13	1	POS
Chloridazon	4.036	222 → 76.9	33	222 → 51	77	1	POS
Imidacloprid	4.088	256.1 → 208.8	17	256.1 → 175	17	1	POS
Cymiazol	4.125	219.1 → 171.2	28	219.1 → 100	17	1	POS
Dimethoate	4.199	230 → 125	17	230 → 47.1	41	1	POS
Fenobucarb	4.259	206.1 → 66.1	21	NA	NA	1	NEG
Acetamiprid	4.265	223.1 → 126	17	223.1 → 73.1	69	1	POS
Metsulfuron	4.501	368.1 → 325.2	17	368.1 → 231.2	5	1	POS
Flumetsulam	4.523	326.1 → 129	21	326.1 → 109	73	1	POS
4-Nitrophenol D_4 (IS)	4.608	142 → 112	17	142 → 46	45	1	NEG
Tebuthiuron	4.656	229.1 → 172.1	13	229.1 → 116	33	1	POS
4-Nitrophenol	4.737	138 → 107.9	17	138 → 46	57	1	NEG
Thiacloprid	4.743	253 → 125.9	17	253 → 73	73	1	POS
Nicosulfuron	4.761	411.1 → 182	22	411.1 → 106	32	1	POS
Simazine-D_{10} (IS)	4.925	212.2 → 137.1	25	212.2 → 44	49	1	POS
Thidiazuron	4.946	221.1 →101.9	13	221.1 → 51.1	80	1	POS
Secbumeton	5.051	226.2 → 170.1	17	226.2 → 113.9	24	1	POS
Imazalil	5.103	297.1 → 158.9	25	297.1 → 69	21	1	POS
Bentazon	5.127	239.1 → 197	21	239.1 → 132.1	29	1	NEG
Oxasulfuron	5.129	407.1 → 150.1	17	407.1 → 107	57	1	POS
Carfentrazone-ethyl	5.165	388.1 → 204.9	29	388.1 → 167.1	17	2	POS
Lenacil	5.216	235.2 → 153	13	235.2 → 136	37	1	POS
Metribuzin	5.315	215.1 → 49.1	214	215.1 → 47	80	1	POS
Cyazofamid	5.334	325.1 → 233	21	325.1 → 231.2	29	1	POS
Propoxur	5.348	210.1 → 111.1	9	210.1 → 64.9	41	1	POS

Target Name	RT (min)	First MRM Transition (*m/z*)	CE (V)	Second MRM Transition (*m/z*)	CE (V)	Delta RT (min)	Polarity
Phenmedipham	5.371	301.1 → 281.2	17	301.1 → 238.1	33	1	POS
2,4-D	5.417	221 → 163.1	13	219 → 161.1	17	1	NEG
Chlorsulfuron	5.481	358 → 167.1	17	358 → 141.2	21	2	POS
Methabenzthiazuron	5.498	222.1 → 165.1	17	222.1 → 150	45	1	POS
Dioxacarb	5.498	224.1 → 167.1	12	224.1 → 123.1	20	1	POS
Carbofuran	5.498	222.1 → 165.1	9	222.1 → 123.1	25	1	POS
2,4,5-TP	5.551	266.9 → 198.8	9	266.9 → 141	17	1	NEG
MCPA	5.552	201 → 143.1	13	199 → 141.1	13	1	NEG
Cycluron	5.561	199.2 → 72	29	199.2 → 69.1	21	1	POS
Amidosulfuron	5.591	370.1 → 261.1	9	370.1 → 218	25	1	POS
Flutriafol	5.592	302.1 → 123	25	302.1 → 70.1	13	1	POS
Carbaryl	5.596	202.1 → 145.1	9	202.1 → 127.2	33	1	POS
Chlorotoluron	5.597	213.1 → 72.1	29	213.1 → 46.1	17	1	POS
Pyracarbolid	5.634	218.1 → 124.9	13	218.1 → 43.1	65	1	POS
Fluometuron	5.645	233.1 → 72	17	233.1 → 46	17	1	POS
Atrazine-D_5 (IS)	5.660	221.1 → 137.1	17	221.1 → 44.1	57	1	POS
Forchlorfenuron	5.669	248.1 → 129	13	248.1 → 93.1	41	1	POS
Fosthiazate	5.692	284.1 →227.9	9	284.1 → 103.9	25	1	POS
Azaconazole	5.778	300 → 231.1	13	300 → 159.1	29	1	POS
Methoprotryne	5.779	272.2 → 198.1	21	272.2 → 170.1	29	1	POS
DEET	5.783	192.1 → 118.9	21	192.1 → 91	33	1	POS
Fenpropidin	5.803	274.3 → 147.1	29	274.3 → 117	61	1	POS
Carboxin	5.842	236.1 → 143	13	236.1 → 42.9	49	1	POS
Diuron	5.855	233 → 72.1	17	233 → 46.1	21	1	POS
2,4,5-T	5.896	254.9 → 197	9	252.9 → 195	9	1	NEG
Spiroxamine	5.901	298.3 → 144.1	21	298.3 → 100	33	1	POS
Dichlorprop	5.957	233 → 175.1	9	233 → 160.9	17	1	NEG
Mecoprop	6.056	213 → 141	13	213 → 71	9	1	NEG
Metobromuron	6.063	259 → 170	13	259 → 90.9	45	1	POS
Dimethomorph I	6.183	388.1 → 300.9	24	388.1 → 165	36	1	POS
Dimethachlor	6.223	256.1 → 224	9	256.1 → 148.1	29	1	POS
Chlorantraniliprole	6.266	482 → 284	33	482 → 112	80	1	POS
Clomazone	6.284	240.1 → 125	32	240.1 → 89.1	68	1	POS
Dimethomorph II	6.303	388.1 → 300.9	24	388.1 → 165	36	1	POS
Cyproconazole	6.325	292.1 → 125	45	292.1 → 70	17	1	POS
Furalaxyl	6.539	302.1 → 242.1	13	302.1 → 95.1	33	1	POS
Chloroxuron	6.591	291.1 → 72.1	21	291.1 → 45.9	27	1	POS
Iprovalicarb	6.601	321.2 → 119	21	321.2 → 91.1	65	1	POS
Halofenozide	6.620	329.1 → 120.9	21	329.1 → 77.1	37	1	NEG
Spinosad A	6.622	732.5 → 142.1	33	732.5 → 98.1	77	1	POS
Linuron	6.630	249 → 159.9	13	249 → 133.1	37	1	POS
Fenamiphos	6.653	304.1 → 216.9	21	304.1 → 201.9	37	1	POS
Promecarb	6.668	208.1 → 109	13	208.1 → 41	49	1	POS
Myclobutanil	6.718	289.1 → 125	41	289.1 → 70.2	21	1	POS
Mandipropamid	6.737	412.1 → 328.2	9	412.1 → 125.1	53	1	POS
Azoxystrobin	6.737	404.1 → 372	13	404.1 → 344.1	25	1	POS
Fenamidone	6.766	312.1 → 92.1	29	312.1 → 65	65	1	POS

Target Name	RT (min)	First MRM Transition (*m/z*)	CE (V)	Second MRM Transition (*m/z*)	CE (V)	Delta RT (min)	Polarity
Boscalid	6.855	343 → 307	17	343 → 139.9	17	1	POS
Fluopicolide	6.944	383 → 173	33	383 → 108.9	80	1	POS
Spinosad D	6.966	746.5 → 142.2	33	746.5 → 98.1	65	1	POS
Isoxaben	6.971	333.2 → 165.1	17	333.2 → 106.9	77	1	POS
Bifenazate	6.985	301.2 → 198.1	9	301.1 → 170.2	17	1	POS
Penconazole	7.008	284.1 → 159.9	33	284.1 → 70	1	1	POS
Pyridat	7.025	389.1 → 59.1	17	379.1 → 42	77	1.5	POS
Diflubenzuron	7.058	311 → 158.1	13	311 → 141.1	37	1	POS
Ethoxyquin	7.169	218.2 → 174.1	33	218.2 → 160.1	37	2	POS
Fluoxastrobin	7.186	459.1 → 427	17	459.1 → 188	41	1	POS
Prochloraz	7.201	376 → 308	9	376 → 70.1	21	1	POS
Isoprothiolane	7.204	291.1 → 231.1	5	291.1 → 188.9	21	1	POS
Flufenacet	7.225	364.1 → 194.1	9	364.1 → 152.1	17	1	POS
Rotenone	7.233	395.2 → 213.1	25	395.2 → 192.2	21	1	POS
Dimoxystrobin	7.239	327.2 → 205.1	9	327.2 → 116	29	1	POS
Cyprodinil	7.277	226.1 → 93	45	226.1 → 51.1	80	1	POS
Moxidectin	7.295	640.4 → 478.1	8	640.4 → 413.1	25	1	POS
Azinphos-ethyl	7.311	346.1 → 289.1	4	346.1 → 132	16	1	POS
Tebufenozide	7.352	351.2 → 149	21	351.2 → 105.1	37	1	NEG
Flubendiamide	7.354	683 → 408	8	683 → 273.9	40	1	POS
Beflubutamid	7.406	356.1 → 91	33	356.1 → 65.2	80	1	POS
Hydramethylnon	7.465	495.2 → 323.2	33	495.2 → 151.1	80	1	POS
Dinoseb	7.470	239.1 → 192.9	25	239.1 → 134	50	1	NEG
Kresoxim-methyl	7.502	314.1 → 267.1	5	314.1 → 221.9	9	1	POS
Picoxystrobin	7.524	368.1 → 205.1	9	368.1 → 145.1	29	1	POS
Pyraclostrobin	7.804	388.1 → 193.9	12	388.1 → 163	25	1	POS
Isofenphos-methyl	7.805	332.1 → 231	17	332.1 → 120.9	44	1	POS
Diflufenican	8.033	395.1 → 266.1	25	395.1 → 217.8	57	1	POS
Trifloxystrobin	8.075	409.1 → 186.1	13	409.1 → 144.9	65	1	POS
Metrafenone	8.185	409.1 → 226.9	21	109.1 → 209.1	9	1	POS
Metaflumizone	8.215	507.1 → 178.1	25	507.1 → 178.1	65	2	POS
Cycloate	8.222	216.1 → 83.2	13	216.1 → 55.2	29	1	POS
Fluazinam	8.299	462.9 → 415.9	21	462.9 → 397.9	17	1	NEG
Temephos	8.488	467 → 419	21	467 → 125	37	1	POS
Fenazaquin	8.619	307.2 → 160.9	13	307.2 → 56.9	25	1	POS
Pyriproxyfen	8.627	322.2 → 227.1	14	322.2 → 95.9	17	1	POS
Hexythiazox	8.843	353.1 → 228.1	9	353.1 → 168.1	21	1	POS
Tralkoxydim	8.862	330.2 → 138	17	330.2 → 96.1	33	1	POS
Buprofezin	8.893	306.2 → 201	9	306.2 → 57.2	25	1	POS
Fenpyroximate	8.966	422.2 → 366.1	16	422.2 → 135.1	36	1	POS
Proquinazid	9.255	373 → 331	13	373 → 289.1	25	1	POS
Pyridaben	9.531	365.2 → 309.1	13	365.2 → 147	25	1	POS
Spirodiclofen	9.638	411.1 → 71.2	13	411.1 → 42.9	65	1	POS

B	dMRM Conditions on GC/MS/MS						
Pesticide	RT (min)	First MRM Transition (*m/z*)	CE (V)	Second MRM Transition (*m/z*)	CE (V)	Delta RT (Min)	MS1 and MS2 Resolution
Dichlorvos	5.047	109 → 79	5	184 → 93	10	1.5	Wide
Dichlobenil	5.686	171 → 100	25	171 → 136.1	15	1.5	Wide
Mevinphos	6.049	127 → 109	10	127 → 95	15	1.5	Wide
Propham	6.309	136.9 → 93	10	119 → 91	10	1.5	Wide
Methacrifos	6.542	207.9 → 180.1	5	124.9 → 47.1	10	1.5	Wide
2-Phenylphenol	6.853	169.1 → 115.1	25	170.1 → 141.1	25	1.5	Wide
Molinate	7.017	126.2 → 55.1	10	126.2 → 83.1	5	1.5	Wide
Diphenylamine	7.634	169 → 168.2	15	168 → 167.2	15	1.5	Wide
Ethalfluralin	7.638	275.9 → 202.1	15	315.9 → 275.9	10	1.5	Wide
Sulfotep	7.896	201.8 → 145.9	10	237.8 → 145.9	10	1.5	Wide
β-BHC	8.302	216.9 → 181	5	218.9 → 183	5	1.5	Wide
Hexachlorobenzene	8.387	283.8 → 213.9	30	283.8 → 248.8	15	1.5	Wide
Demeton-S	8.394	88 → 60	5	126 → 65	10	1.5	Wide
Simazine	8.508	201.1 → 173.1	5	173 → 172.1	5	1.5	Wide
Atrazine-D_5 (IS)	8.539	219.9 → 58.1	10	219.9 → 200.2	5	1.5	Wide
Atrazine	8.574	214.9 → 58.1	10	214.9 → 200.2	5	1.5	Wide
Propetamphos	8.732	138 → 110	10	138 → 64	15	1.5	Wide
Trietazine	8.783	229 → 200.2	5	214.2 → 186.2	10	1.5	Wide
Terbuthylazine	8.810	228.9 → 173.1	5	172.9 → 172	5	1.5	Wide
Terbufos	8.837	230.9 → 129	20	230.9 → 175	10	1.5	Wide
Lindane	8.852	216.9 → 181	5	181 → 145	15	1.5	Wide
Diazinon	8.869	137.1 → 84	10	137.1 → 54	20	1.5	Wide
Pyrimethanil	9.024	198 → 118.1	35	198 → 183.1	15	1.5	Wide
Chlorothalonil	9.088	263.8 → 168	25	263.8 → 229	20	1.5	Wide
Pirimicarb	9.307	238 → 166.2	10	166 → 55.1	20	1.5	Wide
Phosphamidon	9.577	127 → 95	10	127 → 109	10	1.5	Wide
Metribuzin	9.764	198 → 82	15	198 → 55	30	1.5	Wide
Chlorpyrifos-methyl	9.774	124.9 → 47	15	142.9 → 78.9	5	1.5	Wide
Fenitrothion	9.916	125.1 → 47	15	125.1 → 79	5	1.5	Wide
Tolclofos-methyl	9.917	265 → 250	15	265 → 93	25	1.5	Wide
Heptachlor	10.128	271.7 → 236.9	15	273.7 → 238.9	15	1.5	Wide
Pirimiphos-methyl	10.215	290 → 125	20	232.9 → 151	5	1.5	Wide
Propargite	10.220	135 → 107.1	10	149.9 → 135.1	5	1.5	Wide
Malathion	10.422	172.9 → 99	15	126.9 → 99	5	1.5	Wide
Dichlofluanid	10.472	223.9 → 123.1	20	123 → 77	20	1.5	Wide
Diethofencarb	10.545	151 → 123	10	207 → 151	15	1.5	Wide
Metolachlor	10.576	238 → 162.2	10	162.2 → 133.2	15	1.5	Wide
Tetraconazole	10.731	336 → 217.9	20	170.9 → 136	10	1.5	Wide
Aldrin	10.786	262.9 → 192.9	35	254.9 → 220	20	1.5	Wide
Triadimefon	10.788	208 → 181.1	5	208 → 111	20	1.5	Wide
Pendimethalin	11.189	251.8 → 162.2	10	251.8 → 161.1	15	1.5	Wide
Metazachlor	11.261	133.1 → 132.1	10	132.1 → 117.1	15	1.5	Wide
Chlorfenvinphos	11.358	266.9 → 159.1	15	322.8 → 266.8	10	1.5	Wide
Mecarbam	11.382	158.9 → 131	5	130.9 → 74	5	1.5	Wide
Tolylfluanid	11.386	237.9 → 137	15	136.9 → 91.1	20	1.5	Wide

Pesticide	RT (min)	First MRM Transition (*m/z*)	CE (V)	Second MRM Transition (*m/z*)	CE (V)	Delta RT (Min)	MS1 and MS2 Resolution
Quinalphos	11.505	146 → 118	10	146 → 91	30	1.5	Wide
Triflumizole	11.545	206 → 179	15	206 → 186	10	1.5	Wide
Triadimenol	11.559	168 → 70	10	128 → 65	25	1.5	Wide
Procymidone	11.562	284.8 → 96	10	282.8 → 96	30	1.5	Wide
Captan	11.607	149 → 79.1	10	151 → 79.1	15	1.5	Wide
Methidathion	11.786	144.9 → 85	5	144.9 → 58.1	15	1.5	Wide
Paclobutrazole	11.941	236 → 125.1	10	125.1 → 89	20	1.5	Wide
Mepanipyrim	12.044	223.2 → 222.2	10	222.2 → 207.2	15	1.5	Wide
Endosulfan I	12.162	194.9 → 159	5	194.9 → 160	5	1.5	Wide
Fludioxonil	12.227	248 → 154.1	20	248 → 182.1	10	1.5	Wide
Hexaconazole	12.297	256 → 82	10	231 → 175	10	1.5	Wide
Profenofos	12.375	338.8 → 268.7	15	207.9 → 63	30	1.5	Wide
Oxadiazon	12.394	174.9 → 112	15	174.9 → 76	35	1.5	Wide
Tricyclazole	12.455	189 → 162.1	10	189 → 161.1	15	1.5	Wide
DDE	12.466	246.1 → 176.2	30	315.8 → 246	15	1.5	Wide
Uniconazole-P	12.473	234.1 → 164.9	10	234.1 → 136.9	15	1.5	Wide
Bupirimate	12.519	272.9 → 193.1	5	272.9 → 108	15	1.5	Wide
Flusilazole	12.528	233 → 165.1	15	233 → 91	20	1.5	Wide
Dieldrin	12.650	262.9 → 193	35	277 → 241	5	1.5	Wide
Endrin	13.052	262.8 → 193	35	244.8 → 173	30	1.5	Wide
Iprodione	13.130	187 → 124	25	313.8 → 55.9	20	1.5	Wide
Diniconazole	13.167	269.9 → 232	10	267 → 232.1	10	1.5	Wide
Oxadixyl	13.192	163 → 132.1	5	163 → 117.1	25	1.5	Wide
Ethion	13.204	230.9 → 175	10	152.9 → 96.9	10	1.5	Wide
Endosulfan II	13.231	194.9 → 159	5	194.9 → 160	5	1.5	Wide
DDD	13.244	234.9 → 165.1	20	236.9 → 165.1	20	1.5	Wide
Triazophos	13.471	161.2 → 134.2	5	161.2 → 106.1	10	1.5	Wide
Propiconazole I	13.769	172.9 → 109	15	172.9 → 145	15	1.5	Wide
Quinoxyfen	13.827	271.9 → 237.1	10	NA	NA	1.5	Wide
Propiconazole II	13.885	172.9 → 109	30	172.9 → 145	15	1.5	Wide
DDT-D_8 (IS)	13.903	243 → 173.1	20	245 → 173.1	20	1.5	Wide
DDT	13.951	235 → 165.2	20	237 → 165.2	20	1.5	Wide
Fenhexamid	13.967	177.1 → 78	25	177.1 → 113	15	1.5	Wide
Tebuconazole	14.195	250 → 125	20	125 → 89	15	1.5	Wide
TPP (IS)	14.242	325.9 → 169	30	325.9 → 233	27	1.5	Wide
Zoxamide	14.422	189 → 161.1	15	187 → 159.1	15	1.5	Wide
Epoxiconazole	14.435	192 → 138.1	10	192 → 111	25	1.5	Wide
Spiromesifen	14.475	272 → 254.2	5	272 → 209.2	10	1.5	Wide
Bifenthrin	14.738	181.2 → 165.2	25	181.2 → 166.2	10	1.5	Wide
Bromuconazole I	14.759	173 → 145	15	173 → 109	30	1.5	Wide
Phosmet	14.801	160 → 77.1	20	160 → 133.1	20	1.5	Wide
EPN	14.828	169 → 77	25	169 → 141.1	5	1.5	Wide
Picolinafen	14.829	376 → 238.1	20	376 → 239.1	10	1.5	Wide
Fenoxycarb	14.844	255.2 → 186.2	10	186.2 → 158.2	5	1.5	Wide
Methoxychlor	14.927	227.1 → 169.1	25	227.1 → 121.1	10	1.5	Wide
Tebufenpyrad	15.041	275.9 → 171.1	10	332.9 → 171	15	1.5	Wide

Pesticide	RT (min)	First MRM Transition (*m/z*)	CE (V)	Second MRM Transition (*m/z*)	CE (V)	Delta RT (Min)	MS1 and MS2 Resolution
Bromuconazole II	15.167	173 → 109	30	173 → 145	15	1.5	Wide
Metoconazole	15.189	125 → 89	20	125 → 99	20	1.5	Wide
Azamethiphos	15.451	183 → 112	15	215 → 171.1	10	1.5	Wide
Phosalone	15.451	182 → 111	15	182 → 102.1	15	1.5	Wide
Ipconazole	15.893	125 → 89	20	125 → 99	20	1.5	Wide
Mirex	16.016	271.8 → 236.8	15	273.8 → 238.8	15	1.5	Wide
Fenarimol	16.017	219 → 107.1	10	251 → 139.1	10	1.5	Wide
Bitertanol	16.503	170.1 → 115	40	170.1 → 141.1	20	1.5	Wide
Permethrin	16.670	183.1 → 168.1	10	183.1 → 153.1	15	1.5	Wide
Coumaphos	16.693	361.9 → 109	15	210 → 182	10	1.5	Wide
Fluquinconazole	16.707	340 → 107.8	40	340 → 298	15	1.5	Wide
Fenbuconazole	17.097	197.9 → 129	5	128.9 → 102.1	15	1.5	Wide
Etofenprox	17.742	163 → 135	10	163 → 107.1	20	1.5	Wide
Flumiloxazin	18.308	287 → 258.7	15	354 → 325.9	5	1.5	Wide
Pyraclostrobin	18.440	164 → 132.1	35	164 → 77.1	10	1.5	Wide
Difenoconazole	18.870	322.8 → 264.8	15	264.9 → 202	20	1.5	Wide
Deltamethrin	19.208	252.9 → 93	25	181 → 152.1	25	1.5	Wide

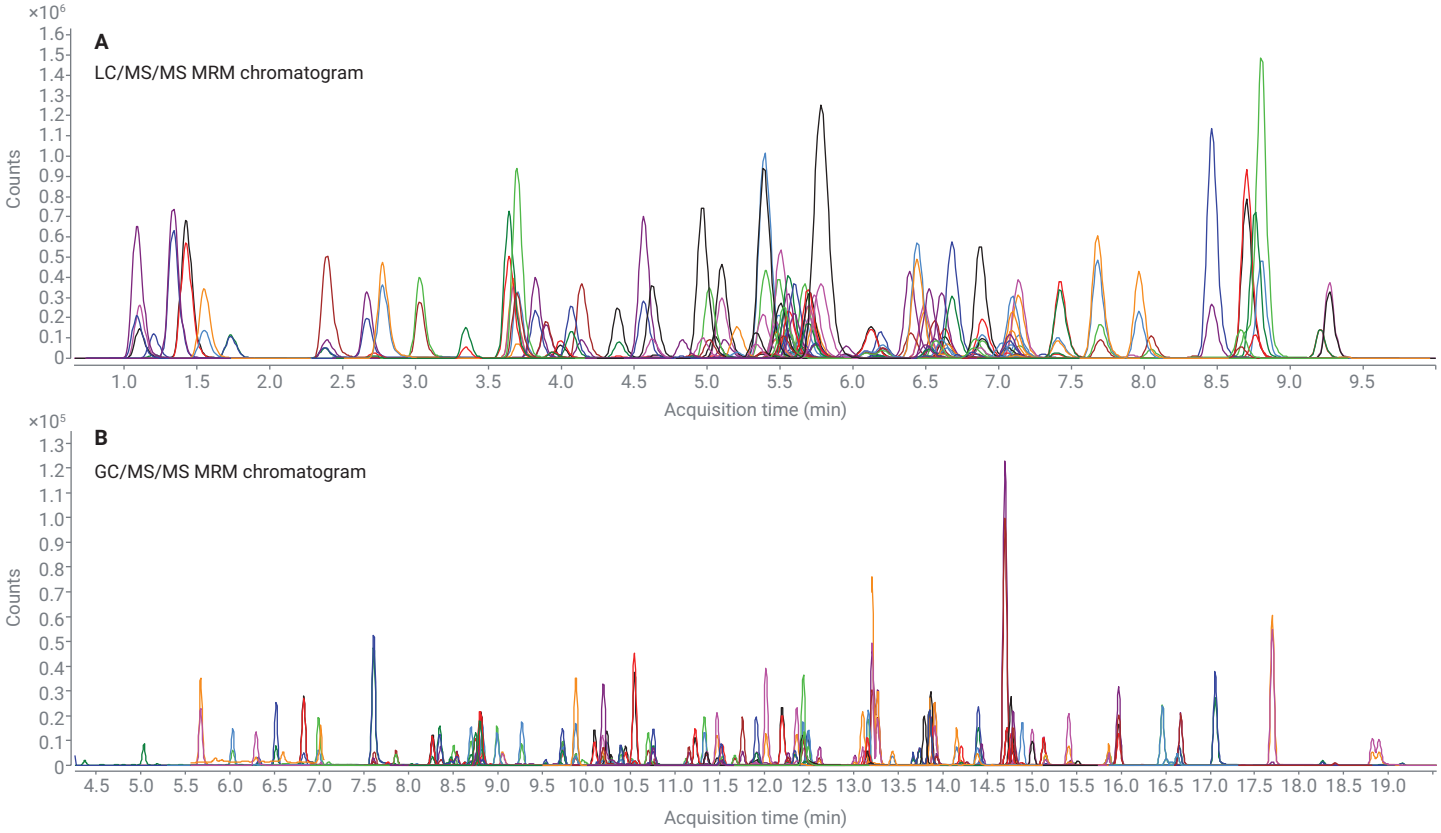

Figure 1. (A) LC/MS/MS and (B) GC/MS/MS MRM chromatograms for extracted bell pepper sample fortified with 100 ng/g of 230 targeted pesticides. The sample was prepared using the Agilent Bond Elut QuEChERS AOAC extraction kit, followed by Agilent Captiva EMR–GPF passthrough cleanup. Refer to Table 3 for peak identification based on retention time order.

Sample preparation

The fresh, organic mixed bell peppers were purchased from local grocery stores. Samples were chopped and frozen in a –20 °C freezer overnight, then homogenized with a grinder. The grinded matrix samples were then weighed at 15 g in the 50 mL centrifuge tubes and stored in the –20 °C freezer until extraction. The weighed mixed bell pepper samples (15 g) were prethawed, then extracted following the QuEChERS AOAC method. The crude extract was then loaded into the Captiva EMR–GPF 3 mL cartridge for passthrough cleanup. For LC/MS/MS analysis, sample eluent was diluted with water five times to generate the final sample in 20/80 ACN/water for instrument injection. For GC/MS/MS analysis, the cleaned sample eluent was dried by anhydrous $MgSO_4$ to completely remove the remaining water residue for instrument injection. The drying procedure was done by addition of anhydrous $MgSO_4$ powder semi-quantitatively, and based on the critical but visual indicators for complete water residue removal during vortexing and after centrifugation, as explained previously.[3] The detailed sample preparation procedure is shown in Figure 2. For a batch of ~30 samples, the entire procedure usually takes approximately 40 to 50 minutes.

Method performance evaluation

The performance of the developed sample preparation method using Captiva EMR–GPF passthrough cleanup was compared to (a) traditional dSPE cleanup using the QuEChERS Universal dispersive SPE kit with GCB (U-dSPE with GCB), (b) a competition polymer phase based dSPE (vendor 1 dSPE), and (c) another synthetic carbon phase based dSPE (vendor 2 dSPE). The comparison was based on a thorough method performance evaluation in terms of matrix cleanup and targets recovery and reproducibility, for pesticides analysis in mixed bell peppers by both LC/MS/MS and GC/MS/MS. The matrix cleanliness evaluation included assessment of pigment removal, matrix effect on LC/MS/MS, and matrix interferences on GC/MS/MS.

For evaluation of recovery and reproducibility, data were at the spiking level of 10 ng/g in the mixed bell pepper sample homogenate in replicates of six. The new method was validated for quantitative analysis of 230 pesticides in bell pepper using both LC/MS/MS and GC/MS/MS, including the matrix matched dynamic calibration range and calibration curve linearity, and method accuracy and precision at both low (10 ng/g) and high (100 ng/g) spiking levels. Analyte identification, confirmation, and quantitation were determined from retention times and MRM transitions.

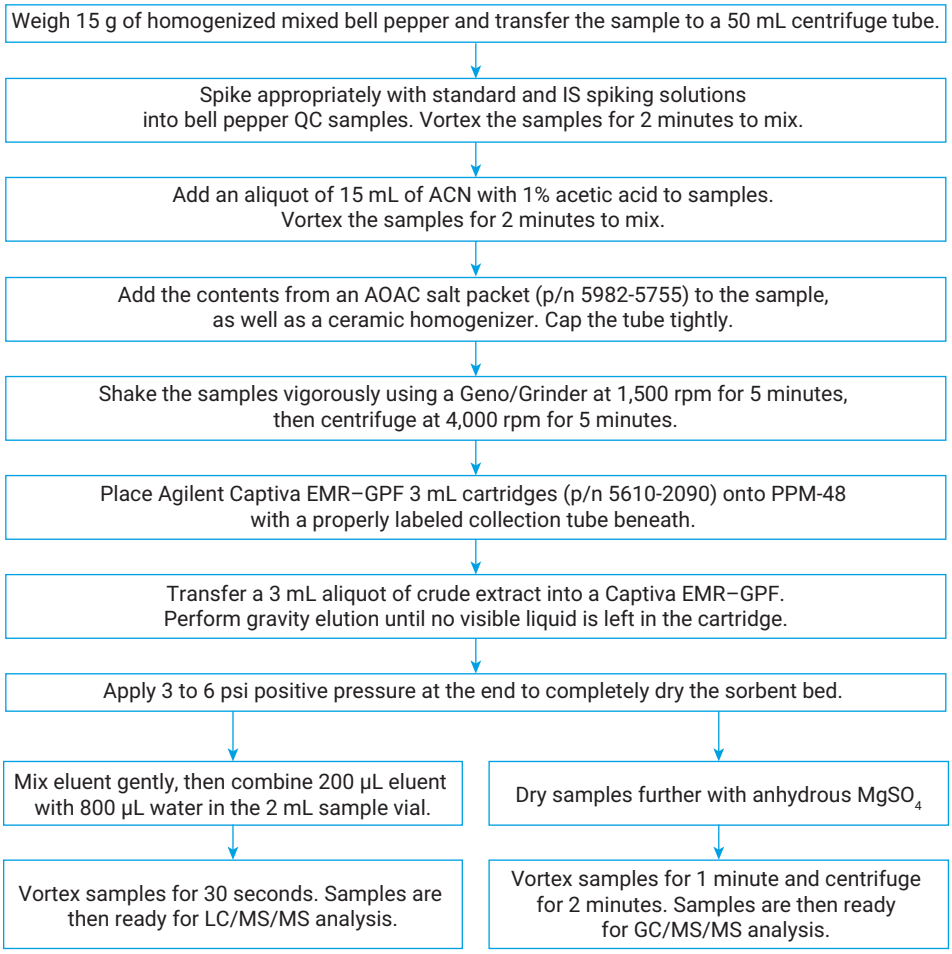

Figure 2. Sample preparation procedure for bell pepper samples using the Agilent Bond Elut QuEChERS AOAC extraction kit followed by Agilent Captiva EMR–GPF passthrough cleanup for both LC/MS/MS and GC/MS/MS analysis.

Results and discussion

Carbon S sorbent and Captiva EMR passthrough cleanup

Agilent Carbon S sorbent is an advanced hybrid carbon material that delivers an excellent balance between analyte recovery and matrix pigment removal efficiency. Captiva EMR passthrough cleanup methodology offers high selectivity and efficiency at removing matrix interferences, making this a convenient, rapid, and reliable sample matrix cleanup technique. This sample cleanup methodology is especially suitable for multiclass, multiresidue analysis, as the matrix cleaning is based on selective retention of unwanted matrix interferences, and thus has minimal impact on target recoveries. Compared to traditional dSPE cleanup, the passthrough cleanup provides simplified workflow steps, such as the elimination of uncapping and capping the dSPE tubes, vortexing, centrifuging. Passthrough cleanup using Captiva EMR products has been used for food analysis, such as in fatty matrices by Agilent Captiva EMR–Lipid[3,4], and in fresh produce by Captiva EMR–GPF[5] and Captiva EMR–HCF.[6] The detailed description of all the Captiva EMR cartridges and their recommendations for plant-origin matrices are shown in Table 4.

The sorbents formula was carefully and thoroughly optimized based on multiresidue target recoveries and matrix cleanup efficiency. Depending on different matrices, these Captiva EMR cartridges provide selective, efficient matrix cleanup, including organic acids, pigments, lipids/fats and other hydrophobic interferences. The commonly used anhydrous $MgSO_4$ powder in dSPE kits is not included in any Captiva EMR cartridges because our investigations showed that the simultaneous water removal by $MgSO_4$ during the cleanup procedure can significantly compromise the buffering effect and result in the loss of some labile pesticides. The simplified workflow provided efficient and selective matrix cleanup; improved target recoveries, and reproducibility; reduced matrix effect and cleaner matrix background. This makes it a relatively consistent sample preparation method for both LC/MS/MS and GC/MS/MS detection, without further modification needs required by different detection methods.

Sample preparation procedure

For fresh fruit and vegetable matrices, QuEChERS extraction has been adopted widely as the standard sample extraction procedure. In this study, the standard QuEChERS extraction method was applied using the QuEChERS AOAC extraction kit. After extraction, 3 mL of crude extract was loaded into the Captiva EMR–GPF cartridges for passthrough cleanup. The elution was performed by gravity, and the entire elution took 5 to 10 minutes for 3 mL of crude sample extract. An aliquot of 200 µL eluent was mixed with 800 µL of water for LC/MS/MS analysis. The rest of the eluent was further dried by anhydrous $MgSO_4$ for complete water residue removal. The gravity elution on Captiva EMR–GPF cartridges is straightforward and requires little effort. While elution is taking place, the analyst can prepare for next steps, such as the following sample dilutions. Usually, it takes approximately 40 to 50 minutes to get 30 samples ready for both LC/MS/MS and GC/MS/MS analysis.

Performance comparison of sample cleanup methods

Captiva EMR–GPF cartridges are designed for general pigmented fresh sample matrix passthrough cleanup, containing the optimized blended sorbents formula of Carbon S, primary secondary amine (PSA) and end-capping C18 (EC-C18). The novel passthrough cleanup methods were compared thoroughly with traditional U-dSPE with GCB, as well as two corresponding vendor dSPEs (vendor 1 and 2).

Table 4. Agilent Captiva EMR cartridges and their recommendations for different plant-origin matrices.

Agilent Product Name	Sorbents	Sample Loading Volume	Recommendations Based on Sample Matrices	Examples of Applicable Sample Matrix
Captiva EMR–Lipid	Carbon EMR–Lipid	2.5 to 3 mL for 3 mL cartridges 5 to 6 mL for 6 mL cartridges	High fatty oily matrices	Edible oils
Captiva EMR–HCF1	Carbon S/NH_2	3 mL	High chlorophyll fresh leafy vegetables	Spinach, parsley, alfalfa
Captiva EMR–HCF2	Carbon S/PSA	3 mL	High chlorophyll fresh leafy vegetables	Spinach, parsley, alfalfa
Captiva EMR–GPF	Carbon S/PSA/EC-C18	3 mL	General pigmented fresh plant-origin matrix	Berries, peppers, broccoli, grapes, celery
Captiva EMR–GPD	Captiva EMR–Lipid/PSA/EC-C18/Carbon S	2.5 to 3 mL	General pigmented dry plant-origin matrix	Spices, tea, coffee
Captiva EMR–LPD	Captiva EMR–Lipid/PSA/EC-C18/Carbon S	2.5 to 3 mL	Low/none pigmented dry plant-origin matrix	Nuts, light pigmented spices, tobacco

Sample matrix cleanup

The sample matrix cleanliness evaluation includes the visual color appearance comparison, samples' LC-UV absorption at 450 nm, and GC/MS full scan background comparison. Figure 3 shows the bell pepper matrix cleanliness comparison using various cleanup methods after QuEChERS extraction, with: (A) the visual color comparison on the final extract, (B) the UV absorption comparison at wavelength of 450 nm, and (C) the GC/MS full scan background comparison. The mixed bell pepper crude extract was brown in color. Samples after either Captiva EMR–GPF or vendor 2 dSPE cleanup were clear with a very light-yellow color. Samples with U-dSPE with GCB cleanup were also light yellow in color, but a little darker than the previous two samples. All three of these cleanup methods were confirmed to have performed >95% pigment removal based on the UV absorption of the pigment components. In comparison, samples after vendor 1 dSPE cleanup were only a little lighter brown in color than the crude extract without any cleanup. The UV absorption confirmed this sample had undergone only approximately 50% pigment removal based on UV absorption. These results are also in alignment with the GC/MS full scan background comparison, where the sample extracts after Captiva EMR–GPF, vendor 2 dSPE, and U-dSPE with GCB cleanup delivered a similar low full scan background. The sample after vendor 1 dSPE cleanup not only had a similar background to the sample without cleanup, but also had significant additional interferences contributing to background at the retention time window of 3 to 7 minutes, indicating there was contamination introduced during the sample cleanup. The results clearly demonstrate the highly efficient matrix cleanup provided by Captiva EMR–GPF passthrough cleanup, compared to existing dSPE cleanup products. The vendor 2 dSPE cleanup in particular showed the poorest performance for matrix/pigment removal.

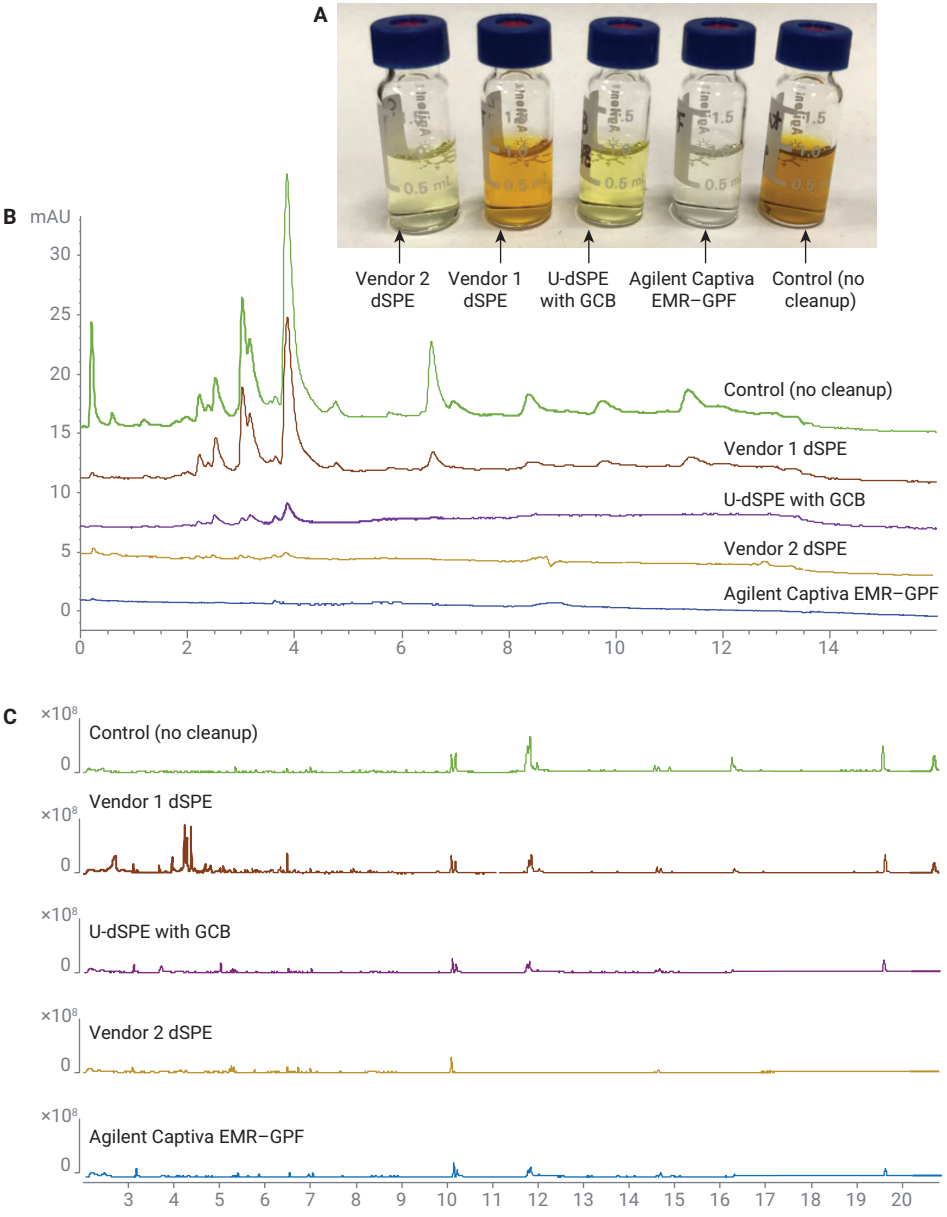

Figure 3. Mixed bell pepper matrix sample cleanliness evaluation. (A) Extracted samples color comparison. (B) LC-UV (λ = 450 nm) stacked chromatograms for samples' UV adsorption. (C) GC/MS full scan background chromatogram.

Analyte recovery and reproducibility

Analyte recovery and reproducibility are key considerations when determining whether a sample preparation method is acceptable or not. High matrix cleanup efficiency is desired, but it cannot be accepted if it comes with a significant compromise to target recovery. Therefore, the developed method was thoroughly evaluated for the sample cleanup impact to the targets' recoveries using a large panel of pesticides, including 230 LC and GC amenable pesticides. The 10 ng/g spiking level was used in the recovery study, as this level is usually beneath the regulatory maximum residue level (MRL) of pesticides in fresh produce. Additionally, low-level analysis challenges the method performance more than high-level analysis. Figure 4A shows a summary of the average recovery distribution of 230 targets at 10 ng/g fortification level, using four types of cleanup methods. Captiva EMR–GPF cleanup was demonstrated to be the best cleanup method with the lowest targets recovery failure rate, while the other three cleanup methods showed the higher recovery failure rates. Vendor 1 dSPE cleanup delivered the second-best recovery results, with approximately 5% failure rate, but the compromise was poor efficiency of sample matrix cleaning. Both vendor 2 dSPE and U-dSPE with GCB cleanup compromised significantly on target recoveries, even with acceptable matrix cleanup efficiency.

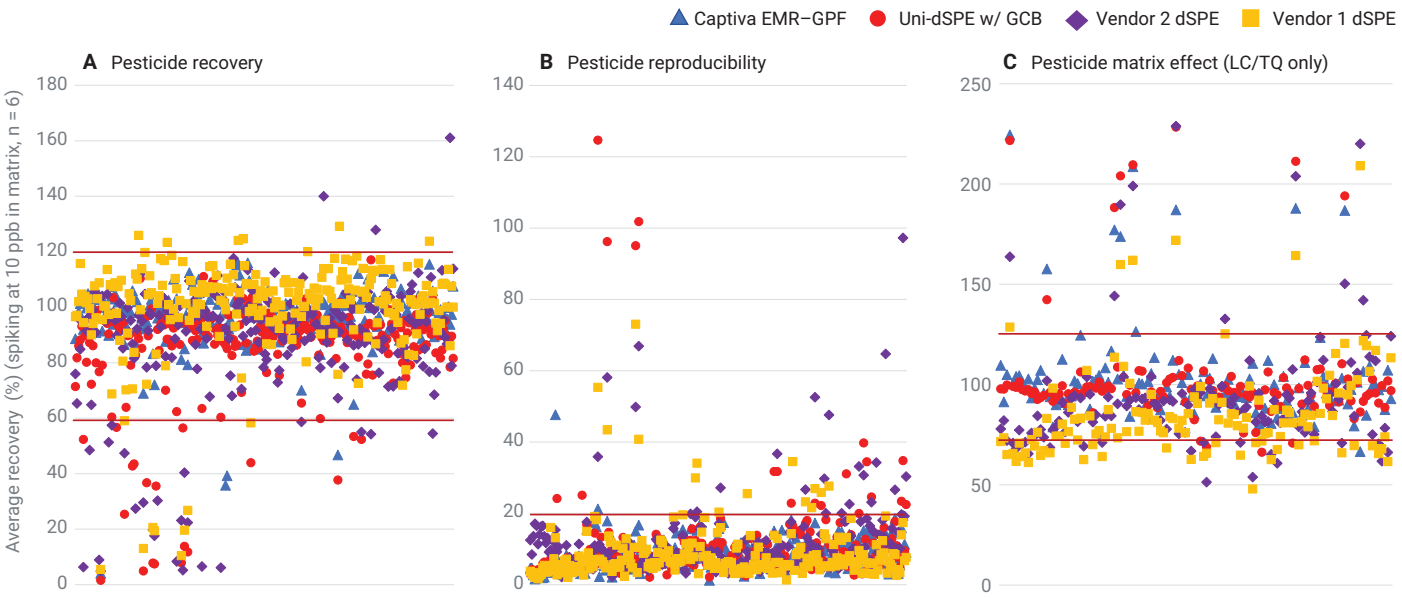

Figure 4. Quantitative analysis of 230 pesticides in mixed bell pepper at 10 ng/g fortification level (n = 6) based on (A) targets recovery, (B) targets reproducibility (RSD%), and (C) matrix effect (LC/TQ only), using different cleanup methods. Refer to Table 3 for target details.

Sensitive pesticides may be lost during sample cleanup, including planar pesticides, acidic pesticides, and other sensitive compounds. This is due to unwanted interactions occurring between these compounds and the sorbents used for matrix cleanup. Figure 5 shows the individual sensitive compound recovery comparison after the four types of cleanup methods. The results confirm that U-dSPE with GCB and vendor 2 dSPE caused extensive loss of planar, acidic, weak acidic, and weak basic pesticides. Vendor 1 dSPE provided better recoveries for planar pesticides, but still caused significant loss of acidic and basic pesticides. In comparison, Captiva EMR–GPF provided significant improvement to recovery of these sensitive pesticides.

Method reproducibility is another critical consideration for sample preparation method acceptability. Poor reproducibility is usually related to either the inconsistent interactions between targets and sorbent, or the inconsistent matrix impact on target responses. Typically, an analyte's relative standard deviation (RSD) is used for method reproducibility assessment with replicates of three to six, and RSD% ≤20 is the acceptance criterion. Figure 4B shows the 230 targeted pesticide RSD summary using six replicates of fortified bell pepper at 10 ng/g spiking level. Results confirm again that Captiva EMR–GPF passthrough cleanup provided the best method reproducibility.

Matrix impact to method performance

Sample matrix impact on method performance was evaluated based on matrix effect on LC/MS/MS and matrix interferences on GC/MS/MS. Matrix effect (ME) on LC/MS/MS is usually caused by matrix coeluted interferences that cause the target response suppression or enhancement. ME is usually calculated by the ratio of analyte response in matrix matched spiked sample to that in the neat standard at the corresponding level. The closer the ME to 100%, the lesser the impact on target analysis from the sample matrix. Even without a strict acceptance criterion for matrix effect, it is an important assessment of the impact of sample matrix cleanliness on the reliability of method quantitation. Figure 4C shows a summary of ME comparison of samples prepared by different cleanup methods, using 129 LC-amenable pesticides. The high matrix cleaning efficiency provided by Captiva EMR–GPF passthrough cleanup, U-dSPE with GCB, and vendor 2 dSPE cleanup generally delivered good matrix effect results. Several pesticides showed significant matrix enhancement, which mostly attributed to the positive contributions from the sample matrix blank. Considering the poor matrix cleanup efficiency in samples prepared by vendor 1 dSPE cleanup, it is not surprising that many analytes experienced more matrix ion suppression.

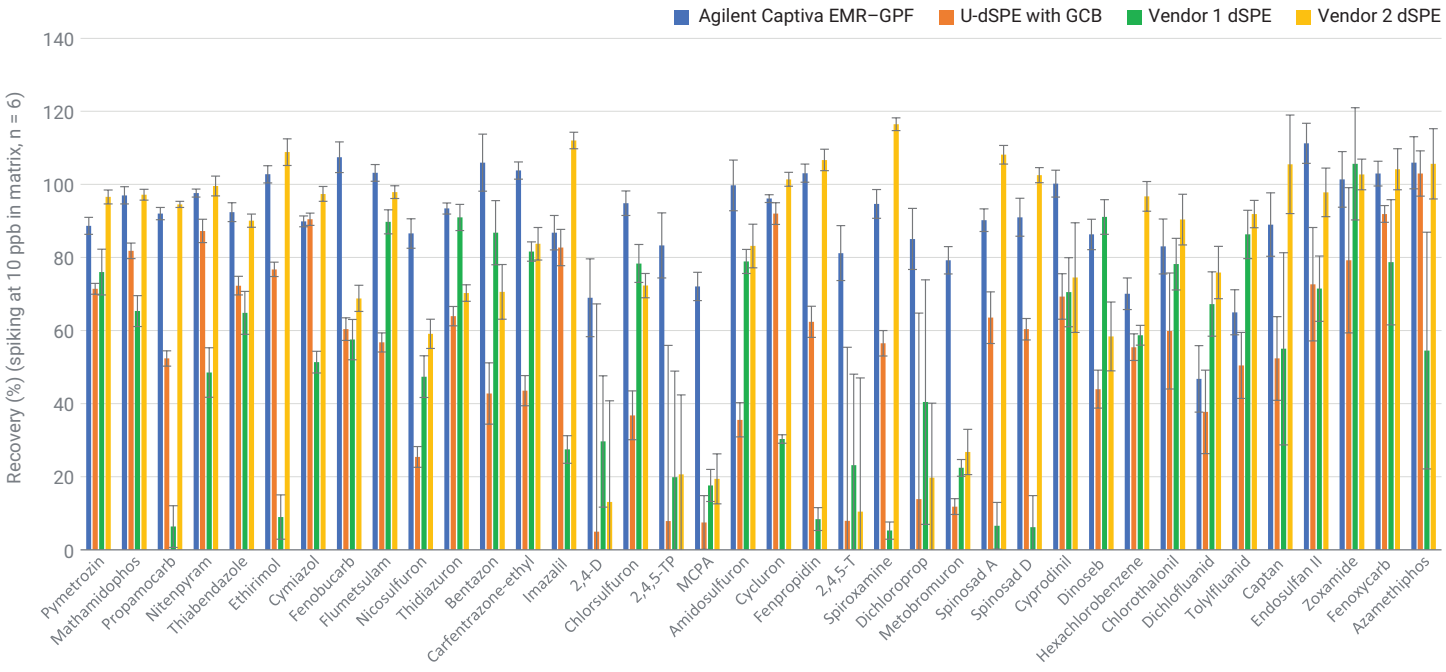

Figure 5. Sensitive pesticide recovery in mixed bell pepper at 10 ng/g fortification level in the matrix (n = 6), compared between the different cleanup methods.

Matrix impact on GC/MS/MS analysis is exhibited as matrix interferences shown in the targets' acquisition window. Given the nature of ionization in GC/MS/MS, targets' MRM transitions are not as selective as those in LC/MS/MS. Therefore, for a complex sample matrix, without efficient cleanup, it is very common that accurate integration can be significantly impacted by the matrix interference peaks shown from the sample background. Figure 6 shows the MRM chromatogram comparison of 10 ng/mL postspiked matrix samples using different cleanup methods. As an example, one pesticide, molinate, was extracted for a specific comparison example. The comparison results clearly demonstrate a cleaner analyte background, provided by Captiva EMR–GPF cleanup, and the resulting analyte integration accuracy.

Target analysis failure rate

For multiclass, multiresidue analysis of a large panel of pesticides, it is unrealistic to have a perfect method where all targets meet the strict acceptance criteria. Compromises always must be made to balance acceptable quantitation, matrix cleanliness, and the impacts on the detection instrumentation. The best balance is always to achieve the desired quantitation results for most targets with minimal compromise.

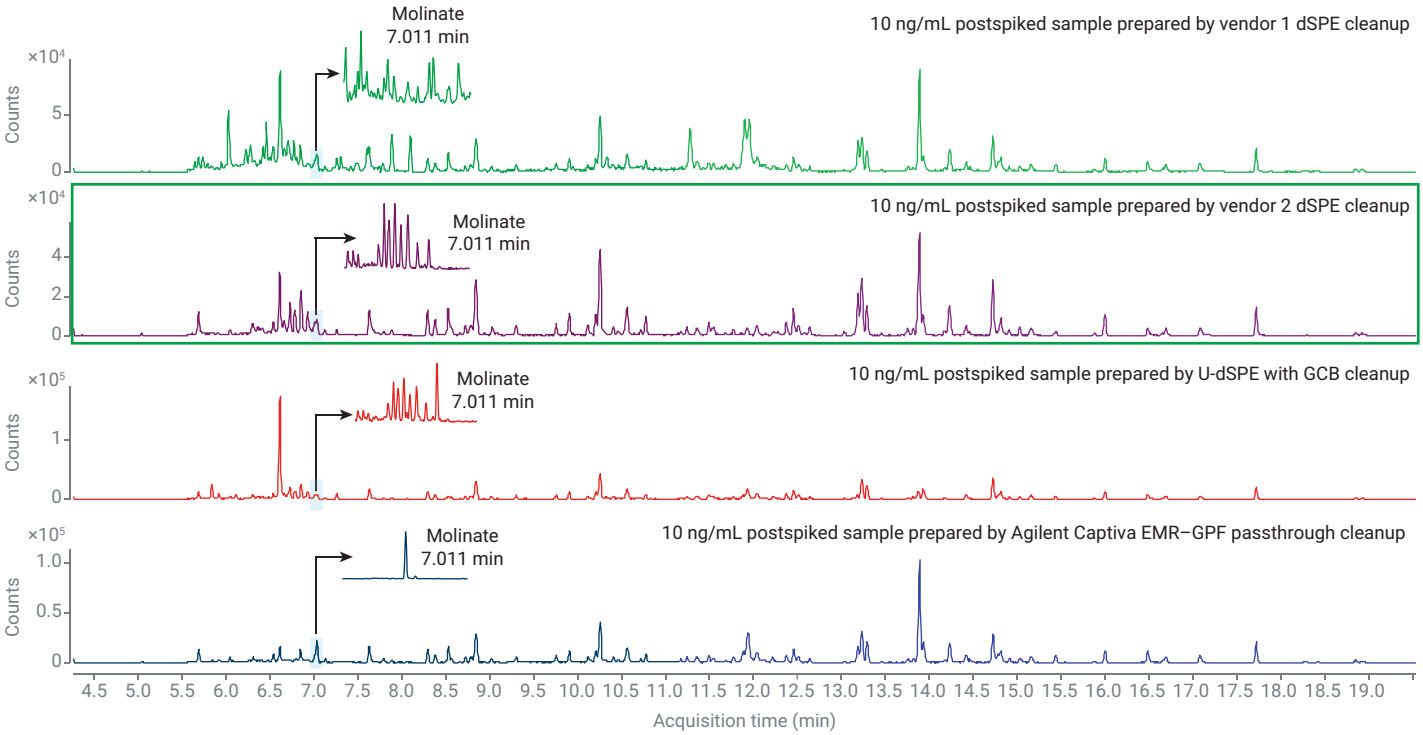

Figure 6. GC/MS/MS MRM chromatograms for the bell pepper extracted samples postspiked at 10 ng/mL. Expanded chromatograms show the MRM chromatograms for molinate in a 1.5-minute acquisition window.

Figure 7 shows the failure rate of the 230 targets based on the recovery, reproducibility, and matrix effect acceptance criteria for quantitative analysis. Results confirmed that the lowest failure rate was achieved for quantitative analysis of a large panel of pesticides when using the newly developed Captiva EMR–GPF passthrough cleanup after traditional QuEChERS extraction, compared to the other cleanup methods.

Method quantitation verification

Method quantitation performance was verified in mixed bell pepper using QuEChERS extraction followed with Captiva EMR–GPF passthrough cleanup for two levels of prespiked QCs: 10 ng/g and 100 ng/g. Nine matrix matched calibration standards were prepared to cover the dynamic range of 0.5 to 500 ng/g on LC/MS/MS, and 1 to 500 ng/g on GC/MS/MS. The calibration curves were generated using linear regression and $1/x^2$ weighting. Three ISs (atrazine-D_5, DDT-D_8, and TPP) were used at 100 ng/g for quantitation on GC/MS/MS. Similarly, two ISs (4-nitrophenol-D_4 and Simazin-D_{10}) were used at 50 ng/g for quantitation on LC/MS/MS. The results for target quantitation accuracy and precision (RSDs), as well as calibration curve linearity, are statistically summarized in Figure 8. The following pesticides were outliers: quinmerac, bifenazate, and dichlofluanid for low recovery, quinmerac and 2,4-D for high RSDs, and dimethomorph I, spinosad D, prochloraz, moxidectin, metribuzin, malathion, triadimefon, zoxamide, azamethiphos, coumaphos, and pyraclostrobin for calibration curve R^2 between 0.98 to 0.99.

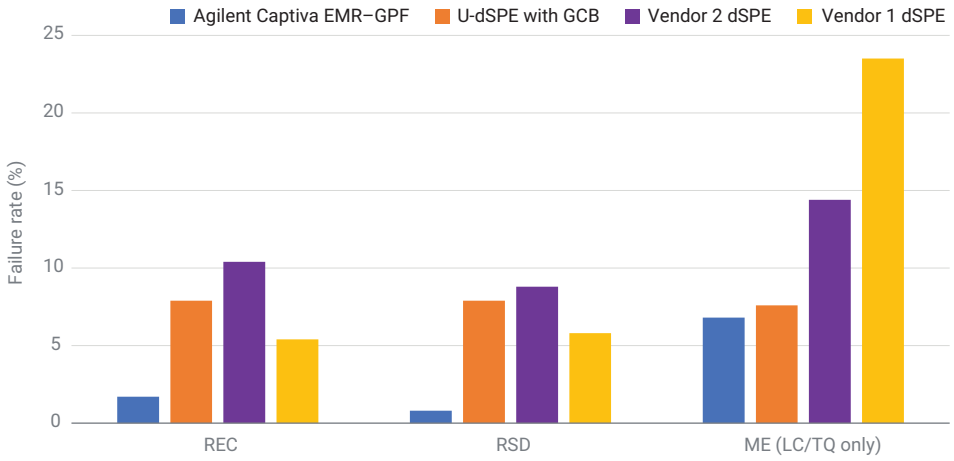

Figure 7. Failure rate of a large panel of multiclass, multiresidue pesticides (230 compounds), compared between the different methods. The acceptance criterion for recovery is 60 to 120%, for RSD is ≤20%, and for matrix effect (ME) on LC/MS/MS is 70 to 130%.

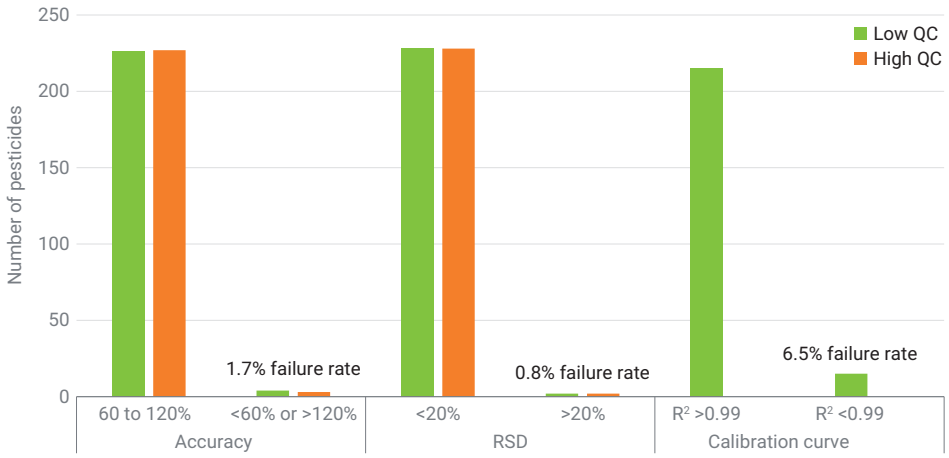

Figure 8. Method verification results summary for quantitative determination of 230 pesticides in mixed bell pepper by LC/MS/MS and GC/MS/MS. Samples were prepared using the Agilent Bond Elut QuEChERS AOAC extraction kit followed by Agilent Captiva EMR–GPF passthrough cleanup.

Conclusion

A simple, rapid, and reliable method using the Agilent Bond Elut QuEChERS AOAC extraction kit followed by Agilent Captiva EMR–GPF cartridge passthrough cleanup was developed and validated for 230 LC and GC amenable pesticides in mixed bell pepper by LC/MS/MS and GC/MS/MS. Compared to traditional dSPE with GCB cleanup and as well as two other vendor dSPE cleanups, this novel method provides a convenient and simplified sample passthrough cleanup. The method offers selective and efficient pigment/matrix removal, improved target recovery and reproducibility, and reduced matrix effect and interferences. The result is an improved quantitation pass rate for the analysis of a large panel of pesticides in fresh produce matrices.

References

1. González-Curbelo, M. Á. *et al. Trends in Anal. Chem*. **2015**, *71*, 169–185.
2. Varela-Martínez, D. A. *et al. Liquid-Phase Extraction Handbooks in Separation Science* **2020**, Chp 14, 399–437.
3. Zhao, L. *et al.* Multiclass Multiresidue Analysis of Veterinary Drugs in Meat using Enhanced Matrix Removal Lipid Cleanup and Liquid Chromatography-Tandem Mass Spectrometry. *J. Chromatog. A* **2018**, *1549*, 14–24.
4. Lucas D.; Zhao L. Multiclass Mycotoxin Analysis in Cheese Using Agilent Captiva EMR-Lipid Cleanup and LC/MS/MS, *Agilent Technologies application note*, publication number 5991-8694EN, **2017**.
5. Zhao L.; Wei T. Determination of Multiclass Multiresidue Pesticides in Berries using Captiva EMR–GPF Passthrough Cleanup by GC/MS/MS, *Agilent Technologies application note*, publication number 5994-4764EN, **2022**.
6. Zhao L.; Wei T. Determination of Multiclass Multiresidue Pesticides in Spring Leaf Mix using Captiva EMR–HCF Passthrough Cleanup by LC/MS/MS, *Agilent Technologies application note*, publication number 5994-4765EN, **2022**.

www.agilent.com

DE21688961

This information is subject to change without notice.

Printed in the USA, April 27, 2022
5994-4767EN

Agilent

Trusted Answers