Application Note

Food & Beverage Testing

Determination of Multiclass, Multiresidue Pesticides in Spring Leaf Mix

Using Capitva EMR-HCF passthrough cleanup and LC/MS/MS

Authors

Limian Zhao and Ta-Chen Wei
Agilent Technologies, Inc.

Abstract

This application note presents the development and validation of a method for the analysis of multiclass multiresidue pesticides in spring leaf mix. The method involves extraction with the Agilent Bond Elut QuEChERS AOAC extraction kit, followed by passthrough cleanup with Agilent Captiva Enhanced Matrix Removal–High Chlorophyll Fresh (EMR–HCF), then LC/MS/MS. Two types of Captiva EMR–HCF (EMR–HCF1 with NH_2, and EMR–HCF2 with PSA) were used for method performance evaluation. They both provided highly efficient chlorophyll pigment removal, and significantly reduced unwanted interactions with targets, especially for sensitive compounds such as planar pesticides. These two types of EMR–HCF cartridges were designed for high-chlorophyll leaf vegetable matrix, with optimized formula and bed mass. The results for both Captiva EMR–HCF cartridges demonstrated that over 96% of the pesticides were identified with 70 to 120% recovery, RSD <20%, and calibration curves with R^2 >0.99, within the calibration range. The pigment removal assessment by LC-UV also confirmed that >96% of green/yellow pigment interferences are removed by the EMR–HCF cleanup.

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 using organic solvents. Without the further removal of pigment co-extractives, the direct injection of highly pigmented sample extract into detection instrumentation, such as LC/MS/MS or GC/MS/MS, could result in multiple matrix effects. The impacts include matrix ion suppression on LC/MS/MS, matrix interferences on GC/MS/MS, accumulated matrix deposition on 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.

Graphized carbon black (GCB) has been used in sample preparation for efficient pigment removal.[1,2] GCB has been used in many dispersive solid phase extraction (dSPE) kits after the commonly used QuEChERS extraction for fresh produce analysis. For high-chlorophyll fresh matrices such as dark green leafy vegetables, a relatively high amount of GCB has been used in dSPE kits to achieve enhanced pigment removal efficiency. The Agilent Bond Elut QuEChERS Pigmented Fruits and Vegetables dispersive SPE kit, AOAC method (AP-dSPE) it is the most suitable for high-chlorophyll fresh matrix cleanup, which contains 50 mg of GCB per 1 mL of dark green extract. However, AP-dSPE cleanup could cause large loss of planar pesticides, such as hexachlorobenzene, thiabendazole, and so on. The Agilent Bond Elut QuEChERS Pigmented Fruits and Vegetables dSPE kit, EN method contains much less GCB (7.5 mg) per 1 mL of crude dark green extract, so planar pesticide recoveries can be improved, but with significant compromise to chlorophyll pigment removal. Balancing planar pesticide recovery and chlorophyll pigment removal is challenging with only adjusting the amount of GCB in the dSPE kit.

For high-chlorophyll fresh matrices, two kinds of Captiva EMR–HCF cartridges are available, with the Carbon S sorbent blended at a ratio of 1:1 with either NH_2 for the EMR–HCF1 cartridges, or with primary secondary amine (PSA) for the EMR–HCF2 cartridges using the optimized bed mass. Two versions of Captiva EMR–HCF are provided for use in the similar applications using identical procedure except the different cartridges. However, both cartridges perform excellently in terms of chlorophyll pigment removal and analyte recovery, so the current protocols can easily be transferred to corresponding currently used products with high confidence. In this study, sample preparation using both Captiva EMR–HCF1 and EMR–HCF2 cartridges for passthrough cleanup was demonstrated for the analysis of 138 common pesticides in spring leafy mix by LC/MS/MS. Spring mix is a common leaf vegetable mix, which contains tender baby lettuce, spinach, red and green romaine, oak leaf, chard, arugula, endive, and so on. This matrix was selected to represent the high-chlorophyll leaf matrix, which is the most challenging pigmented fresh matrix.

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), 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

A combined standard spiking solution (138 pesticides) and a combined IS (two IS compounds) spiking solution were prepared at 10 µg/mL in ACN and 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 study was performed using an Agilent 1290 Infinity LC system coupled to an Agilent 6490 triple quadrupole LC/MS system. The Agilent 1290 Infinity LC system consists 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 coupled Agilent triple quadrupole LC/MS (G6490) is equipped with an Agilent Jet Stream iFunnel electrospray ion source. 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–HCF1, with

NH_2, 3 mL (part number 5610-2088), Agilent Captiva EMR–HCF2, with PSA, 3 mL (part number 5610-2089)

Instrument conditions

Table 1 lists the LC/MS/MS conditions, and Table 2 lists the targets' dynamic multiple reaction monitoring (dMRM) parameters. Figure 1 shows a typical MRM chromatogram of targeted pesticides in the fortified spring mix sample at the level of 100 ng/g using the LC/MS/MS conditions in Table 1, prepared by QuEChERS AOAC extraction followed by Captiva EMR–HCF1 cleanup.

Table 1. Agilent 1290 Infinity LC and Agilent 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. Targeted pesticides dMRM conditions.

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

Target Name	RT (min)	First MRM Transition (*m/z*)	CE (V)	Second MRM Transition (*m/z*)	CE (V)	Delta RT (min)	Polarity
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	N/A	N/A	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 D4 (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-D10 (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
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-D5 (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

Target Name	RT (min)	First MRM Transition (*m/z*)	CE (V)	Second MRM Transition (*m/z*)	CE (V)	Delta RT (min)	Polarity
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
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

Target Name	RT (min)	First MRM Transition (*m/z*)	CE (V)	Second MRM Transition (*m/z*)	CE (V)	Delta RT (min)	Polarity
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

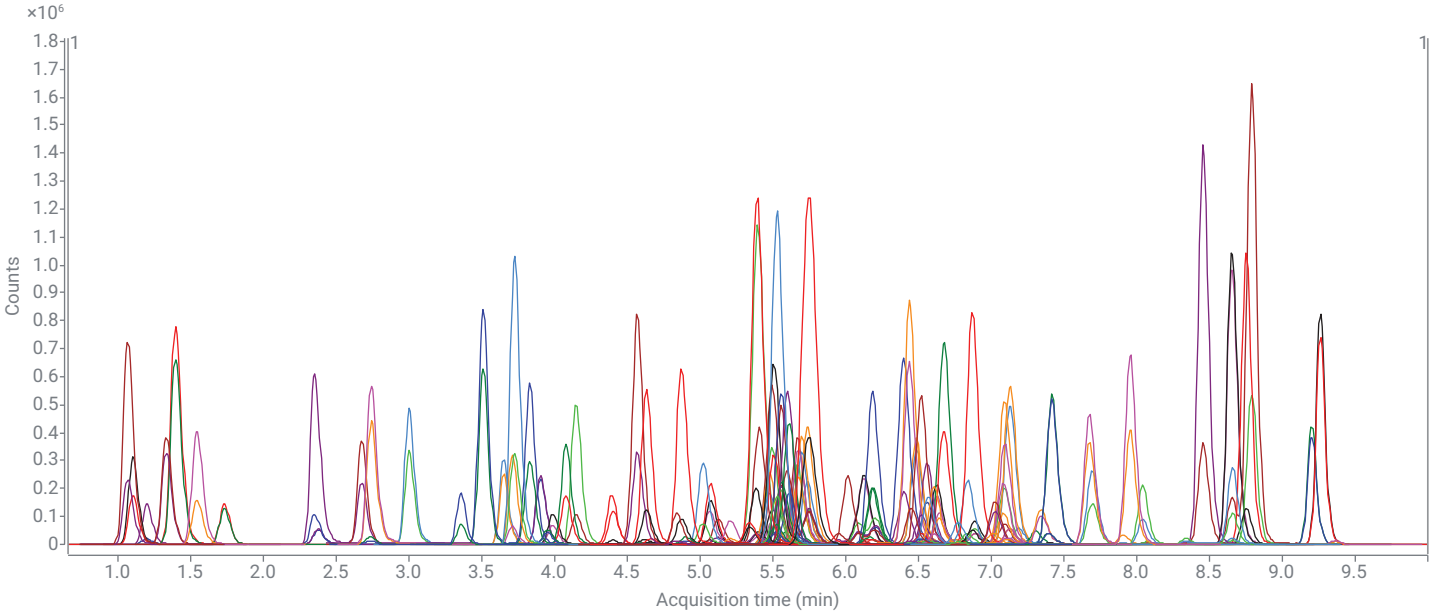

Figure 1. LC/MS/MS MRM chromatogram for extracted spring mix sample fortified with 100 ng/g of 130 targeted pesticides. The sample was prepared using the Agilent Bond Elut QuEChERS AOAC extraction kit, followed by Agilent Captiva EMR–HCF1 cleanup.

Sample preparation

The fresh organic spring mix was purchased from a local grocery store. Samples were frozen at –20 °C overnight, then homogenized with a grinder. The ground matrix samples were then weighed at 15 g, placed into a 50 mL centrifuge tube, and stored in the –20 °C freezer until extraction. The weighed spring mix samples (15 g) were thawed and then extracted following the QuEChERS AOAC method. The crude extract was then loaded into the 3 mL Captiva EMR–HCF1 cartridges or Captiva EMR–HCF2 cartridges for passthrough cleanup. The sample eluent was then diluted with water five times to generate the final sample in 20:80 ACN/water. The diluted sample was then ready for LC/MS/MS analysis. The detailed sample preparation procedure is shown in Figure 2. For a batch of ~30 samples, the entire procedure usually takes approximately 25 to 30 minutes.

Method performance evaluation

The novel sample preparation method performance was evaluated in terms of matrix pigment removal; target recovery and reproducibility; and matrix matched calibration curve linearity and limits of quantitation (LOQs) in spring mix, by Captiva EMR–HCF1 and EMR–HCF2 cleanup, respectively. To evaluate recovery, reproducibility, and matrix effect study, prespiked quality control (PR-QC) samples were prepared at 10 ng/g in spring mix sample homogenate in replicates of six. The spiked samples and matrix blank samples were then prepared following the procedure. Postspiked QCs (PO-QC) were prepared at 10 ng/mL in matrix blank. Neat QCs were directly spiked at 10 ng/mL in reagent blank (extraction solvent). 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 sample preparation method reproducibility RSD calculation. The peak area ratios of corresponding target in PO-QCs versus neat QCs were used for target matrix effect calculation. Matrix matched calibration curve linearity and LOQ were evaluated by postspiking at the levels of 0.5, 1, 5, 10, 50, 100, 250, 400, and 500 ng/mL in spring mix matrix blank extract. Method accuracy and precision verification include two spiking level PR-QCs for quantitation: 10 ng/g (low QC) and 100 ng/g (high QC). Analyte identification, confirmation, and quantitation were determined from retention times and MRM transitions.

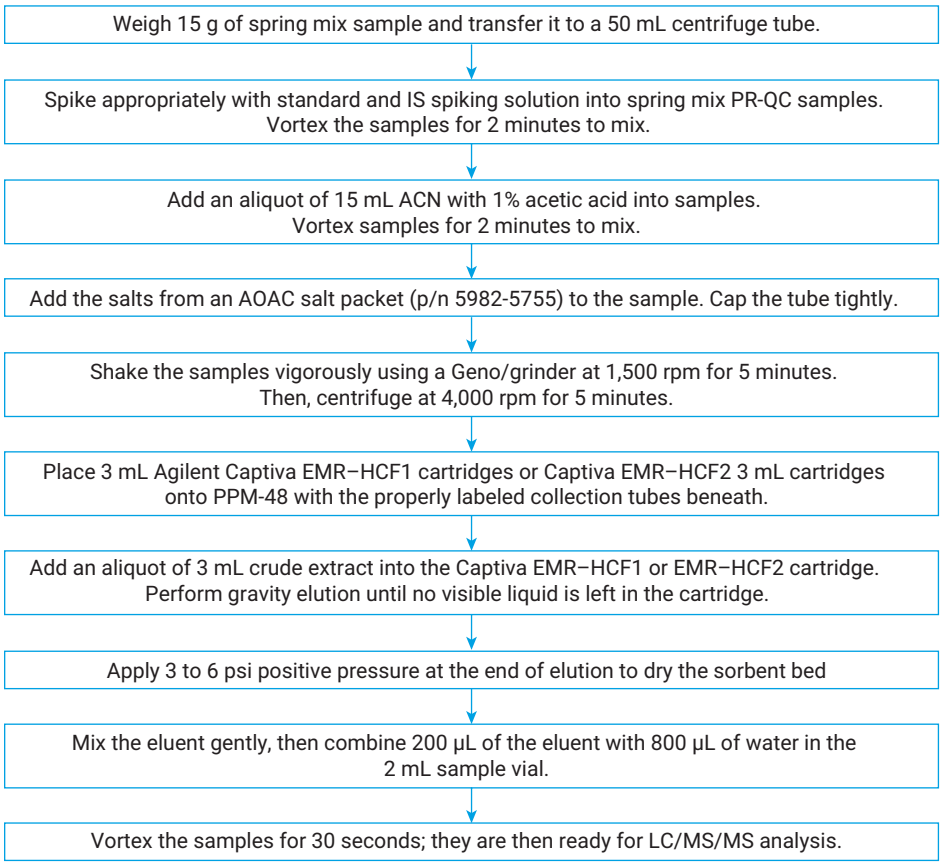

Figure 2. Sample preparation procedure for spring mix samples by Agilent Bond Elut QuEChERS AOAC extraction followed by Agilent Captiva EMR–HCF1 or EMR–HCF2 passthrough cleanup.

Results and discussion

Carbon S sorbent and Captiva EMR passthrough cleanup

Agilent Carbon S sorbent is an advanced hybrid carbon material with optimized carbon content and pore structure. The improved sorbent provides equivalent or better pigment removal from plant-origin sample matrices than GCB sorbent, and significantly improves recoveries of sensitive targeted analytes. As a result, Carbon S sorbent delivers an excellent balance between analyte recovery and matrix pigment removal efficiency.

Captiva EMR passthrough cleanup methodology was introduced by the Captiva EMR–Lipid products. The EMR–Lipid passthrough cleanup methodology offers high selectivity and efficiency at removing lipids, 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 provides 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, and centrifuging. Passthrough cleanup using Captiva EMR–Lipid products has been widely used for food analysis in fatty matrices by LC/MS/MS.[3-5]

The new Carbon S sorbent enables Agilent to further expand the Captiva EMR family and thus provide selective and efficient matrix passthrough cleanup to plant-origin sample matrices, including fresh and dry matrices. Five new Captiva EMR cartridges were developed with optimized formula for various complicated plant-origin sample matrices. A detailed description of all the Captiva EMR cartridges and their recommendations for plant-origin matrices are shown in Table 3.

The sorbents formula was carefully and thoroughly optimized based on multiresidue target recoveries and matrix cleanup efficiency. Depending on different matrices, these 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 has not been included in any EMR cartridges because the investigations showed that the simultaneous water removal by $MgSO_4$ during cleanup procedure can significantly compromise the buffering effect and result in loss of some labile pesticides. For LC and LC/MS analysis, sample eluent after EMR cleanup can either be diluted with water or injected directly.

For the fresh sample matrices in this study, spring mix is considered as a high-chlorophyll fresh matrix. Therefore, both 3 mL Captiva EMR–HCF1 and EMR–HCF2 cartridges were applicable for this sample matrix cleanup after QuEChERS extraction.

Table 3. Agilent Captiva EMR cartridges and their recommendations for pesticide analysis in 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 oil
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
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 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 Bond Elut QuEChERS AOAC extraction kit, followed by either Captiva EMR–HCF1 with NH_2 or Captiva EMR–HCF2 passthrough cleanup, shown in Figure 2. The elution was performed by gravity, which took ~10 minutes for 3 mL of crude spring extract. When preparing the same quantity of samples for cleanup, the EMR passthrough cleanup procedure saves time by 30 to 40% compared to dSPE cleanup. Additionally, the passthrough cleanup is more user friendly with easy operations, thus making sample preparation more efficient.

Sample preparation method performance assessment

Both Captiva EMR–HCF1 and EMR–HCF2 are designed for high-chlorophyll fresh sample matrix passthrough cleanup. Both cartridges were evaluated for the spring mix sample cleanup after QuEChERS extraction. The novel passthrough cleanup methods were compared thoroughly with traditional AOAC Pigmented dSPE with GCB (AP-dSPE with GCB) cleanup, as well as a corresponding competition dSPE cleanup that uses a polymer sorbent for pigment removal. Both Captiva EMR–HCF cleanup methods were then validated in spring mix for quantitation accuracy and precision, and calibration curve linearity and LOQ.

A. Cleanup method performance comparison

The evaluation of various cleanup methods involves comparison of pigment removal efficiency, analyte recovery, reproducibility, and matrix effect. The pigment removal evaluation was based on visual color comparison and LC-UV adsorption at 450 nm, and the results are shown in Figure 3. Visually, all three sample extracts after Captiva EMR–HCF1 and EMR–HCF2 passthrough cleanup and AP-dSPE with GCB cleanup delivered a color range of light to medium yellow, with >95% of pigment removal based on UV 450 nm adsorption. Competition dSPE cleanup still resulted in a green color for the final extract, with <60% of pigment removal based on UV 450 nm adsorption. Captiva EMR–HCF1 cleanup (98%) provided slightly higher pigment removal than Captiva EMR–HCF2 cleanup (97%), and both Captiva EMR–HCF cleanup methods delivered slightly higher pigment removal than AP-dSPE with GCB leanup (95%).

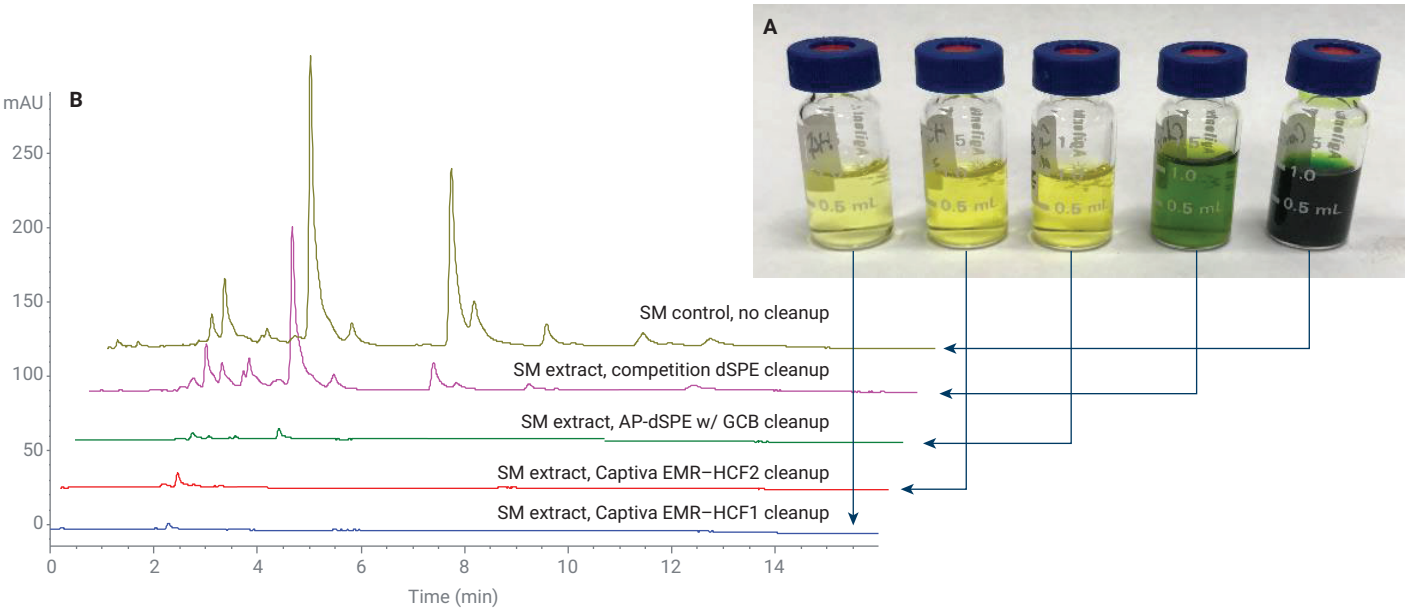

Figure 3. Spring mix (SM) matrix sample pigment removal efficiency demonstration. (A) Extracted samples color comparison. (B) LC-UV (λ = 450 nm) stacked chromatograms for extracted spring mix samples.

The statistical results summary for analyte recovery, RSD, and matrix effect is shown in Figure 4, including the number of passed/failed targets and failure rate (%). The pass/fail criteria for analyte recovery is 70 to 120% versus <70% or >120%; for RSD is ≤20% versus >20%; and for matrix effect is 80 to 120% versus <80% or >120%. Overall, both Captiva EMR–HCF1 and EMR–HCF2 provided the equivalent and best overall target recovery and reproducibility results, with lower failure rate than both traditional AP-dSPE with GCB and competition dSPE. Captiva EMR–HCF1 cartridges provided slightly better matrix effect than Captiva EMR–HCF2 cartridges. In comparison, AP-dSPE with GCB showed significantly higher failure rate on analyte recovery and reproducibility, although the matrix effect results by AP-dSPE with GCB were the best. The results clearly show the significant compromise to analyte recovery using this cleanup method, which certainly causes many concerns in applications. Competition dSPE cleanup showed better target recovery than AP-dSPE with GCB cleanup, but the significant payoff was the pigment/matrix removal efficiency, and the resulting higher matrix effect overall.

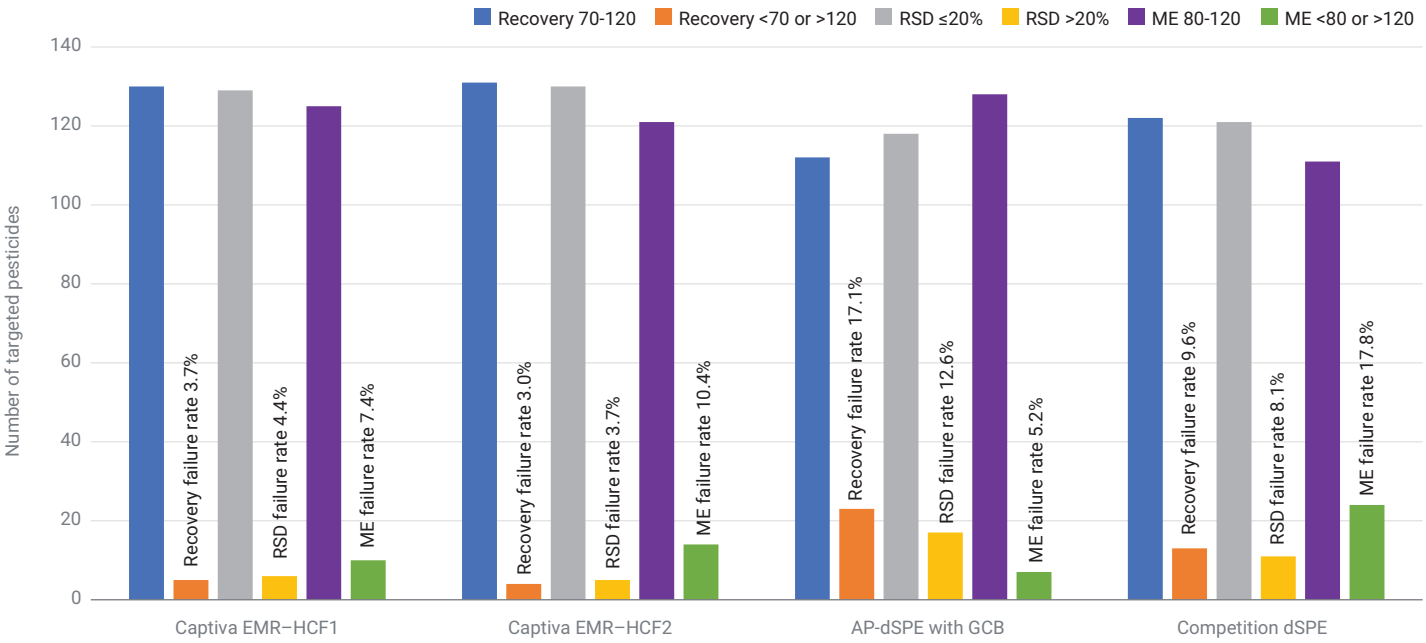

Figure 4. Targeted pesticides statistical comparison for recovery, RSD, and matrix effect (ME) in spring mix samples prepared by Agilent Bond Elut QuEChERS AOAC extraction followed with different cleanup methods. Spiking level at 10 ng/g in spring mix.

Figure 5 shows the individual sensitive targets recovery comparison by four different cleanup methods. Conclusions drawn from these results include: (a) Captiva EMR–HCF1 with NH_2 and Captiva EMR–HCF2 with PSA passthrough cleanup generally delivered equivalent recoveries, with slight differences in a couple of individual sensitive targets. (b) Both Captiva EMR–HCF passthrough cleanup methods provided significant improved recoveries over traditional AP-dSPE with GCB as well as competition dSPE cleanup. The improvement included recovery of not only typical planar compounds, such as thiabendazole, cyprodinil, forchlorfenuron, and so on, but also acidic or basic pesticides, such as 2,4-D, MCPA, nicosulfuron, oxasulfuron, and so on. The improvement in recovery of these sensitive compounds can be attributed to the following two factors: First, 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 better controlled, thus significantly improves the interaction selectivity and reduces the unwanted interactions between sorbent and target molecules. Second, 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 makes the Captiva EMR–HCF passthrough cleanup a more suitable sample cleanup method for multiclass, multiresidue large panel pesticides in food. (c) Competition dSPE cleanup provided better recoveries of some of sensitive targets, but recovery was still low for other sensitive targets. Plus, there were significant compromises to pigment removal efficiency and matrix ion suppression effect.

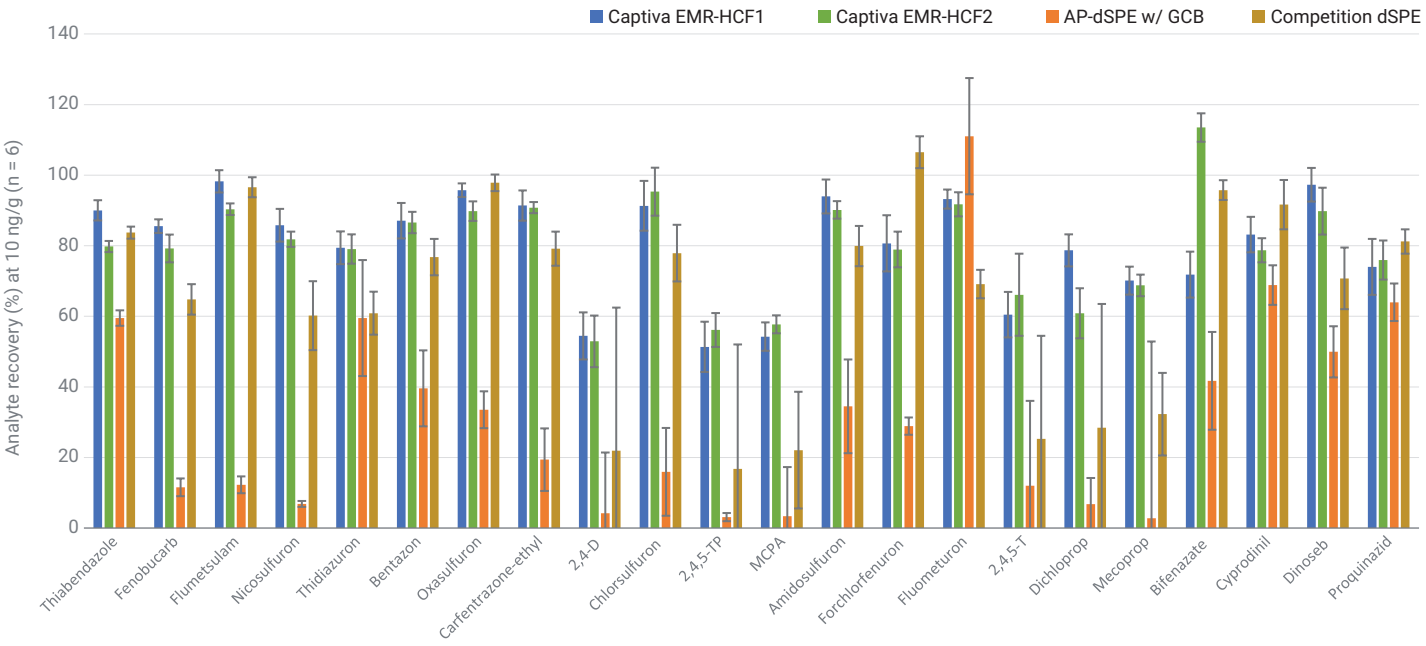

Figure 5. Sensitive target recovery results comparison for samples prepared using different cleanup methods.

B. Method quantitation verification: Method quantitation performance was verified in spring mix with 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 in spring mix. The calibration curves were generated using linear regression and $1/x^2$ weight. Three ISs (4-nitrophenol-D_4, simazine-D_{10}, and atrazine-D_5) were used at 50 ng/g for quantitation. The results of target accuracy and precision (RSD%) at two spiking levels using Captiva EMR–HCF1 with NH_2 and Captiva EMR–HCF2 with PSA cleanup were summarized in Figure 6. Results demonstrated that both Captiva EMR–HCF cleanup methods provided generally equivalent accuracy and precision for >95% of targets at both high and low spiking levels. The outliers are mostly focused on few acidic pesticides, including 2,4-D, 2,4,5-TP, MCPA, 2,4,5-T, dichlorprop, and mecoprop, but still with improved acceptable accuracy (50 to 70%) and RSDs, compared to other cleanup methods. The matrix matched calibration curve linearity and lowest limit of quantitation (LLOQ) are summarized in Table 4.

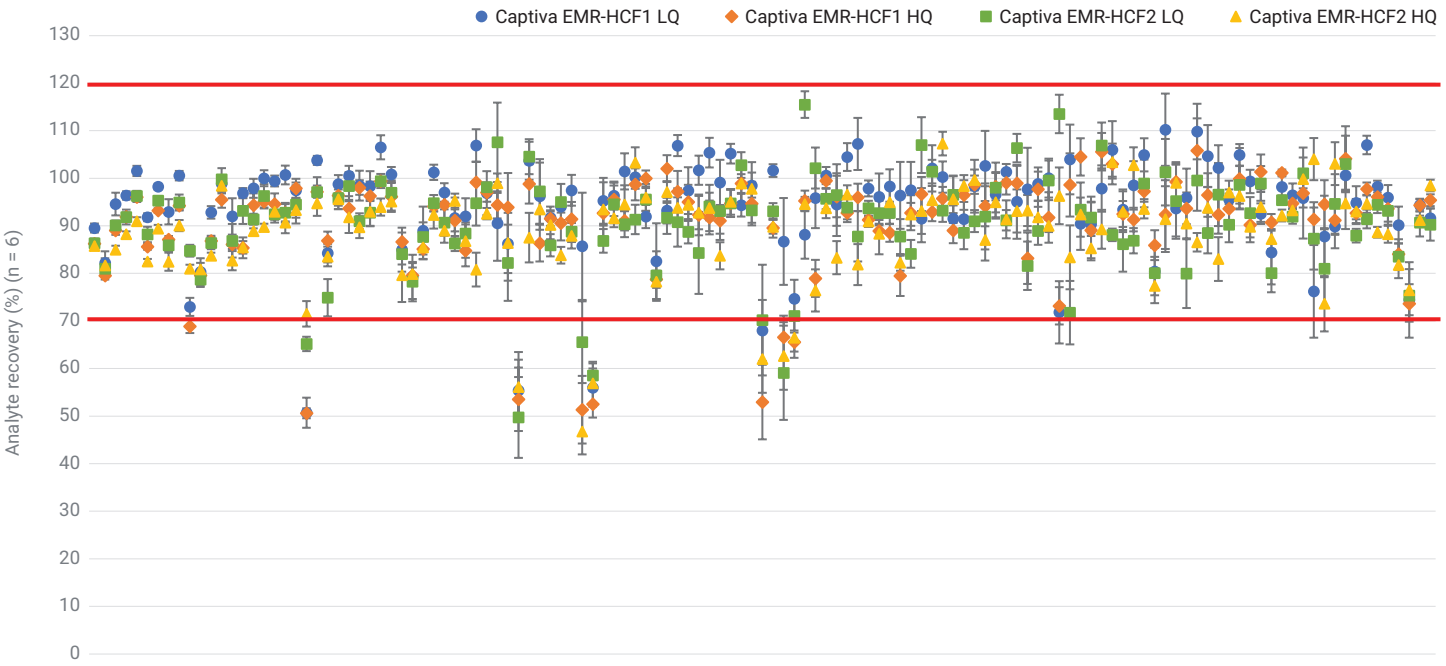

Figure 6. Target accuracy and precision results in spring mix. Two levels of pre-spiking include 10 ng/g for LQ and 100 ng/g for HQ. Samples were prepared using Agilent Bond Elut QuEChERS AOAC extraction kit followed by Agilent Captiva EMR–HCF1 and Captiva EMR–HCF2 cleanup, respectively.

Table 4. Method matrix matched calibration curve and detection limits results summary in spring mix.

Target Name	Agilent Captiva EMR-HCF1 with NH_2 Cleanup			Agilent Captiva EMR-HCF2 with PSA Cleanup		
	LLOQ (ng/g)	HLOQ (ng/g)	R^2	LLOQ (ng/g)	HLOQ (ng/g)	R^2
Methamidophos	0.5	500	0.9985	0.5	500	0.9962
Pymetrozine	0.5	500	0.9991	0.5	500	0.9974
Acephate	0.5	500	0.9971	0.5	500	0.9962
Omethoate	0.5	500	0.9978	0.5	500	0.9963
Aminocarb	0.5	500	0.9978	0.5	500	0.9963
Propamocarb	0.5	500	0.9949	0.5	500	0.9962
Dinotefuran	0.5	500	0.9947	0.5	500	0.9976
Carbendazim	0.5	500	0.9971	0.5	500	0.9962
Monocrotophos	0.5	500	0.9986	0.5	500	0.9971
Nitenpyram	0.5	500	0.9981	0.5	500	0.9979
Thiabendazole	0.5	500	0.9975	0.5	500	0.9936
Fuberidazole	0.5	500	0.9983	0.5	500	0.9958
Thiamethoxam	0.5	500	0.9979	0.5	500	0.9956
Cymoxanil	0.5	500	0.9981	0.5	500	0.9983
Mexacarbate	0.5	500	0.9988	0.5	500	0.9966
Ethirimol	0.5	500	0.9955	0.5	500	0.9963
Metamitron	0.5	500	0.9954	0.5	500	0.9960
Fenuron	0.5	500	0.9958	0.5	500	0.9958
Chloridazon	0.5	500	0.9959	0.5	500	0.9959
Imidacloprid	0.5	500	0.9973	0.5	500	0.9977
Cymiazol	0.5	500	0.9980	0.5	500	0.9939
Dimethoate	0.5	500	0.9969	0.5	500	0.9923
Fenobucarb	0.5	500	0.9923	0.5	500	0.9943
Acetamiprid	0.5	500	0.9966	0.5	500	0.9956
Metsulfuron	0.5	500	0.9921	0.5	500	0.9938
Flumetsulam	0.5	500	0.9993	0.5	500	0.9972
Tebuthiuron[1]	0.5	250	0.9980	0.5	500	0.9912
4-Nitrophenol	0.5	500	0.9961	0.5	500	0.9948
Thiacloprid	0.5	500	0.9967	0.5	500	0.9966
Nicosulfuron	0.5	500	0.9961	0.5	500	0.9947
Thidiazuron	0.5	500	0.9938	0.5	500	0.9912
Secbumeton	0.5	500	0.9903	0.5	500	0.9927
Imazalil	0.5	500	0.9924	0.5	500	0.9975
Bentazon	0.5	500	0.9954	0.5	500	0.9940
Oxasulfuron	0.5	500	0.9965	0.5	500	0.9905
Carfentrazone-ethyl	0.5	500	0.9959	0.5	500	0.9966
Lenacil	0.5	500	0.9932	0.5	500	0.9911
Metribuzin	0.5	500	0.9902	0.5	500	0.9917
Cyazofamid	0.5	500	0.9913	0.5	500	0.9907
Propoxur	0.5	500	0.9909	0.5	500	0.9912
Phenmedipham	0.5	500	0.9938	0.5	500	0.9922
2,4-D[1]	1	500	0.9968	1	500	0.9949
Chlorsulfuron[1]	0.5	500	0.9931	0.5	400	0.9932
Methabenzthiazuron[1]	0.5	250	0.9962	0.5	250	0.9937

Target Name	Agilent Captiva EMR-HCF1 with NH_2 Cleanup			Agilent Captiva EMR-HCF2 with PSA Cleanup		
	LLOQ (ng/g)	HLOQ (ng/g)	R^2	LLOQ (ng/g)	HLOQ (ng/g)	R^2
Dioxacarb	0.5	250	0.9906	0.5	500	0.9967
Carbofuran[1]	0.5	250	0.9977	0.5	500	0.9936
2,4,5-TP	1	100	0.9956	1	250	0.9940
MCPA[1]	0.5	250	0.9936	0.5	500	0.9962
Cycluron	0.5	500	0.9933	0.5	500	0.9900
Amidosulfuron	0.5	500	0.9967	0.5	500	0.9907
Flutriafol	0.5	500	0.9963	0.5	500	0.9937
Carbaryl	0.5	500	0.9919	0.5	500	0.9924
Chlorotoluron[1]	0.5	250	0.9901	0.5	250	0.9921
Pyracarbolid[1]	0.5	100	0.9914	0.5	100	0.9965
Fluometuron	0.5	500	0.9937	0.5	500	0.9925
Forchlorfenuron	0.5	500	0.9948	0.5	500	0.9940
Fosthiazate	0.5	500	0.9958	0.5	500	0.9936
Azaconazole	0.5	500	0.9943	0.5	500	0.9946
Methoprotryne	0.5	500	0.9936	0.5	500	0.9941
DEET	0.5	500	0.9919	0.5	250	0.9936
Fenpropidin	0.5	500	0.9922	0.5	500	0.9949
Carboxin	0.5	500	0.9933	0.5	500	0.9940
Diuron	0.5	500	0.9918	0.5	500	0.9935
2,4,5-T[1]	1	500	0.9960	1	500	0.9913
Spiroxamine	0.5	500	0.9928	0.5	500	0.9933
Dichlorprop[1]	5	500	0.9955	5	500	0.9954
Mecoprop	0.5	500	0.9930	0.5	500	0.9924
Metobromuron	0.5	500	0.9916	0.5	250	0.9964
Dimethomorph I	0.5	500	0.9924	0.5	250	0.9951
Dimethachlor	0.5	500	0.9952	0.5	500	0.9956
Chlorantraniliprole	0.5	500	0.9936	0.5	500	0.9944
Clomazone	0.5	500	0.9924	0.5	500	0.9935
Dimethomorph II	0.5	500	0.9928	0.5	500	0.9964
Cyproconazole	0.5	500	0.9919	0.5	500	0.9937
Furalaxyl	0.5	250	0.9984	0.5	250	0.9927
Chloroxuron	0.5	500	0.9952	0.5	500	0.9932
Iprovalicarb	0.5	500	0.9904	0.5	500	0.9919
Halofenozide	0.5	500	0.9923	0.5	250	0.9927
Spinosad A[2]	10	500	0.9935	10	500	0.9913
Linuron[1]	0.5	250	0.9944	0.5	500	0.9945
Fenamiphos	0.5	500	0.9948	0.5	500	0.9939
Promecarb	0.5	500	0.9968	0.5	500	0.9936
Myclobutanil	0.5	500	0.9970	0.5	500	0.9916
Mandipropamid[1]	0.5	500	0.9963	0.5	250	0.9909
Azoxystrobin	0.5	500	0.9969	0.5	500	0.9929
Fenamidone[1]	0.5	100	0.9941	0.5	250	0.9931
Boscalid[1]	0.5	250	0.9926	0.5	400	0.9960
Fluopicolide[1]	0.5	500	0.9957	0.5	250	0.9982
Spinosad D[1]	1	500	0.9968	1	500	0.9941
Isoxaben	0.5	500	0.9948	0.5	500	0.9883

Target Name	Agilent Captiva EMR-HCF1 with NH_2 Cleanup			Agilent Captiva EMR-HCF2 with PSA Cleanup		
	LLOQ (ng/g)	HLOQ (ng/g)	R^2	LLOQ (ng/g)	HLOQ (ng/g)	R^2
Bifenazate	0.5	500	0.9913	0.5	500	0.9937
Penconazole	0.5	500	0.9936	0.5	500	0.9935
Pyridat	0.5	500	0.9953	0.5	500	0.9923
Diflubenzuron[1]	0.5	250	0.9905	0.5	500	0.9937
Ethoxyquin[2]	100	500	0.9887	100	500	0.9887
Fluoxastrobin	0.5	500	0.9918	0.5	500	0.9973
Prochloraz	0.5	500	0.9939	0.5	500	0.9926
Isoprothiolane	0.5	500	0.9977	0.5	500	0.9938
Flufenacet[1]	0.5	250	0.9909	0.5	250	0.9879
Rotenone	0.5	500	0.9836	0.5	500	0.9901
Dimoxystrobin[1]	0.5	250	0.9959	0.5	500	0.9935
Cyprodinil	0.5	500	0.9940	0.5	500	0.9919
Moxidectin[1]	10	500	0.9947	10	500	0.9976
Azinphos-ethyl	0.5	500	0.9931	0.5	500	0.9924
Tebufenozide	0.5	500	0.9973	0.5	500	0.9950
Flubendiamide	0.5	400	0.9912	0.5	500	0.9931
Beflubutamid	0.5	500	0.9970	0.5	500	0.9921
Hexaflumuron[1]	5	500	0.9966	5	500	0.9955
Dinoseb	0.5	500	0.9930	0.5	500	0.9971
Kresoxim-methyl	0.5	500	0.9946	0.5	500	0.9963
Picoxystrobin[1]	0.5	500	0.9961	0.5	250	0.9970
Pyraclostrobin	0.5	500	0.9928	0.5	500	0.9930
Isofenphos-methyl	0.5	500	0.9901	0.5	500	0.9956
Diflufenican	0.5	500	0.9970	0.5	500	0.9965
Trifloxystrobin[1]	0.5	500	0.9927	0.5	250	0.9925
Metrafenone	0.5	500	0.9948	0.5	500	0.9972
Metaflumizone[1]	5	500	0.9993	5	500	0.9945
Cycloate	0.5	500	0.9959	0.5	500	0.9946
Fluazinam	0.5	500	0.9954	0.5	500	0.9948
Temephos	0.5	500	0.9935	0.5	500	0.9934
Fenazaquin	0.5	250	0.9943	0.5	500	0.9952
Pyriproxyfen	0.5	250	0.9937	0.5	250	0.9902
Hexythiazox	0.5	100	0.9926	0.5	250	0.9927
Tralkoxydim	0.5	500	0.9957	0.5	500	0.9947
Buprofezin	0.5	250	0.9973	0.5	500	0.9944
Fenpyroximate	0.5	250	0.9907	0.5	250	0.9956
Proquinazid	0.5	500	0.9963	0.5	500	0.9961
Pyridaben	0.5	500	0.9977	0.5	500	0.9953
Spirodiclofen	0.5	500	0.9954	0.5	500	0.9954

[1] Modified dynamic calibration range either due to analyte sensitivity in the matrix or failure of acceptance criteria at the high end.

[2] Raised LLOQ due to the positive contribution from matrix.

Conclusion

Two simple, rapid, and reliable methods using Agilent Bond Elut QuEChERS AOAC extraction, followed by either Agilent Captiva EMR–HCF1 or Captiva EMR–HCF2 cartridge passthrough cleanup, were developed and verified for 138 LC-amenable pesticides in spring mix by LC/MS/MS. Both novel Captiva EMR–HCF cleanup methods provide convenient and simplified sample passthrough cleanup, selective and efficient pigment removal from high chlorophyll leafy matrices, and significant improved recovery and reproducibility of sensitive pesticides. In terms of acceptance criteria, the quantitation results demonstrated that a >95% pass rate was achieved by either Captiva EMR–HCF cleanup method when considering the combined results for target recovery and RSD. The Captiva EMR–HCF cleanup methods were compared to the traditional Agilent Bond Elut QuEChERS Pigmented Fruits and Vegetables dSPE kit with GCB, AOAC method, as well as competition dSPE cleanup for pigment removal. Captiva EMR–HCF passthrough cleanup was shown to provide improved sensitive pesticide recovery and reproducibility, improved or equivalent pigment removal efficiency, reduced matrix effect, and overall increased pass rates for large panel target analysis in high-chlorophyll leaf matrices. Both Captiva EMR–HCF cleanup methods demonstrated equivalent use and can be adopted easily based on current preparation protocol.

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