Application Note

Food & Beverage Testing

Determination of Multiclass, Multiresidue Pesticides in Berries

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

Authors

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Abstract

This application note presents the development and validation of a multiresidue method for the analysis of pesticide residues in blackberry, blueberry, and raspberry. The method involves extraction with the Agilent Bond Elut QuEChERS EN extraction kit, followed by Agilent Captiva Enhanced Matrix Removal–General Pigmented Fresh (EMR–GPF) cartridge passthrough cleanup, then GC/MS/MS analysis. An Agilent advanced synthetic carbon sorbent, Carbon S, is used in Captiva EMR–GPF cartridges. The newly designed Captiva EMR–GPF cartridge is optimized to deliver a convenient passthrough cleanup for general pigmented fresh vegetable and fruit matrices such as berries, peppers, grapes, citrus fruits, and so on. The results demonstrated that over 96% of the pesticides were identified with 60 to 120% recovery, RSD <20%, using the simple passthrough cleanup with no additional elution steps. The pigment removal assessment by LC/UV confirmed that >99% of pigment interferences are removed by the EMR–GPF cleanup. Comparing to traditional cleanup by QuEChERS dispersive SPE kit with GCB, Captiva EMR–GPF cleanup provided significantly improved recoveries for sensitive pesticides, and equivalent pigment removal efficiency.

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 solvent. 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, 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. Therefore, it is important to apply enhanced cleanup to remove pigment co-extractives before instrument analysis.

Graphitized carbon black (GCB) has been widely 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 dispersive solid phase extraction (dSPE) kits and has been recommended for pigment removal. Although GCB demonstrates efficiency for pigment removal, it also causes unwanted loss of analytes, especially compounds with a planar structure, such as hexachlorobenzene, thiabendazole, etc. Therefore, many QuEChERS dSPE kit formulas have been carefully adjusted to contain a limited amount of GCB sorbent to achieve acceptable target recoveries. However, improvement in the recovery of sensitive analytes results in significant compromises in matrix pigment removal efficacy.

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 delivers a better balance between analyte recovery and matrix pigment removal efficiency than traditional GCB sorbent. The Carbon S sorbent is used in various dSPE kits as an alternative to GCB and has demonstrated equivalent or improved performance. It has also been used for the Captiva EMR products expansion, where a convenient passthrough cleanup is adopted for efficient and selective matrix removal with significant improvement in sensitive pesticide recoveries.

This study evaluates sample preparation using Captiva EMR–GPF cartridge passthrough cleanup for the GC/MS/MS analysis of 108 common pesticides in three typical berry matrices: blackberry, blueberry, and raspberry.

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 AccuStandard (New Haven, CT, USA), or as individual standard stock solutions or powder from Sigma-Aldrich (St. Louis, MO). HPLC grade acetonitrile (ACN) was from Honeywell (Muskegon, MI). Reagent grade acetic acid, ammonium acetate, and ammonium fluoride were also from Sigma-Aldrich.

Solutions and standards

A combined standard spiking solution (108 pesticides) and a combined internal standard (three 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 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, 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.

The following equipment was also used for sample preparation: 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 EN extraction kit (part number 5982-5650), Agilent Captiva EMR–GPF cartridge, 3 mL (part number 5610-2090), Agilent Bond Elut QuEChERS EMR–Lipid polish pouch, 3.5 g anhydrous $MgSO_4$ (part number 5982-0102). Ceramic homogenizers, 50 mL tubes, 100/pk (part number 5982-9313).

Instrument conditions

The GC/MS/MS instrument conditions were established based on previously published methods using equivalent instruments. Table 1 lists the conditions of GC/MS/MS operation, and Table 2 lists the target MRM parameters.

Table 1. Agilent 8890 GC and Agilent 7000D GC/MS/MS conditions.

Parameter	Value
Columns	Agilent HP-5ms UI, 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	4 mm id Ultra Inert liner single taper with wool (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, and 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 2. Targeted pesticides dMRM conditions.

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
Menvinphos	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
BHC-beta	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

Pesticide	RT (min)	First MRM Transition (*m/z*)	CE (V)	Second MRM Transition (*m/z*)	CE (V)	Delta RT (min)	MS1 and MS2 Resolution
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
Progargite	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
Marcarbam	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
Quinalphos	11.505	146 → 118	10	146 → 91	30	1.5	Wide
Triflunizole	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
Fludixonil	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

Pesticide	RT (min)	First MRM Transition (*m/z*)	CE (V)	Second MRM Transition (*m/z*)	CE (V)	Delta RT (min)	MS1 and MS2 Resolution
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
Quinozyfen	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
Spiromasifen	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
Methozychlor	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
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
Fluquinoconazole	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
Ethofenprox	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 shows an MRM chromatogram of targeted pesticides in the fortified blackberry sample at the level of 100 ng/g using the above GC/MS/MS conditions.

Sample preparation

Fresh, organic blackberries, blueberries, and raspberries were purchased from local grocery stores. Samples were frozen in a –20 °C freezer overnight, then homogenized with a grinder. The ground matrix samples were then weighed for 10 g in the 50 mL centrifuge tubes and stored in the –20 °C freezer until extraction. The weighed berry samples (10 g) were prethawed, then extracted following the QuEChERS EN method. The crude extract was then loaded into the 3 mL Captiva EMR–GPF cartridges for passthrough cleanup. The cleaned sample eluent was dried by anhydrous $MgSO_4$ to completely remove the remaining water residue in the sample extract. The dried sample was then ready for GC/MS/MS analysis. The detailed sample preparation procedure is shown in Figure 2. For a batch of approximately 30 samples, the entire procedure usually takes approximately 40 to 45 minutes.

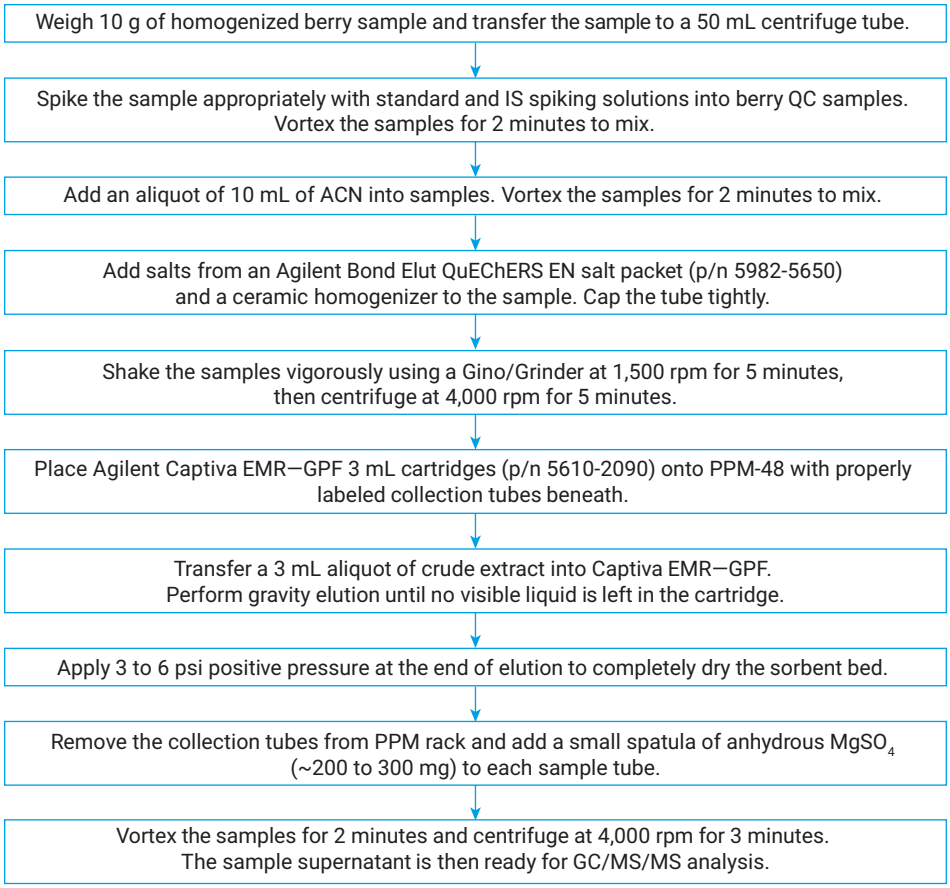

Figure 2. Sample preparation procedure for berry samples by Agilent Bond Elut QuEChERS EN extraction followed with Agilent Captiva EMR–GPF cleanup.

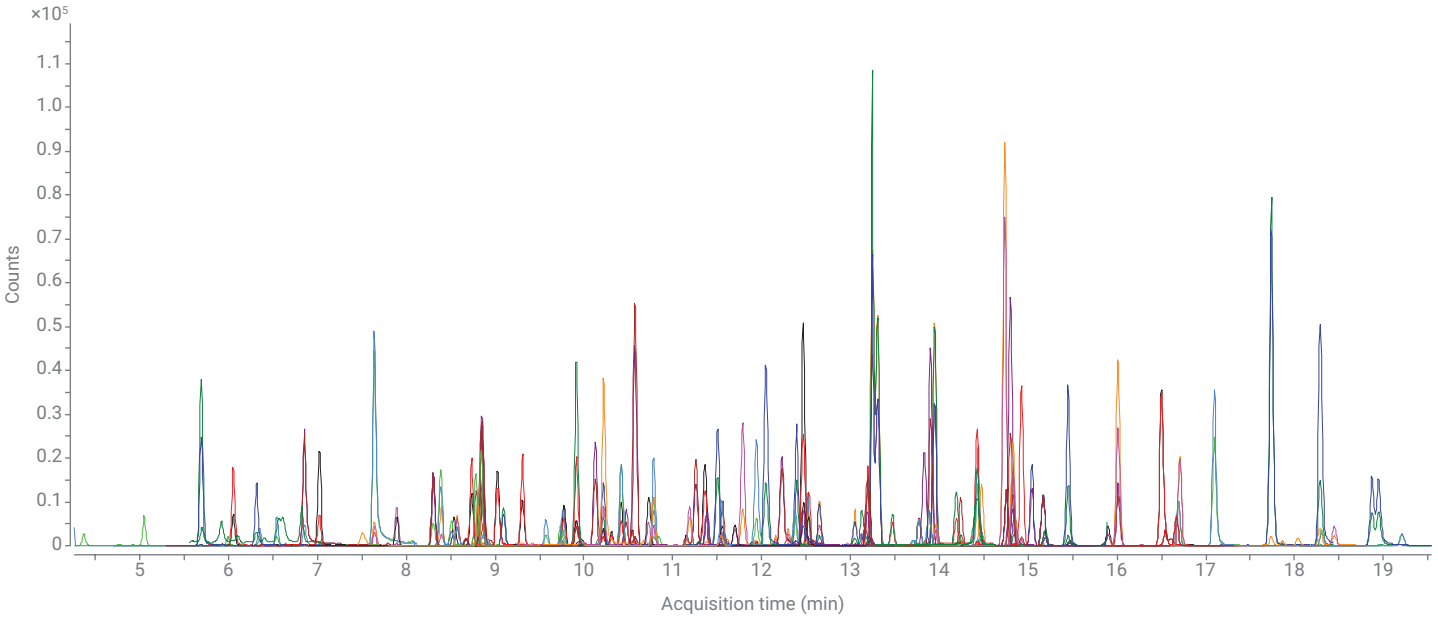

Figure 1. GC/MS/MS MRM chromatogram for extracted blackberry sample fortified with 100 ng/g of 108 targeted pesticides. The sample was prepared using an Agilent Bond Elut QuEChERS EN extraction kit, followed by Agilent Captiva EMR–GPF cleanup.

Captiva EMR–GPF provides convenient passthrough cleanup. The elution can be done by gravity elution. For sample analysis by LC/MS/MS, the sample eluent can either be injected directly onto the LC/TQ instrument or diluted further with water before injection. For sample analysis on GC/MS/MS, the sample eluent is then needed for further drying by anhydrous $MgSO_4$ powder. The addition of $MgSO_4$ can be as simple as a small spatula of anhydrous $MgSO_4$ powder (~200 to 300 mg) from the Agilent Bond Elut QuEChERS EMR–Lipid polish pouch. The added $MgSO_4$ does not have to be exact, and the complete water residue removal can be confirmed by two simple indications. First, a milky white homogenous sample mixture should be visible during vortex. Second, the salts should settle down as powder, rather than coagulated chunks, at the bottom after settling down. Figure 3 shows the pictured steps for sample drying after Captiva EMR–GPF cleanup but before GC/MS/MS analysis.

Method performance evaluation

The novel sample preparation method performance was evaluated in terms of matrix pigment removal, target recovery and reproducibility, matrix matched calibration curves linearity, and limits of quantitation (LOQs) in three berry matrices: blackberry, blueberry, and raspberry. To evaluate recovery and reproducibility, two levels of quality control (QC) samples were prespiked at 10 and 100 ng/g in berry sample homogenate. The spiked samples and matrix blank samples were then prepared following the procedure. The final matrix blank extract was then postspiked at 10 and 100 ng/mL, correspondingly. Six replicates of prespiked QCs were prepared at each level. The peak area ratio of corresponding target in prespiked versus postspiked QC samples was then used to calculate target recovery through the sample preparation procedure. In addition, the traditional dSPE cleanup using Bond Elut QuEChERS Universal dSPE with GCB (U-dSPE with GCB) was used to compare target recovery and reproducibility in blackberry. The matrix matched calibration curve linearity and LOQ were evaluated by postspiking at the levels of 1, 2, 5, 10, 50, 100, 250, 400, and 500 ng/mL in three kinds of matrix blank extract. Analyte identification, confirmation, and quantitation were determined from retention times and MRM transitions.

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 first 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

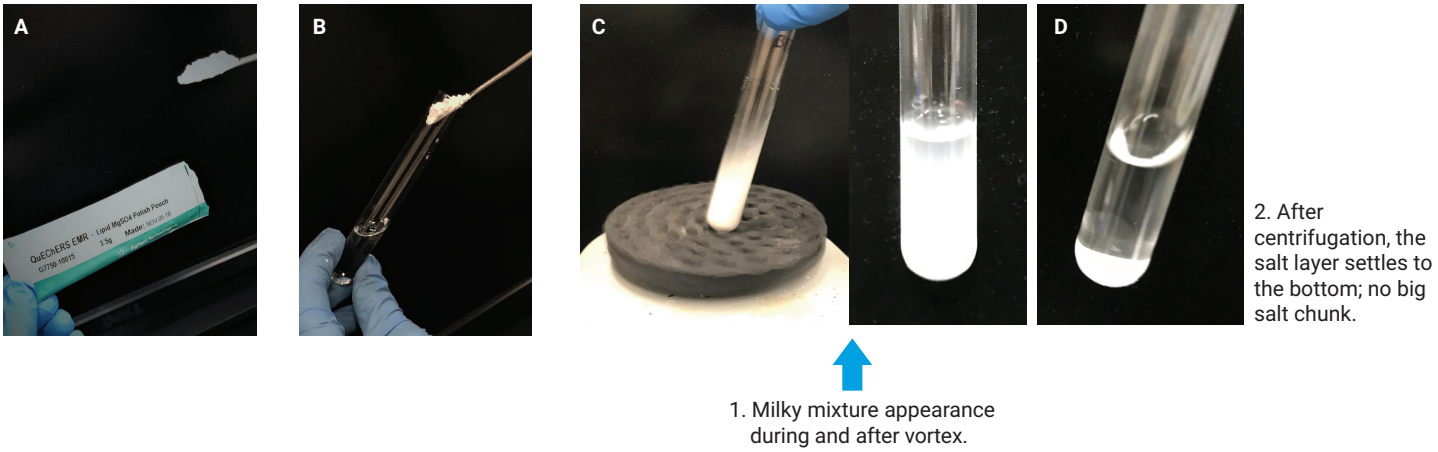

Figure 3. Sample drying after Agilent Captiva EMR–GPF cleanup for GC/MS/MS analysis. (A) Take out a spatula of $MgSO_4$ anhydrous powder from the Agilent Bond Elut QuEChERS EMR–Lipid polish pouch. (B) Add the $MgSO_4$ powder to the collection tube containing the sample eluent after cleanup. (C) Vortex sample for 2 to 3 minutes. (D) Centrifuge sample for 3 minutes. (1) and (2) are critical indicators for complete water residue removal.

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 widely been used for food analysis in fatty matrices by GC/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 for plant-origin sample matrices, including fresh and dry matrices. Five new Captiva EMR cartridges were developed with optimized formulas for various complicated plant sample matrices. Table 3 shows the detailed description of all the Captiva EMR cartridges, and their recommendations.

The sorbent formulas were carefully and thoroughly optimized based on multiresidue target recoveries and matrix cleanup efficiency. Depending on different matrices, these EMR cartridges provide selective and 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 EMR cartridges because investigations showed that the simultaneous water removal by $MgSO_4$ during the cleanup procedure can compromise the buffering effect and result in significant loss of some labile pesticides. For GC and GC/MS analysis, further drying is thus required after EMR cleanup to completely remove the water residue.

For the fresh berry matrices in this study (blackberry, blueberry, and raspberry) Captiva EMR–GPF 3 mL cartridges were used for passthrough cleanup after QuEChERS extraction. Efficient matrix pigment removal was achieved in all three berry matrices. Figure 4 shows the before and after passthrough cleanup to demonstrate the visual appearance. The before and after samples were also analyzed by LC/UV detection at 450 nm to demonstrate >99% pigment removal.

Table 3. Agilent Captiva EMR cartridges and their recommendations for different plant-origin matrices.

Product Name	Sorbents	Sample Loading Volume	Recommendations Based on Sample Matrices	Examples of Applicable Sample Matrix
Captiva EMR—Lipid	Captiva 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
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

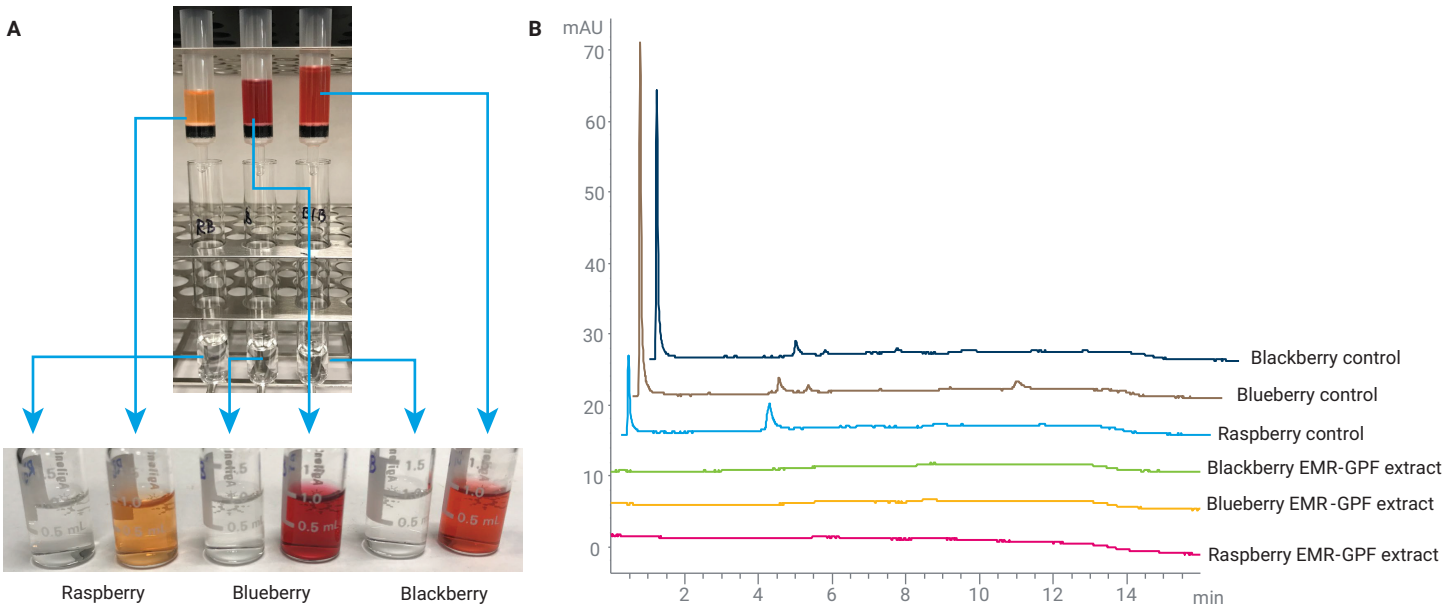

Figure 4. Berry matrix sample pigment removal efficiency demonstration. (A) Extracted samples color comparison before and after Captiva EMR–GPF cleanup. (B) LC-UV (λ = 450 nm) stacked chromatograms for extracted berry samples before and after Captiva EMR–GPF cleanup.

Sample preparation procedure

For fresh fruits and vegetables 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 EN extraction kit. After extraction, 3 mL of crude extract was loaded to into the 3 mL Captiva EMR–GPF cartridge for passthrough cleanup. The elution was performed by gravity, and the entire elution took 5 to 10 minutes for 3 mL of crude berry extract. For GC/MS/MS analysis, the sample eluent then was dried by anhydrous $MgSO_4$ powder. The addition of anhydrous $MgSO_4$ does not have to be exact or accurate. The drying procedure and indications of complete water removal are shown in Figure 3.

Sample preparation method performance assessment

The sample preparation method using Captiva EMR–GPF cleanup was evaluated thoroughly using the following experiments:

A. Analyte recovery comparison between Captiva EMR–GPF cleanup versus U-dSPE with GCB cleanup

B. Method quantitation accuracy and precision verification

C. Method cross verification in three berry matrices (blackberry, blueberry, and raspberry) for analyte recovery and reproducibility at 10 ng/g spiking level (n = 6), and calibration curve linearity for the dynamic range of LOQ to 500 (or 400) ng/g in matrix

A. Analyte recovery comparison between Captiva EMR–GPF cleanup versus U-dSPE with GCB cleanup

The novel developed method using Captiva EMR–GPF cleanup was compared to traditional U-dSPE with GCB cleanup for analytes recovery and reproducibility. Considering the commonly accepted criteria of 70 to 120% recovery range, the use of the Captiva EMR–GPF cleanup resulted in all 108 targets falling into the recovery acceptance range with 0% failure rate. In comparison, when using U-dSPE with GCB cleanup, five targets fell out of the recovery acceptance range, with 4.6% failure rate. Within the five failed targets, four of them gave significantly low recoveries, and much lower responses in postspiked samples. This indicated that the U-dSPE with GCB cleanup caused the significant loss of these sensitive pesticides. The different matrix impact on these targets also resulted in the low responses of targets on GC/MS/MS instrument. Figure 6 shows the chromatographic comparison of pre- and postspiked 10 ppb samples in blackberry, using Captiva EMR–GPF cleanup versus U-dSPE with GCB cleanup.

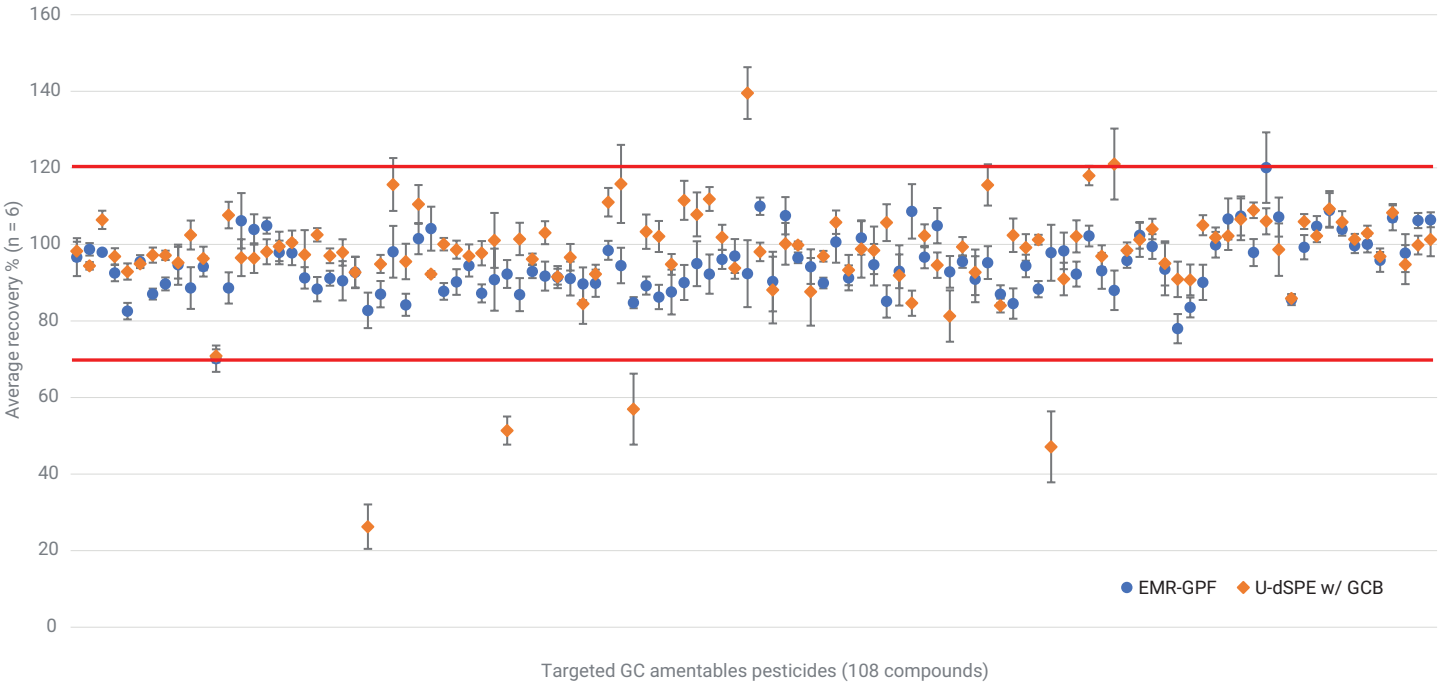

Figure 5. Targeted pesticides recovery comparison between Agilent Captiva EMR–GPF and QuEChERS U-dSPE with GCB cleanup. Spiking level at 10 ng/g in blackberry. Pesticides are in the order of retention time upon the elution on GC/MS/MS. Refer to Table 2 for targeted pesticides labelling.

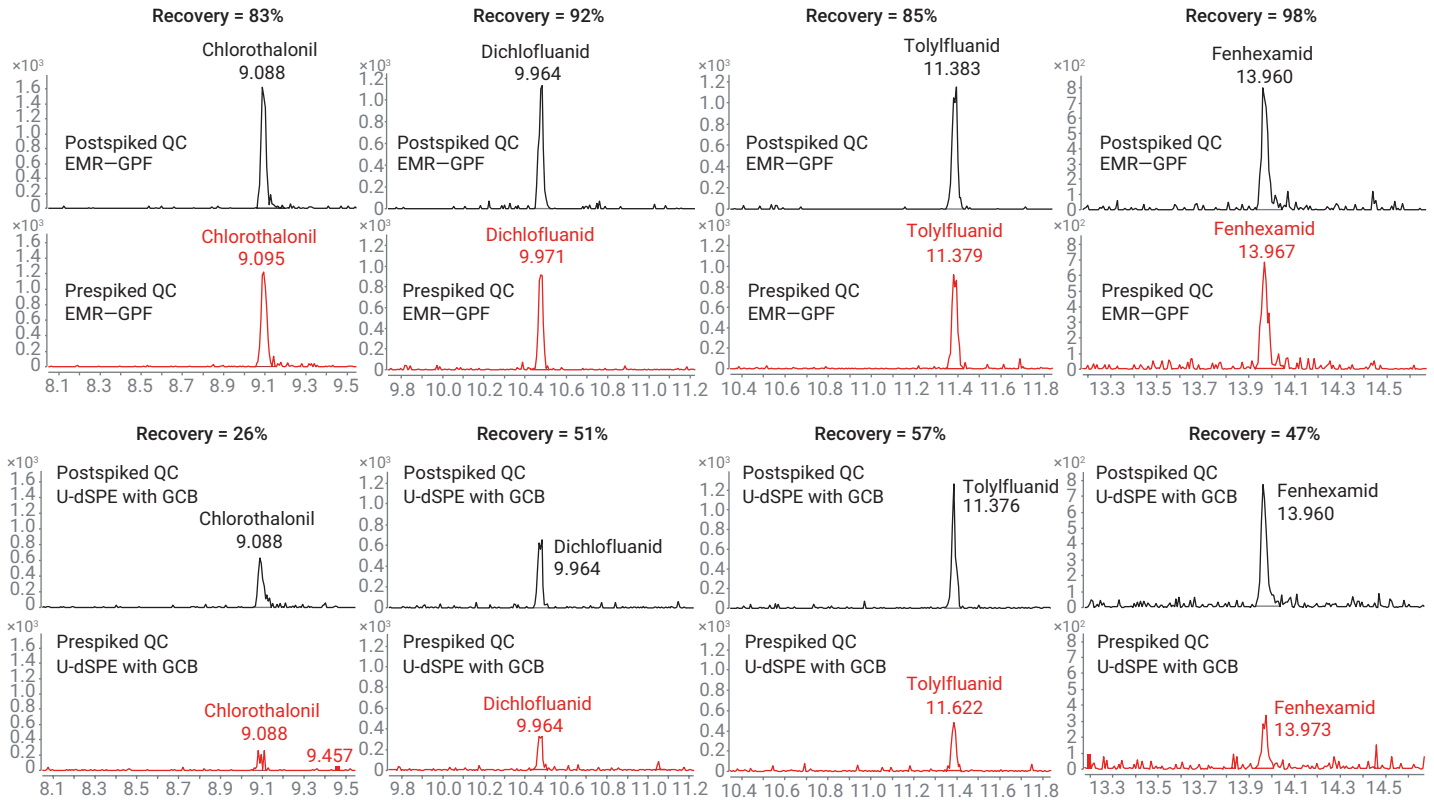

Figure 6. Sensitive targets chromatographic comparison for samples prepared using different cleanup methods.

B. Method quantitation accuracy and precision verification

Method quantitation accuracy and precision was verified in blackberry with two levels of prespiked QCs, at 10 and 100 ng/g. Nine matrix matched calibration standards were prepared to cover the dynamic range of 1 to 500 ng/g in blackberry. The calibration curves were generated using linear regression and $1/x^2$ weight. Three ISs (atrazine-D_5, DDT-D_8, and TPP) were used at 100 ng/g for quantitation. The quantitation results are summarized in Table 4. Out of 108 targets, 84 targets made the dynamic calibration range of 1 to 500 ng/g in blackberry with acceptable accuracy criteria; 23 targets had increased lowest limit of quantitation (LLOQ), due to either lack of sensitivity in the matrix, or failure to achieve the acceptance accuracy criteria. Malathion had significantly raised LLOQ due to the positive contribution from sample matrix blank. The confirmed positive incurrence of malathion also directly resulted in greatly higher calculated concentration for 10 ng/g low QC, and thus failed for accuracy acceptance criteria.

Table 4. Method quantitative verification in blackberry results summary.

Pesticide	Calibration Curve			Accuracy and Precision			
				Low QC (10 ng/g, n = 6)		High QC (100 ng/g)	
	LLOQ (ng/g)	HLOQ (ng/g)	R^2	Avg. Accuracy (%)	RSD%	Avg. Accuracy (%)	RSD%
Dichlorvos	1	500	0.9968	107	3.7	103	4.2
Dichlobenil	1	500	0.9993	101	3.1	95	2.7
Menvinphos	1	500	0.9965	98	4.9	102	4.4
Propham	1	500	0.9936	108	5.8	100	1.7
Methacrifos	1	500	0.9957	94	3.5	97	2.2
2-Phenylphenol	1	500	0.9949	96	4.2	100	5.3
Molinate	1	500	0.9993	99	3.5	98	1.7
Diphenylamine	1	500	0.9985	92	4.1	95	4.7
Ethalfluralin	1	500	0.9881	94	5.6	105	3.5
Sulfotep	1	500	0.9984	94	7.2	102	3.9
BHC-beta	1	500	0.9972	98	6.4	100	5.4
Hexachlorobenzene	1	500	0.9985	82	7.9	74	3.9
Demeton-S [1]	2	500	0.9921	94	4.8	98	6.6
Simazine [1]	2	500	0.9951	103	12.7	99	6.4
Atrazine	1	500	0.9919	92	6.5	104	5.5
Propetamphos	1	500	0.9990	106	5.9	100	5.9
Trietazine	1	500	0.9989	93	3.0	99	1.1
Terbuthylazine	1	500	0.9980	86	9.3	98	4.1
Terbufos	1	500	0.9949	89	4.2	100	3.1
Lindane	1	500	0.9928	99	9.1	101	2.0
Diazinon	1	500	0.9961	101	9.4	104	1.7
Pyrimethanil	1	500	0.9932	84	6.5	96	1.6
Chlorothalonil	1	500	0.9945	60	5.6	77	2.6
Pirimicarb	1	500	0.9988	90	7.2	98	4.2
Phosphamidon [1]	2	500	0.9935	80	8.5	102	9.7
Metribuzin [1]	2	500	0.9928	105	9.8	99	3.4
Chlorpyrifos-methyl	1	500	0.9919	91	4.5	104	7.6
Fenitrothion	1	500	0.9986	99	9.5	100	4.3
Tolclofos-methyl	1	500	0.9972	98	4.6	100	2.8
Heptachlor	1	500	0.9970	101	7.5	100	4.0
Pirimiphos-methyl	1	500	0.9966	94	4.2	99	2.9
Progargite [1]	5	500	0.9964	96	5.0	101	4.7
Malathion [2]	50	500	0.9961	147	11.1	103	0.8
Dichlofluanid	1	500	0.9911	61	8.4	78	7.3
Diethofencarb [1]	10	500	0.9918	91	11.4	103	8.7
Metolachlor	1	500	0.9980	95	8.2	100	3.9
Tetraconazole	1	500	0.9950	92	2.8	103	7.8
Aldrin	1	500	0.9966	98	2.8	96	3.6
Triadimefon	1	500	0.9932	92	6.87	103	6.0
Pendimethalin	1	500	0.9942	102	12.0	106	6.6
Metazachlor	1	500	0.9981	96	3.7	102	3.3
Chlorfenvinphos	1	500	0.9925	99	2.9	102	4.1
Marcarbam [1]	5	500	0.9909	103	5.5	97	5.3
Tolylfluanid [1]	2	500	0.9929	84	1.4	97	3.7

Pesticide	Calibration Curve			Accuracy and Precision			
				Low QC (10 ng/g, n = 6)		High QC (100 ng/g)	
	LLOQ (ng/g)	HLOQ (ng/g)	R^2	Avg. Accuracy (%)	RSD%	Avg. Accuracy (%)	RSD%
Quinalphos	1	500	0.9950	83	8.1	97	3.0
Triflunizole	1	500	0.9952	104	3.2	105	5.6
Triadimenol [1]	5	500	0.9988	100	5.7	103	8.4
Procymidone	1	500	0.9918	94	7.5	104	2.1
Captan [1]	5	500	0.9903	86	5.6	81	13.0
Methidathion	1	500	0.9916	89	5.1	102	5.1
Paclobutrazole	1	500	0.9995	102	5.0	103	5.8
Mepanipyrim	1	500	0.9953	86	7.7	95	5.1
Endosulfan I [1]	5	500	0.9980	94	15.6	103	5.1
Fludixonil [1]	2	500	0.9980	89	8.3	102	4.8
Hexaconazole [1]	2	500	0.9923	89	15.6	100	8.7
Profenofos	1	500	0.9916	80	6.0	103	2.6
Oxadiazon	1	500	0.9973	96	7.8	102	4.4
Tricyclazole [1]	5	500	0.9952	94	8.2	92	6.4
DDE	1	500	0.9987	94	4.2	95	2.5
Uniconazole-P	1	500	0.9934	88	4.0	100	4.9
Bupirimate	1	500	0.9956	100	5.9	101	2.8
Flusilazole	1	500	0.9919	104	5.9	103	3.3
Dieldrin [1]	2	500	0.9907	95	6.6	102	2.0
Endrin [1]	2	500	0.9961	108	13.5	104	2.5
Iprodione	1	500	0.9948	99	7.0	97	3.0
Diniconazole	1	500	0.9988	95	5.9	105	1.6
Oxadixyl	1	500	0.9949	103	5.1	105	0.9
Ethion	1	500	0.9942	82	7.2	100	2.1
Endosulfan II [1]	5	500	0.9937	101	10.9	106	7.2
DDD	1	500	0.9967	92	6.5	103	1.7
Triazophos [1]	2	500	0.9946	87	0.7	102	3.6
Propiconazole I [1]	2	500	0.9934	108	5.0	100	3.2
Quinozyfen	1	500	0.9972	89	7.0	90	1.9
Propiconazole II	1	500	0.9926	96	8.1	100	2.1
DDT	1	500	0.9967	91	3.9	100	1.0
Fenhexamid [1]	2	500	0.9911	76	12.5	87	7.8
Tebuconazole	1	500	0.9960	97	9.4	104	1.7
Zoxamide	1	500	0.9914	77	5.9	105	9.1
Epoxiconazole	1	500	0.9911	87	4.5	93	4.0
Spiromasifen	1	500	0.9980	85	3.1	96	4.8
Bifenthrin	1	500	0.9988	91	8.8	97	4.5
Bromuconazole I	1	500	0.9942	101	9.0	101	4.7
Phosmet	1	500	0.9939	85	9.4	102	2.6
EPN [1]	2	500	0.9983	87	3.9	89	3.6
Picolinafen	1	500	0.9953	77	4.9	86	4.9
Fenoxycarb	1	250	0.9906	101	4.7	87	1.7
Methozychlor	1	500	0.9913	87	3.8	96	4.1
Tebufenpyrad	1	500	0.9973	89	4.5	97	3.5
Bromuconazole II	1	500	0.9943	88	5.3	99	4.1

Pesticide	Calibration Curve			Accuracy and Precision			
				Low QC (10 ng/g, n = 6)		High QC (100 ng/g)	
	LLOQ (ng/g)	HLOQ (ng/g)	R^2	Avg. Accuracy (%)	RSD%	Avg. Accuracy (%)	RSD%
Metoconazole	1	500	0.9971	89	7.5	96	6.3
Azamethiphos	1	500	0.9964	89	3.2	88	7.5
Phosalone	1	500	0.9935	72	3.9	83	6.0
Ipconazole	1	500	0.9935	86	5.6	100	8.1
Mirex	1	500	0.9985	97	6.3	108	5.6
Fenarimol	1	500	0.9973	86	6.3	100	5.9
Bitertanol	1	500	0.9969	95	7.4	105	6.7
Permethrin	1	500	0.9939	91	6.5	99	5.4
Coumaphos	1	500	0.9954	81	9.0	97	7.5
Fluquinoconazole	1	500	0.9932	89	8.2	100	5.7
Fenbuconazole	1	500	0.9969	94	8.8	102	4.6
Ethofenprox	1	500	0.9979	90	6.8	97	3.1
Flumiloxazin [1]	2	500	0.9929	96	9.6	96	4.6
Pyraclostrobin	1	500	0.9902	100	7.3	108	5.6
Difenoconazole	1	500	0.9992	89	7.3	101	6.0
Deltamethrin	1	500	0.9990	96	7.9	111	5.8

[1] Raised LLOQ due to analytes sensitivity in the matrix or failure of acceptance criteria.
[2] Raised LLOQ due to the positive contribution from matrix.

C. Method cross verification in other berry matrices

The developed method was extended to other two berry matrices, blueberry and raspberry, for cross verification, including the verification of recovery and reproducibility at the prespiking level of 10 ng/g in the matrix, and calibration curve linearity over 1 to 500 ng/g. Figure 7 shows the statistical summary of the quantitation results in all three berry matrices. Excellent quantitation results were achieved in both blackberry and raspberry, with <1% of failure rate for recovery, reproducibility, and calibration curve linearity acceptance criteria. Failure rates in blueberry were a little higher, with 3.7, 6.5, and 1.8% of targets failing recovery, RSD, and calibration curve linearity acceptance criteria. Clearly, sample matrix complexity directly impacts the method quantitation results. However, for over 100 multiresidue pesticides analysis, the >90% pass rate is acceptable.

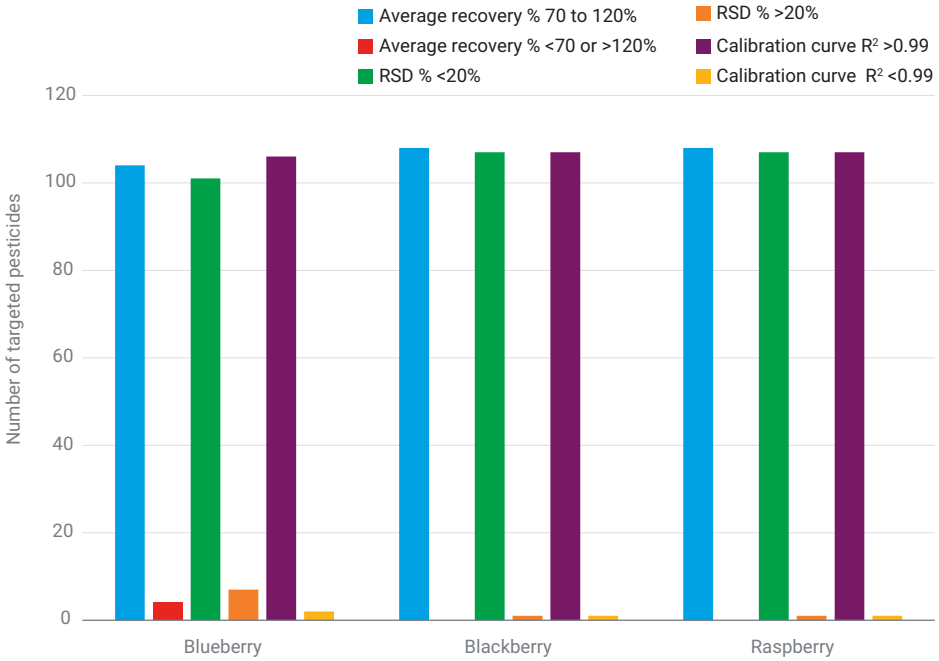

Figure 7. Quantitation results statistic summary in blueberry, blackberry, and raspberry.

Conclusion

A simple, rapid, and reliable method using the Agilent Bond Elut QuEChERS EN extraction kit followed by Agilent Captiva EMR–GPF cartridge passthrough cleanup was developed and validated for 108 GC-amenable pesticides in berries by GC/MS/MS. When compared to traditional dSPE cleanup, the Captiva EMR–GPF cartridges provide convenient and simplified sample passthrough cleanup, selective and efficient pigment removal from berry matrices, improved sensitive target recovery and reproducibility, and a higher pass rate for multiclass multiresidue pesticides analysis. In terms of acceptance criteria, the quantitation results demonstrated a >93% pass rate in blueberry, and >99% pass rate in blackberry and raspberry when considering the combined results for target recovery, RSD, and calibration linearity. In addition, highly efficient pigment removal was also confirmed with the final colorless extract after cleanup, and an >99% reduction in UV adsorption.

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