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Analysis of Pesticide Residues in Spinach Using AOAC Pigmented dSPE with Carbon S Cleanup and LC/MS/MS

Authors

Christopher Ferlin and
Limian Zhao
Agilent Technologies, Inc.

Abstract

This application note describes the use of the Agilent Bond Elut QuEChERS AOAC Pigmented Fruits and Vegetables dispersive solid phase extraction (dSPE) kit with Carbon S cleanup for the analysis of 26 pesticide residues in fresh organic spinach by LC/MS/MS. The selected compounds for analysis represent various commonly used classes of pesticides. Comparing to the traditional AOAC pigmented dSPE kit with graphitized carbon black (GCB), the AOAC pigmented dSPE kit with Carbon S demonstrated with the improved analytes recovery and comparable pigment removal. The two formats (2 and 15 mL) of the AOAC pigmented dSPE kits with Carbon S were confirmed to deliver the consistent performance with >80% average recoveries and <20% RSD for the targets, and >95% pigment removal for spinach pigment matrix.

Introduction

Natural pigments in fresh fruits and vegetables can be highly abundant, such as chlorophyll and lutein from green 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 on a detection instrument, 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 the detection flow path and MS source, and so on. Therefore, it is important to apply efficient cleanup to remove pigment co-extractives prior to instrument analysis.

GCB has regularly been used in QuEChERS dispersive SPE kits as a cleanup step to remove unwanted pigment matrix interferences. Although GCB has a high efficiency for the removal of pigments, it can lower the recoveries of certain pesticides, especially planar compounds. 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, but significantly improves sensitive analytes recoveries.

In this study, 26 representative pesticides were used for evaluating the performance of the Agilent Bond Elut AOAC Pigment dSPE kit with Carbon S (AP-dSPE with Carbon S) as a matrix cleanup step for fresh spinach crude extract, and compared to its counterpart Agilent Bond ELut AOAC Pigment dSPE with GCB (AP-dSPE with GCB). The pesticides chosen are commonly analyzed and represent a variety of pesticide classes. Spinach was selected as the matrix to represent a high level of chlorophyll matrix, which is considered as the significant challenges to pesticides analysis without an efficient cleanup procedure.

Experimental

Chemicals and reagents

Pesticide standards and the internal standard (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). LC/MS grade acetonitrile (ACN) and reagent grade acetic acid, ammonium acetate, and ammonium fluoride were also from Sigma-Aldrich.

Solutions and standards

A combined standard spiking solution (26 pesticides) and IS spiking solutions 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 and 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 1260 Infinity II LC system consisting of an Agilent 1260 Infinity II flexible pump (G7104C), and Agilent 1260 Infinity II vial sampler (G7129C), and an Agilent 1260 Infinity II multicolumn thermostat (G7116A). The LC system was coupled to an Agilent Ultivo triple quadrupole LC/MS system (G6465B) equipped with an Agilent Jet Stream Electrospray ion source. Agilent MassHunter Workstation software was used for data acquisition and analysis.

Other equipment used for sample preparation included: Sorvall ST 16R centrifuge (Thermo Scientific Inc., MA, USA), pipettes (Eppendorf, NY, USA), Agilent Bond Elut QuEChERS AOAC extraction kit (part number 5982-5755), Agilent Bond Elut QuEChERS AOAC Pigmented Fruits and Vegetables dSPE kit with Carbon S, 2 mL (part number 5610-2062), Agilent Bond Elut QuEChERS Pigmented Fruits and Vegetables dSPE kit with Carbon S, 15 mL (part number 5610-2064), Agilent ceramic homogenizers, 50 mL (part number 5982-9313).

Instrument conditions

Table 1 lists the LC/MS/MS conditions, and Table 2 lists the target dMRM parameters. Figure 1 shows a typical MRM chromatogram of targeted pesticides in the fortified spinach mix sample at the level of 100 ng/g prepared by QuEChERS AOAC extraction followed by AP-dSPE with Carbon S cleanup.

Table 1. LC/MS/MS method conditions.

HPLC Conditions	
Column	Agilent ZORBAX RRHD Eclipse Plus C18 column, 2.1 × 100 mm, 1.8 µm, (p/n 959758-902)
Guard Column	Agilent ZORBAX RRHD Eclipse Plus C18 plus UHPLC guard, 2.1 × 5 mm, 1.8 µm (p/n 821725-901)
Column Temperature	35 °C
Injection Volume	2 µL
Mobile Phase	A) 10 mM NH_4OAc, 0.5 mM NH_4F buffer with 0.125% formic acid in H_2O B) 10 mM NH_4OAc, 0.5 mM NH_4F buffer with 0.125% formic acid in 95:5 ACN/H_2O
Needle Wash	1:1:1:1 ACN:MeOH:IPA:H_2O
Gradient	Time (min)　%B　　Flow Rate (mL/min) 0.00　　　15　　0.3 6.00　　　95　　0.3 8.00　　　100　　0.3 10.00　　100　　0.3 10.01　　15　　0.3 13.00　　Stop
Post Run	2 min
Total Cycle Time	15 min

MS Conditions	
Mode	Positive mode
Gas Temperature	120 °C
Gas Flow	12.0 L/min
Nebulizer	40 psi
Capillary	4,500 V (positive and negative)

Table 2. Targeted pesticides dMRM parameters.

| Analyte	MRM Channels (*m/z*)	Fragmentor (V)	CE (V)	RT (min)	Analyte	MRM Channels (*m/z*)	Fragmentor (V)	CE (V)	RT (min)
Methamidophos	1) 142 → 125	90	13	1.16	Propoxur	1) 210.1 → 110.9	75	13	6.00
	2) 142 → 94		13			2) 210.1 → 168		5	
Acephate	1) 184 → 142.9	65	9	1.25	Amidosulfuron	1) 370.1 → 218	104	25	6.09
	2) 184 → 49		21			2) 370.1 → 260.9		10	
Pymetrozine	1) 218.1 → 104.9	115	21	1.25	Carbaryl	1) 202.1 → 145.1	70	9	6.23
	2) 218.1 → 78		53			2) 202.1 → 126.9		33	
Omethoate	1) 214 → 183	100	9	1.36	Forchlorfenuron	1) 248.1 → 129	72	13	6.26
	2) 214 → 124.9		21			2) 248.1 → 93.1		42	
Propamocarb	1) 189.2 → 102	72	4	1.52	Atrazine-d_5 (IS)	1) 220.13 → 69	76	70	6.38
	2) 189.2 → 74		26			2) 220.13 → 178.9		18	
Carbendazim	1) 192.1 → 132	115	33	2.60	Spiroxamine	1) 298.3 → 144.1	76	21	6.50
	2) 192.1 → 159.9		17			2) 298.3 → 58.1		54	
Thiabendazole	1) 202 → 131	145	37	3.14	Chlorantraniliprole	1) 482 → 284	72	33	6.89
	2) 202 → 175.1		29			2) 482 → 177.1		70	
Ethirimol	1) 210.2 → 140.1	96	17	4.38	Penconazole	1) 284.1 → 159	130	37	7.73
	2) 210.2 → 70.1		34			2) 284.1 → 70.1		17	
Imidacloprid	1) 256.1 → 209.1	105	13	4.46	Cyprodinil	1) 226.1 → 92.9	165	41	7.94
	2) 256.1 → 175.1		21			2) 226.1 → 77		53	
Dimethoate	1) 230 → 199	85	5	4.61	Pyraclostrobin	1) 388.1 → 193.9	72	12	8.40
	2) 230 → 124.9		25			2) 388.1 → 133.1		38	
Flumetsulam	1) 326.1 → 129	72	26	4.98	Nicosulfuron	1) 411.1 → 209.1	72	10	9.07
	2) 326.1 → 109.1		62			2) 411.1 → 143.9		18	
Fenobucarb	1) 208.1 → 115.9	74	2	5.36	Proquinazid	1) 373 → 331	72	13	9.92
	2) 208.1 → 89		14			2) 373 → 145		62	
Thidiazuron	1) 221 → 51.1	72	80	5.48	Spirodiclofen	1) 411.1 → 42.9	72	64	10.05
	2) 221 → 102.1		14			2) 411.1 → 70.9		14	
Imazalil	1) 297.1 → 159	140	21	5.87					
	2) 297.1 → 41.2		37						
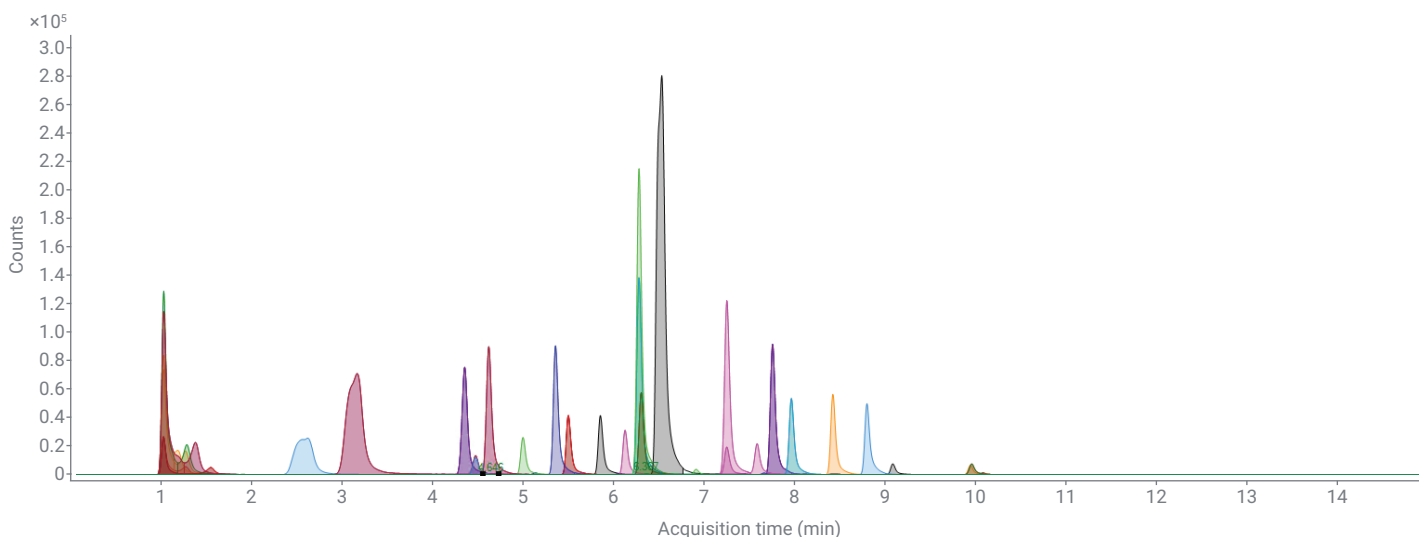
Figure 1. LC/MS/MS MRM chromatogram for extracted spinach sample fortified with 100 ng/g of 26 targeted pesticides with IS. Sample was prepared using QuEChERS AOAC extraction kit followed with AP-dSPE kit with Carbon S cleanup. Refer to Table 2 for peaks identification based on elution order.

Sample preparation

Fresh organic spinach 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 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. For the AP-dSPE with Carbon S 2 mL tube, a 1 mL aliquot of the crude extract was pipetted. For the AP-dSPE with Carbon S tube 15 mL, an 8 mL aliquot of the crude extract was transferred. For the AP-dSPE with GCB, the equivalent volume of the same crude extract was transferred to a 2 mL or 15 mL tube. All the tubes were capped tightly and vortexed for 2 to 3 minutes, followed by centrifugation. The sample supernatant was then diluted with water five times to generate the final sample in 20:80 ACN/water. The diluted sample was injected for LC/MS/MS analysis. The detailed sample preparation procedure is shown in Figure 2.
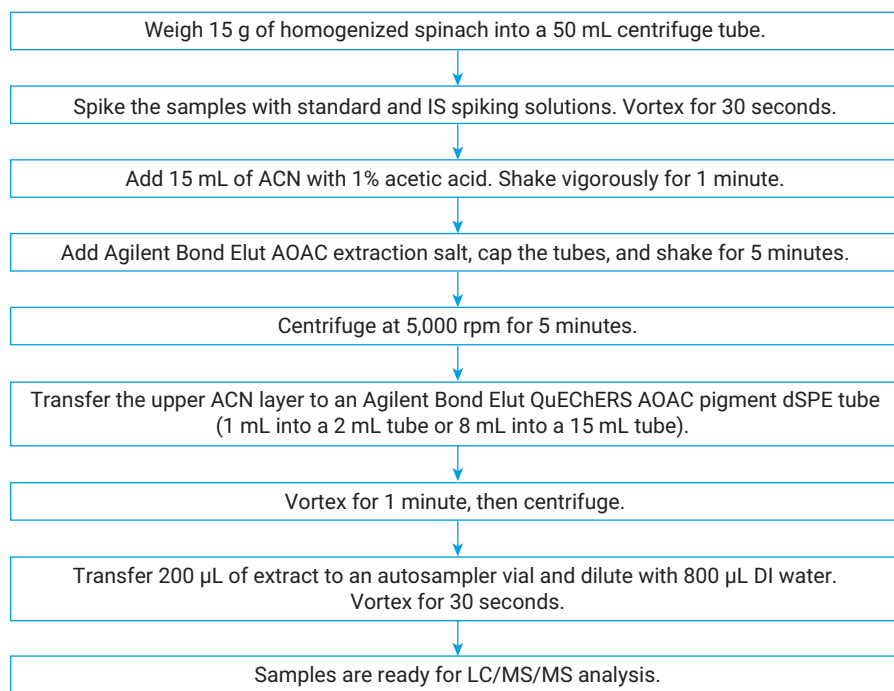

Figure 2. Sample preparation procedure for fresh spinach samples by QuEChERS AOAC extraction kit followed with AP-dSPE cleanup.

Results and discussion

AP-dSPE with Carbon S versus AP-dSPE with GCB

The Bond Elut AP-dSPE with Carbon S was compared to the corresponding AP-dSPE with GCB for targets recovery and reproducibility. Considering the volume of crude extract from the same sample extraction, the comparison study was conducted using the 2 mL dSPE tubes. The samples were prepared in replicates of six. Each sample was prespiked with 10 ng/g of targets as well as a 100 ng/g of IS (atrazine-d_5). Figure 3 shows the recovery and RSD (%) for 26 targets in the spinach matrix.

Generally, the AP-dSPE with Carbon S had a higher average recovery (72%), ranging from 40 to 97% for individual analytes. In comparison, the AP-dSPE with GCB had a lower average recovery (64%), ranging from 9 to 106%. Significant improvement on the recovery was obtained for typical planar pesticides, such as pymetrozine, carbendazim, thiabendazole, thidiazuron, forchlorfenuron, cyprodinil, and proquinazid (Figure 3). However, it is noticeable that few planar analytes still demonstrated low recoveries even with the improvement. This was linked to the dSPE cleanup format, as much better recoveries were achieved using the passthrough cleanup format.[1] For nonplanar pesticides, recoveries were similar, with noticeable differences for only a couple of pesticides.
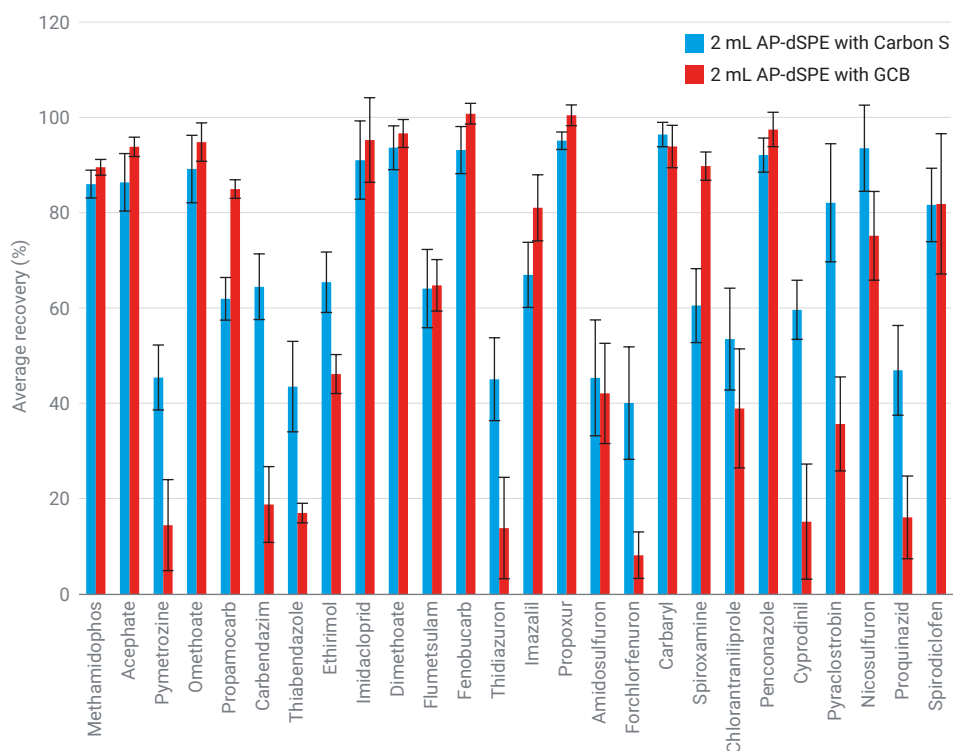

Figure 3. Comparison of Agilent Bond Elut AP-dSPE with Carbon S versus AP-dSPE with GCB for 26 representative pesticides analysis in spinach.

AP-dSPE with Carbon S 2 mL versus 15 mL

The two formats (2 and 15 mL) of AP-dSPE with Carbon S were further investigated for consistency of performance. The average recovery of targets using the 2 mL kit was 80%, while the average recovery of targets using the 15 mL kit was 81%, with <15% RSD on average. The average variability for both formats was acceptable at less than 15% RSD. The ratio of Carbon S was the same through both formats. Figure 4 shows the individual pesticide consistent recoveries when using the two different dSPE kits.

Pigment removal

Agilent Bond Elut AP-dSPE with Carbon S was developed to remove highly pigmented matrixes and evaluated for matrix pigment removal in spinach. Figure 5 demonstrates the matrix pigment removal of AP-dSPE with Carbon S and the counterpart AP-dSPE with GCB by samples' color visual comparison and UV adsorption collected by LC-UV at 450 nm. Results confirmed that the AP-dSPE with Carbon S removed 96% of chlorophyll pigments from the spinach matrix, which was similar to the 98% pigment removal obtained by AP-dSPE with GCB.
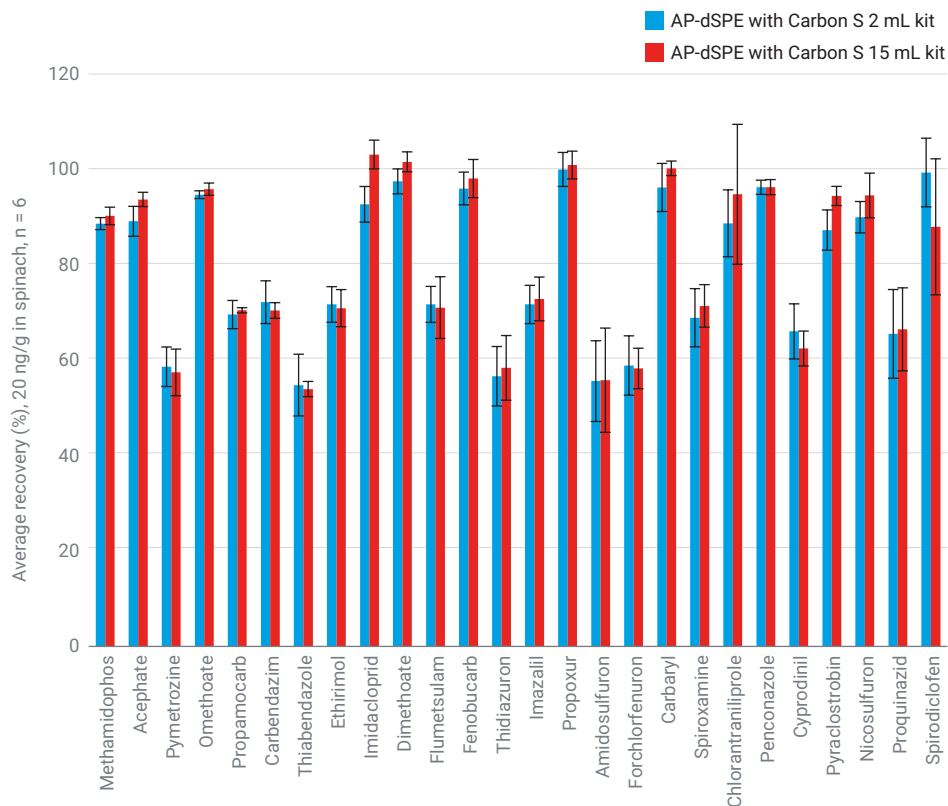

Figure 4. Consistent performance on pesticides analysis in spinach using AP-dSPE with Carbon S 2 mL and 15 mL kits.
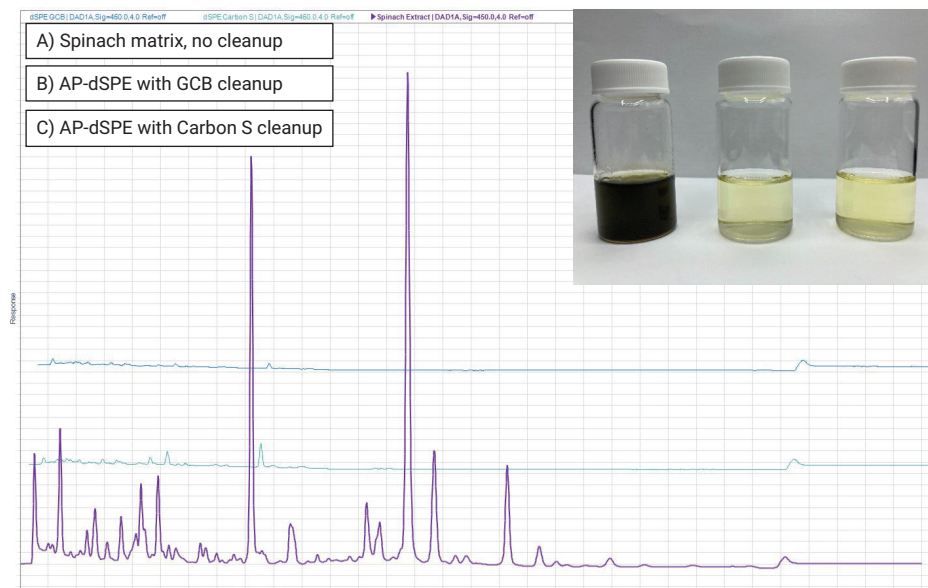

Figure 5. Comparison of matrix pigment removal using the Agilent Bond Elut AP-dSPE with Carbon S and AP-dSPE with GCB. Chromatograms were collected by LC-UV at 450 nm.

Method validation

The method was validated in spinach at two prespiked levels of 10 and 100 ng/g in spinach in replicates of four. Atrazine-D$_5$ was used as an IS and was spiked at 100 ng/g for each QC sample. Nine calibration standards were spiked for the dynamic range of 5 to 2,500 ng/mL in the spinach matrix blank prepared using the 15 mL Bond Elut AP-dSPE with Carbon S, corresponding to a range of 5 to 2,500 ng/g in spinach. Seven or more calibration levels were used to generate the calibration curve for each compound. Each curve used a linear regression and 1/x^2 weighting. The target calibration curve linearity was demonstrated with correlation coefficients (R^2) >0.99 for the most of targets, shown in Table 3.

Target accuracy ranged from 43 to 110% for nonplanar analytes and 35 to 69% for planar pesticides (Table 3). Low accuracy results for planar targets were certainly related to the low recoveries with dSPE cleanup, even with the significant improvement provided by the replacement of GCB with Carbon S sorbent. Better quantitation results on the sensitive pesticides can be achieved with the format and methodology improvements.[1] The RSDs for all analytes were <16%, with single-digit RSDs for many targets. The improved reproducibility demonstrates as another benefit when using Carbon S sorbent instead of GCB in the dSPE kit.

The results demonstrated that Bond Elut AP-dSPE with Carbon S provided improved method accuracy and reproducibility for pesticide analysis in spinach, and thus can be a direct replacement to the currently used AP-dSPE with GCB.

Table 3. Calibration range and accuracy of two spiking levels in spinach using AP-dSPE with Carbon S.

Analytes	Calibration Range (ng/g)	R^2	10 ng/g Accuracy (%)	RSD (n = 4)	100 ng/g Accuracy (%)	RSD (n = 4)
Methamidophos	5 to 1,250	0.9920	87.2	2.5	97.7	6.1
Acephate	5 to 1,250	0.9927	88.0	2.4	93.0	6.1
Pymetrozine	5 to 1,250	0.9956	62.3	4.5	40.2	6.2
Omethoate	5 to 500	0.9962	95.4	2.6	100.6	6.3
Propamocarb	5 to 500	0.9942	71.6	3.1	69.6	7.0
Carbendazim	5 to 500	0.9920	68.6	3.5	54.7	6.8
Thiabendazole	5 to 500	0.9907	49.2	4.3	45.0	5.5
Ethirimol	5 to 1,250	0.9882	70.6	2.8	64.7	6.8
Imidacloprid	5 to 1,250	0.9885	100.7	6.1	107.0	5.3
Dimethoate	5 to 2,500	0.9840	97.8	2.3	99.2	6.6
Flumetsulam	5 to 1,250	0.9891	71.1	5.2	65.3	7.0
Fenobucarb	5 to 1,250	0.9850	106.6	1.1	110.5	6.1
Thidiazuron	5 to 1,250	0.9933	53.9	3.5	45.3	4.4
Imazalil	5 to 2,000	0.9901	75.5	4.7	83.2	4.4
Propoxur	5 to 1,250	0.9904	104.4	2.1	110.1	6.6
Amidosulfuron	5 to 2,000	0.9952	87.6	6.9	67.9	8.0
Forchlorfenuron	5 to 2,000	0.9989	41.8	4.9	35.1	7.4
Carbaryl	5 to 2,500	0.9979	97.1	4.8	108.6	5.6
Spiroxamine	5 to 1,250	0.9894	69.3	4.1	62.8	5.7
Chlorantraniliprole	5 to 1,250	0.9947	94.8	4.1	69.5	8.5
Penconazole	5 to 2,000	0.9985	94.1	1.9	105.9	6.1
Cyprodinil	5 to 500	0.9948	59.9	5.6	51.3	7.9
Pyraclostrobin	5 to 500	0.9982	81.1	6.3	70.0	5.7
Nicosulfuron	5 to 500	0.9977	82.5	4.7	87.7	6.2
Proquinazid	5 to 2,000	0.9960	43.1	6.2	33.2	7.4
Spirodiclofen	5 to 2,000	0.9881	107.5	10.6	90.5	6.2

Conclusion

The Agilent Bond Elut QuEChERS AOAC Pigmented Fruits and Vegetables dSPE kit with Carbon S demonstrated equivalent removal of high-chlorophyll matrix pigment, and improved recoveries of planar pesticides compared to the traditional Agilent Bond Elut QuEChERS AOAC Pigmented Fruits and Vegetables dSPE kit with GCB. It is therefore considered as a direct replacement to AP-dSPE with GCB kit. Both 2 mL and 15 mL formats of the AP-dSPE with Carbon S were confirmed with consistent target quantitation results and pigment removal.

Reference

1. Zhao, L.; Wei, T. Determination of Multiclass, Multiresidue Pesticides in Spring Leaf Mix by Captiva EMR−HCF Passthrough Cleanup and LC/MS/MS, *Agilent Technologies application note*, publication number 5994-4765EN, **2022**.

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