



Analysis of Pesticide Residues in Spinach Using Agilent SampliQ QuEChERS EN Kit by LC/MS/MS Detection

Application Note

Food Safety

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Abstract

This application note describes the use of a quick, easy, cheap, effective, rugged, and safe (QuEChERS) EN sample preparation approach for extraction and cleanup of 13-pesticide residues representing various classes in spinach. Because spinach is considered a highly pigmented matrix, the EN dispersive SPE kit for highly pigmented fruits and vegetables is selected. Graphitized carbon black (GCB) in the amount of 7.5 mg/mL of ACN extract is added to the kit. The target pesticides in the spinach extracts are then determined by liquid chromatography coupled to an electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) operating in positive ion multiple reaction monitoring (MRM) mode. GCB is reported to have a significantly negative impact on the extraction of pesticides with planar structure. However, with the small amount of GCB addition in the EN dispersive SPE kit, our results show that the impact of GCB on planar pesticides is negligible and acceptable quantitation results are obtained. The 5 ng/g limit of quantitation (LOQ) for pesticides in spinach shown in this application is well below the maximum residue limits (MRLs). The spiking levels for the recovery experiments are 10, 50, and 200 ng/g. Mean recoveries range between 60 and 99% (85.4% on average), with an RSD below 11% (5.5% on average).

Introduction

The EN QuEChERS method has been widely employed in the analysis of pesticides in food, especially in Europe. [1-2] The method uses acetonitrile extraction, followed by salting out water from the sample using anhydrous magnesium sulfate (MgSO_4), NaCl and buffering citrate salts to induce liquid-liquid partitioning. For cleanup, a dispersive solid phase extraction (dispersive SPE) is conducted using a combination of primary secondary amine (PSA) to remove fatty acids from among other components, and anhydrous MgSO_4 to reduce the remaining water in the extract. According to different food matrices, other ingredients may be added in this step, such as graphitized carbon black (GCB) to remove pigments and sterol, or C18 to remove more lipids and waxes.

Spinach is considered to be a highly pigmented vegetable since it contains high levels of chlorophyll. Therefore, the EN dispersive SPE kits for highly pigmented commodities were selected for this application. In these kits, besides 25 mg of PSA and 150 mg of MgSO_4 , 7.5 mg of GCB is added per mL of ACN extracts. GCB adsorbs planar molecules like pigments and sterols; hence it is very helpful in cleaning up pigmented matrices like spinach. The efficiency of cleanup is dependent upon the amount of GCB used. The more GCB used, the more planar molecules are absorbed, and therefore, a cleaner sample matrix is obtained. The main difference between the EN method and AOAC method for cleaning up the highly pigmented matrix is the amount of GCB used in the dispersive SPE step. Instead of the relatively high amount of GCB used in AOAC method (50 mg of GCB per mL of ACN extracts), a much lower amount of GCB was used in the EN methods (2.5 mg of GCB per mL of ACN extracts for "pigmented" produce, or 7.5 mg of GCB per mL of ACN extracts for "highly pigmented" produce). The GCB impacted the extraction of planar pesticides differently, depending upon the method used. The AOAC method generated much cleaner final sample matrix but caused significant loss of planar pesticides; the EN method, on the contrary, caused little to no loss of planar pesticides but generated a more complicated sample matrix.

Previously, we described that a modified AOAC method with toluene addition in the dispersive SPE step greatly increased the extraction efficiency of planar pesticides in a pigmented matrix such as spinach. [3] Subsequently, we demonstrated the performance of SampliQ QuEChERS AOAC kit for the analysis of pesticides in spinach using combination of the modified (with toluene addition) and the original AOAC

method (without toluene addition). [4, 5] In this study, 13 pesticides were used for evaluating the performance of the Agilent EN Buffered Extraction kit (p/n 5982-5650) and SampliQ QuEChERS EN Dispersive SPE kits for Highly Pigmented Fruits and Vegetables (p/n 5982-5321 and 5982-5356). The method was validated in terms of recovery and reproducibility. Table 1 shows the chemical and regulatory information for these pesticides in spinach.

Experimental

Reagents and Chemicals

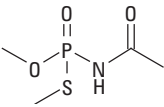
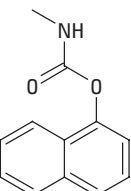
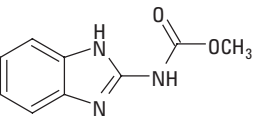
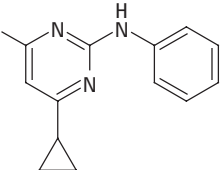
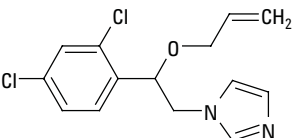
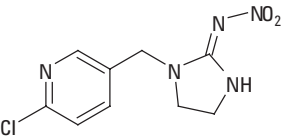
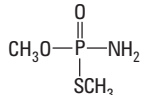
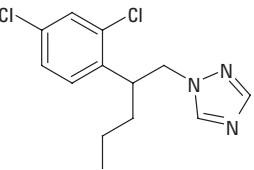
All reagents and solvents were HPLC or analytical grade. Methanol (MeOH), and toluene were from Honeywell (Muskegon, MI, USA). Acetonitrile (ACN), dimethyl sulfoxide (DMSO) and glacial acetic acid (HAc) were from Sigma-Aldrich (St Louis, MO, USA). Ammonium acetate (NH_4OAc) was from Fisher Chemicals (Fair Lawn, NJ, USA). Formic acid (FA) was from Fluka (Sleinheim, Germany). The pesticide standards and internal standard (triphenyl phosphate, TPP) were purchased from Sigma-Aldrich (St Louis, MO, USA), ChemService (West Chester, PA, USA), Ultra (Kingstown, RI, USA), or AlfaAesar (Ward Hill, MA, USA).

Solutions and Standards

A 1 M ammonium acetate pH 5 stock solution was made by dissolving 19.27 g NH_4OAc powder in 250 mL Milli-Q water, pH adjusted to 5 with acetic acid monitored with a pH meter. The solution was stored at 4 °C. A 5 mM ammonium acetate in 20:80 MeOH/ H_2O solution, pH 5, was made by combining 200 mL MeOH and 800 mL Milli-Q water, adding 5 mL of 1 M ammonium acetate pH 5 stock solution and mixing well. A 5 mM ammonium acetate in ACN was prepared by adding 5 mL of 1 M ammonium acetate pH 5 stock solution to 1 L ACN, mixing well and sonicating 5 min. A 1% formic acid in ACN solution was prepared by adding 1 mL of formic acid to 100 mL of ACN, and mixing well.

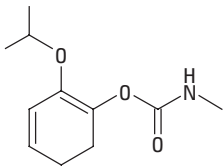
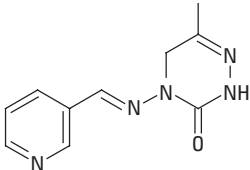

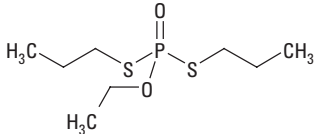
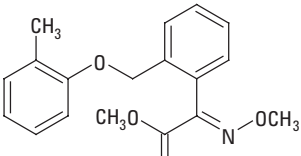
Standard and internal standard (IS) stock solutions (2.0 mg/mL for all except 0.5 mg/mL for carbendazim) were made in MeOH, 0.1% FA in ACN, or DMSO, respectively, and stored at -20 °C. Three QC spiking solutions of 1, 5, and 20 $\mu\text{g}/\text{mL}$, were made fresh daily in 1:1 ACN/ H_2O with 0.1% FA. A 10 $\mu\text{g}/\text{mL}$ standard spiking solution in 1:1 ACN/ H_2O with 0.1% FA was made also for the preparation of a calibration curve in the matrix blank extract by appropriate dilution. A 15 $\mu\text{g}/\text{mL}$ of TPP in 1:1 ACN/ H_2O with 0.1% FA was made as an IS spiking solution.

Table 1. Pesticides Chemical and Regulatory Information [6–8]

Name	Class	Log P	pKa	Structure	MRLs in spinach (ng/g)*
Acephate	Organophosphate	-0.89	8.35		20
Carbaryl	Carbamate	2.36	10.4		50
Carbendazim	Benzimidazole	1.48	4.2		100
Cyprodinil	Anilinopyrimidine	4	4.44		500
Imazalil	Imidazole	3.82	6.53		20
Imidacloprid	Neonicotinoid	0.57	NA		1000
Methamidophos	Organophosphate	-0.79	NA		10
Penconazole	Triazole	3.72	1.51		50

(Continued)

Table 1. Pesticides Chemical and Regulatory Information [6–8]

Name	Class	Log P	pKa	Structure	MRLs in spinach (ng/g)*
Propoxur	Carbamate	0.14	NA		2000
Pymetrozine	Pyridine	-0.19	4.06		600
Thiabendazole	Benzimidazole	2.39	4.73 12.00 0		50
Ethoprophos	Organophosphate	2.99	NA		5
Kresoxim-methyl	Strobilurin	3.4	NA		50

*The MRLs numbers list in the table are for spinach or other vegetables. They could be higher in different commodities.

Equipment and Material

Agilent 1200 Series HPLC with Diode Array Detector (Agilent Technologies Inc., CA, USA).

Agilent 6410 triple quadrupole LC/MS system with Electrospray Ionization (Agilent Technologies Inc., CA, USA).

Agilent SampliQ QuEChERS EN Extraction kits, p/n 5982-5650, and SampliQ QuEChERS EN dispersive SPE kits for Highly Pigmented Fruits and Vegetables, p/n 5982-5321 and 5982-5356 (Agilent Technologies Inc., DE, USA).

CentraCL3R Centrifuge (Thermo IEC, MA, USA)

Bottle top dispenser (VWR, So. Painfield, NJ, USA)

Eppendorf microcentrifuge (Brinkmann Instruments, Westbury, NY, USA)

Instrument Conditions

The previous LC/MS/MS method was used. [9]

HPLC conditions

Column:	Agilent ZORBAX Solvent Saver Plus Eclipse Plus Phenyl-Hexyl, 3.0 x 150 mm, 3.5 μ m (p/n 959963-312)		
Flow rate:	0.3 mL/min		
Column Temperature:	30 °C		
Injection volume:	10 μ L		
Mobile Phase:	A, 5 mM ammonium acetate, pH 5.0 in 20:80 MeOH/H ₂ O B, 5 mM ammonium acetate, pH 5.0 in ACN		
Needle wash:	1:1:1:1 ACN/MeOH/IPA/H ₂ O w/0.2% FA.		
Gradient:	Time	% Acetonitrile	Flow rate (mL/min)
	0	20	0.3
	0.5	20	0.3
	8.0	100	0.3
	10.0	100	0.3
	13.0	STOP	
Post run:	4 min		
Total cycle time:	17 min		

MS conditions

Positive mode	
Gas temp.:	350 °C
Gas flow:	10 L/min
Nebulizer:	40 Psi
Capillary:	4000 V

Other conditions relating to the analytes are listed in Table 2.

Sample Preparation

The sample preparation procedure includes sample comminution, extraction/partitioning and dispersive SPE cleanup. It was described in detail in the previous application notes. [9] The procedure used in spinach was similar to the one used in apple, except that the dispersive SPE kit was for highly pigmented produce rather than general fruits and vegetables.

Briefly, the frozen chopped organic spinach was homogenized thoroughly. A 10 g (\pm 0.1g) of homogenized sample was placed into a 50 mL centrifuge tube. Samples were fortified with appropriate QC spiking solutions (100 μ L) when necessary, and then 66.7 μ L of IS spiking solution (15 μ g/mL of TPP). After vortexing sample for 30 s, 10 mL of ACN was added to each tube using the dispenser. Tubes were then capped and shaken by hand for 1 min. To each tube, an Agilent SampliQ QuEChERS EN extraction salt packet (p/n 5982-5650), containing 4 g anhydrous MgSO₄, 1 g NaCl, 1 g Na₃Citrate, and 0.5 g Na₂HCitrate sesquihydrate, was added directly. Sample tubes were capped tightly, and hand-shaken vigorously for 1 min. Tubes were centrifuged at 4000 rpm for 5 min.

A 1 mL aliquot of upper ACN layer was transferred into Agilent SampliQ QuEChERS EN dispersive SPE 2 mL tube (p/n 5982-5321); or 6 mL aliquot into Agilent SampliQ QuEChERS EN dispersive SPE 15 mL tube (p/n 5982-5356). The 2 mL tube contains 25 mg of PSA, 150 mg of anhydrous MgSO₄ and 7.5 mg of GCB; while the 15 mL tube contains 150 mg of PSA, 900 mg of anhydrous MgSO₄ and 45 mg of GCB. The tubes were capped tightly and vortexed for 1 min. The 2 mL tubes were centrifuged with a micro-centrifuge at 13,000 rpm for 2 min, and the 15 mL tubes in a standard centrifuge at 4000 rpm for 5 min. A 200 μ L aliquot of extract was transferred into an autosampler vial. An aliquot of 10 μ L 1% FA in ACN was added immediately. Then 800 μ L of water or appropriate standard solutions (prepared in water) were added. The samples were capped and vortexed thoroughly for LC/MS/MS analysis.

Table 2. Instrument Acquisition Data Used for the Analysis of 13 Pesticides by LC/MS/MS

Analyte	MRM channels (m/z)	Fragmentor (V)	CE (V)	RT (min)
Acephate	1) 184.0 > 94.9	60	3	2.55
	2) 184.0 > 111.0		15	
Methamidophos	1) 142.0 > 94.0	60	8	2.54
	2) 142.0 > 124.9		8	
Pymetrozine	1) 218.1 > 105.0	115	20	2.97
	2) 218.1 > 78.0		50	
Carbendazim	1) 192.1 > 160.0	95	18	5.07
	2) 192.1 > 105.0		40	
Imidacloprid	1) 256.1 > 209.1	60	12	5.53
	2) 256.1 > 175.0		18	
Thiabendazole	1) 202.1 > 175.0	110	27	5.65
	2) 202.1 > 131.0		38	
Propoxur	1) 210.1 > 111.0	50	12	6.89
	2) 210.1 > 92.9		15	
Carbaryl	1) 202.0 > 145.0	50	3	7.30
	2) 202.0 > 115.0		40	
Ethoprophos	1) 243.1 > 130.9	80	15	8.50
	2) 243.1 > 172.9		15	
Imazalil	1) 297.1 > 158.9	80	22	8.52
	2) 297.1 > 200.9		15	
Penconazole	1) 284.1 > 158.9	80	32	8.95
	2) 284.1 > 172.9		32	
Cyprodinil	1) 226.1 > 93.0	120	35	9.23
	2) 226.1 > 108.0		35	
Kresoxim methyl	1) 314.0 > 222.1	70	10	9.44
	2) 314.0 > 235.0		10	
TPP (IS)	1) 327.1 > 77.0	70	45	9.49
	2) 327.1 > 151.9		45	

1) Quantifier transition channel

2) Qualifier transition channel

Results and Discussion

The QuEChERS method for pesticide residue analysis provides high-quality results in a fast, easy, inexpensive approach. For the pigmented fruits and vegetables, the addition of GCB in the dispersive SPE tube can improve the removal of pigments and sterols. The cleaning efficiency of the method with GCB is related to the amount of GCB added. The more GCB used, the cleaner the matrix after treatment and less matrix interferences remaining in the final sample. Since GCB can also cause the removal of planar pesticides during the extraction procedure, smaller amounts of GCB used in the EN dispersive SPE step has less of an effect on the planar pesticides. Compared to the AOAC method, the EN method for pigmented produce uses much less GCB in the dispersive SPE step. For normal pigmented commodities like carrots and romaine lettuce, 2.5 mg of GCB can be used per mL of ACN extract;

and for highly pigmented commodities like spinach or red sweet pepper, 7.5 mg of GCB can be used per mL of ACN extract. [1]

According to the recommendation, the EN dispersive SPE kit for highly pigmented products was used for spinach in our study. Given the highly pigmented kit, the amount of GCB used in the EN method is still much lower than that used in AOAC method, which is 50 mg of GCB per mL of ACN extract. Therefore, visually, the efficiency of matrix cleanup provided by the EN method was much weaker than that provided by AOAC method. The final sample processed by EN method still appeared dark green in color; while the previous final sample processed by AOAC method showed almost colorless transparency. The matrix blank differences are also shown in the UV chromatogram at $\lambda = 254$ nm shown in Figure 1. More interference peaks appear in the matrix blank processed by the EN method. Also more impurities may have accumulated

in the column or ionization source, which can have negative effects on the column and MS instrument. However, with the powerful selectivity provided by LC/MS/MS, the MRM chromatogram of matrix blank did not show any interference peaks to the target analytes. Figure 2 shows the LC/MS/MS chromatograms of matrix blank (IS spiked) and 50 ng/g fortified spinach extract processed by EN dispersive SPE method.

Four pesticides including Carbendazim, Thiabendazole, Cyprodinil, and Pymetrozine, with planar structure showed significant loss by the original AOAC dispersive SPE method. In addition, the modified method with toluene in the disper-

sive SPE step increased the extraction efficiency. [3,4] In order to investigate the impact of GCB on the planar pesticides, a comparison experiment with and without toluene addition in the dispersive SPE step for spinach samples fortified with the same level of pesticide standard (50 ng/g) was performed. The results showed little to no loss of planar pesticides caused by the small amount of GCB used in the EN method, and no significant improvement obtained by the addition of toluene. Therefore, the original EN method was employed for subsequent experiments. The method was validated in terms of recovery and reproducibility, and the quantitation results are discussed subsequently.

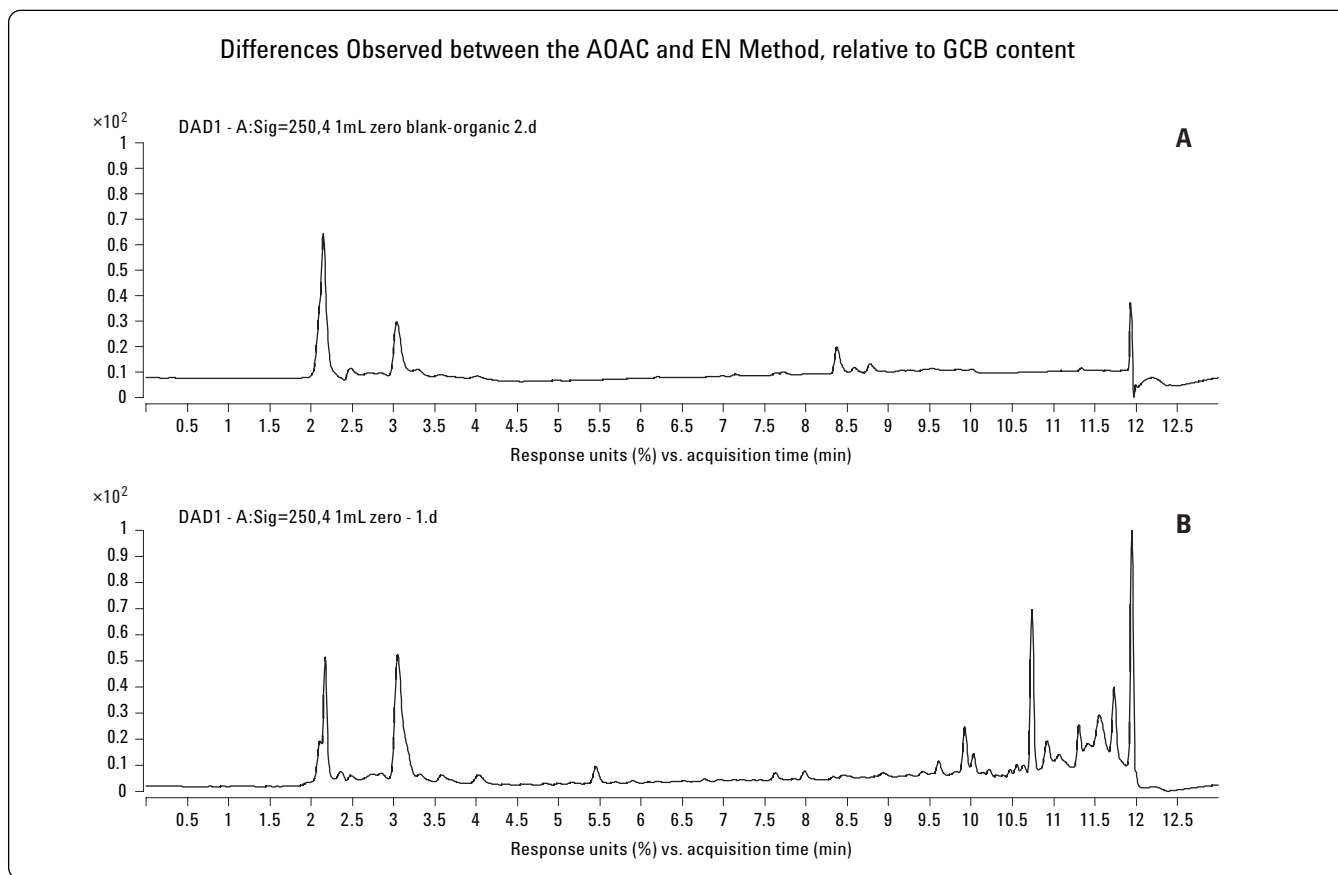


Figure 1. UV chromatogram ($\lambda = 254\text{nm}$) of spinach matrix blank processed by AOAC method (A) and EN method (B).

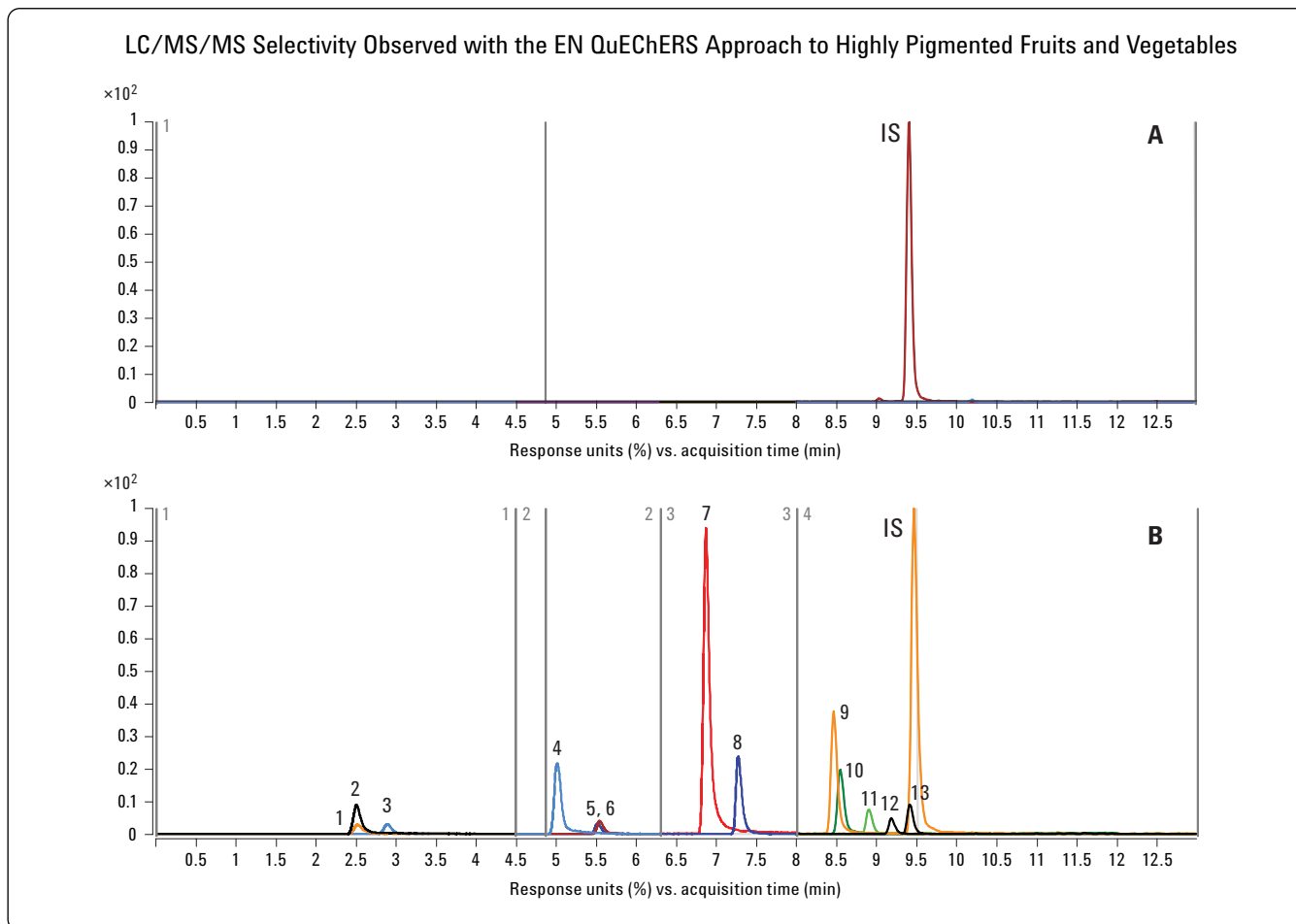


Figure 2. MRM chromatograms of spinach matrix blank (A) and 50 ng/g fortified sample (B) processed by EN method. Peak identification: 1. Methamidophos, 2. Acephate, 3. Pymetrozine, 4. Carbendazim, 5. Imidacloprid, 6. Thiabendazole, 7. Propoxur, 8. Carbaryl, 9. Ethoprophos, 10. Imazalil, 11. Penconazole, 12. Cyprodinil, 13. Kresoxim methyl IS: Internal Standard, TPP.

Linearity and limit of quantification (LOQ)

The linear calibration range for all of the pesticides tested was 5–250 ng/g. Calibration curves, spiked in matrix blanks, were made at levels of 5, 10, 50, 100, 200, and 250 ng/g. The TPP was used as an internal standard at 100 ng/g. The calibration curves were generated by plotting the relative responses of analytes (peak area of analyte/peak area of IS) to the relative concentration of analytes (concentration of analyte/concentration of IS). The 5 ng/g quantification limits LOQ (5 ppb) established for all of the pesticides is lower than the MRLs of these pesticides in fruits and vegetables. Table 3 shows the linear regression equation and correlation coefficient (R^2) for both 1 mL and 6 mL dispersive SPE.

Table 3. Linearity of Pesticides in Spinach Extract

Analytes	1 mL dispersive SPE Regression equation	R ²	6 mL dispersive SPE Regression equation	R ²
Methamidophos	Y = 0.2220X + 0.0005	0.9950	Y = 0.2244X + 0.0003	0.9893
Acephate	Y = 0.0814X + 0.0008	0.9972	Y = 0.0797X + 0.0005	0.9974
Pymetrozine	Y = 0.2063X + 0.0009	0.9559	Y = 0.1544X - 0.0006	0.9946
Carbendazim	Y = 0.9015X + 0.0164	0.9945	Y = 0.8526X + 0.0008	0.9917
Imidacloprid	Y = 0.0630X + 0.0001	0.9814	Y = 0.0682X - 0.0002	0.9952
Thiabendazole	Y = 0.3028X + 0.0059	0.9539	Y = 0.2315X + 0.0007	0.9968
Propoxur	Y = 1.3721X + 0.0018	0.9983	Y = 1.3304X + 0.0003	0.9981
Carbaryl	Y = 0.3459X + 0.0009	0.9968	Y = 0.3224X - 0.0003	0.9963
Ethoprophos	Y = 0.7588X - 0.0011	0.9979	Y = 0.7211X - 0.0023	0.9984
Imazalil	Y = 0.4644X + 0.0007	0.9889	Y = 0.4203X + 0.0002	0.9990
Penconazole	Y = 0.1647X - 0.0010	0.9937	Y = 0.1595X - 0.0008	0.9979
Cyprodinil	Y = 0.2575X + 0.0010	0.9884	Y = 0.2272X + 0.0007	0.9987
Kresoxim methyl	Y = 0.1175X - 0.0003	0.9976	Y = 0.1779X - 0.0008	0.9962

Recovery and Reproducibility

The recovery and reproducibility were evaluated by spiking pesticides standards in comminuted spinach sample at levels of 10, 50 and 200 ng/g. These QC samples were quantitated against the matrix spiked calibration curve. The analysis was performed in replicates of six at each level. The recovery and reproducibility (shown as RSD) data of 1 mL and 6 mL dispersive SPE are shown in Tables 4 and Table 5, respectively. It can be seen from the results that the nine pesticides with non-planar structure give excellent recoveries (average of 90.4% for 1 mL and 94.3% for 6 mL) and precision (average of 4.7% RSD for 1 mL and 5.3% RSD for 6 mL). The four pesticides with planar structure give lower but still acceptable

recovery (average of 71.8% for 1 mL and 79.8% for 6 mL) but good precision (average of 5.8% RSD for 1 mL and 4.8% RSD for 6 mL).

The impact of GCB on planar pesticides is visible and varies with different compounds. Cyprodinil gave excellent recovery and precision. Carbendazim gave excellent recovery and precision for low and mid level QCs, but poorer recovery for high level QC. Pymetrozine and thiabendazole gave lower recovery but still acceptable precision. The data in Table 6 show that the results of planar pesticides generated by EN method and AOAC modified method (with toluene addition) are comparable.

Table 4. Recovery and Reproducibility of Pesticides in Fortified Spinach with 1 mL Dispersive SPE Tube (p/n 5982-5321)

Analytes	10 ng/g fortified QC		50 ng/g fortified QC		200 ng/g fortified QC	
	Recovery	RSD (n=6)	Recovery	RSD (n=6)	Recovery	RSD (n=6)
Methamidophos	85.5	4.1	84.4	3.8	87.5	6.2
Acephate	83.7	8.3	84.6	5.9	91.6	5.8
Pymetrozine *	60.0	6.4	57.8	4.7	61.4	9.1
Carbendazim *	78.0	7.1	87.7	3.9	49.8	6.8
Imidacloprid	96.5	6.2	91.1	4.6	94.6	4.6
Thiabendazole *	64.3	7.0	71.5	6.5	71.5	5.8
Propoxur	93.7	4.7	92.0	4.1	86.7	4.3
Carbaryl	93.8	5.6	89.4	3.6	91.4	4.1
Ethoprophos	97.1	4.6	89.8	2.6	83.7	4.1
Imazalil	86.6	5.7	80.6	4.9	84.2	4.8
Penconazole	107.8	4.9	94.4	3.2	81.2	3.7
Cyprodinil *	89.6	4.4	88.6	4.5	80.8	3.9
Kresoxim methyl	101.5	3.8	94.6	1.4	92.8	3.8

* Pesticides with planar structure.

Table 5. Recovery and Reproducibility of Pesticides in Fortified Spinach with 6 mL Dispersive SPE Tube (p/n 5982-5356)

Analytes	10 ng/g fortified QC		50 ng/g fortified QC		200 ng/g fortified QC	
	Recovery	RSD (n=6)	Recovery	RSD (n=6)	Recovery	RSD (n=6)
Methamidophos	85.0	8.3	87.7	2.7	95.0	9.4
Acephate	88.6	5.1	84.6	3.1	94.6	9.3
Pymetrozine *	68.7	3.7	65.7	1.5	71.9	10.8
Carbendazim *	94.0	5.4	91.4	2.7	53.5	9.3
Imidacloprid	102.0	8.9	85.4	6.1	100.1	7.7
Thiabendazole *	77.2	4.4	77.6	2.4	79.2	9.7
Propoxur	98.2	5.7	96.3	1.8	93.9	7.2
Carbaryl	98.5	3.6	94.0	1.7	97.4	7.2
Ethoprophos	102.3	6.0	95.3	1.7	91.0	6.8
Imazalil	88.8	6.4	86.8	2.8	93.5	7.7
Penconazole	104.5	2.5	96.4	2.0	84.6	5.5
Cyprodinil *	101.5	4.2	92.2	2.4	86.8	7.6
Kresoxim methyl	99.7	6.1	97.4	1.6	95.3	6.9

* Pesticides with planar structure.

Table 6. Results Comparison of Planar Pesticides Generated by EN Method and Modified AOAC Method (With Toluene Addition)*

Analytes	EN method for highly pigmented matrix		Modified AOAC method by toluene addition	
	Mean recovery (%)	Mean RSD (%)	Mean recovery (%)	Mean RSD (%)
Carbendazim	75.7	5.9	98.5	2.5
Cyprodinil	89.9	4.5	63.1	3.2
Pymetrozine	64.3	6.0	65.2	3.7
Thiabendazole	73.2	4.9	69.7	2.7

*The data can be found in reference [4].

Conclusions

Agilent SampliQ QuEChERS EN buffered extraction kits and dispersive SPE kits for highly pigmented fruits and vegetables provide a simple, fast and effective method for the purification of representative pesticides in spinach. The small amount of GCB used in dispersive SPE does not impact the extraction of planar pesticides significantly, which makes the extraction procedure in this highly pigmented matrix as simple as the one used in general fruit and vegetables. The recovery and reproducibility, based on matrix spiked standards, are acceptable for multiclass, multi-residue pesticide determination in spinach. However, the final extract matrix contains more impurities, which may result in more negative impacts on the column and MS instrument. The selected pesticides represent a broad variety of different classes and properties; therefore, the Agilent SampliQ QuEChERS EN Buffered Extraction and Dispersive kits for Highly Pigmented Fruits and Vegetables can be used for other pesticides in similar highly pigmented matrices.

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