Analysis of Pesticide Residues in Spinach using Phenomenex roQ[™] QuEChERS AOAC Kits by LC/MS/MS and GC/MS

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The AOAC official method 2007.01 for pesticide residues in foods is described for the analysis of 18 pesticides and 2 internal standards from spinach using a Phenomenex roQ QuEChERS extraction kit and a PSA/GCB dSPE kit for sample preparation. Analysis was performed by LC/MS/MS using a Luna® C18(2) HPLC column and GC/MS using a Zebron[™] ZB-5MSi column. The roQ PSA/GCB dSPE kit quickly and effectively removed pigment and other matrix interferences from the difficult spinach sample. Recoveries of all 18 pesticides were between 70 % to 120 % with sample-to-sample variability <15 %. The lower limit of quantification (LLOQ) was well below the maximum residue limit reported in the International Maximum Residue Level database. Such results demonstrate the excellent performance of the roQ QuEChERS product for food analysis.

Introduction

QuEChERS (an acronym for Quick, Easy, Cheap, Effective, Rugged, and Safe) has grown over the past decade to become a commonly used sample preparation technique that is employed in most multi-residue pesticide testing laboratories. The method was first introduced at the European Pesticide Residues Workshop in Rome, May 2002 and published in the Journal of the Association of Official Analytical Chemists (JAOAC) in 2003.¹⁻³ Since the publication of the original method, the format and theory of QuEChERS has been adapted and modified accordingly to improve method performance for many different sample matrices and to expand the capacity of the multi-residue method by analyzing more compound classes. In 2005, a USDA validation study covering 229 analytes was reported.⁴ The QuEChERS method was designated as an AOAC Official Method 2007.01 for pesticide residues in foods in 2007.⁵

The QuEChERS method begins with extraction of pesticides from food, in our case spinach, using 1 % acetic acid in acetonitrile. Addition of magnesium sulfate induces phase separation of acetonitrile and water for liquid-liquid partitioning to occur. Sodium acetate buffer is added to control the pH between 4 to 6 for stability of base sensitive pesticides. After extraction, the top organic layer is then treated with a dispersive blend of anhydrous MgSO₄ to remove moisture from acetonitrile, PSA sorbent is to remove fatty acids from the extract while leaving the target analytes in solution, and graphitized carbon black (GCB) to remove matrix interferences in highly pigmented samples. Additional sorbents such as C18 for removal of fats can be added to this blend depending on the sample matrix.

Every food group in our food pyramid requires multi-residue pesticide testing since pesticides are widely used in our agriculture system. These pest-repelling chemicals undoubtedly have a strong presence in the food cycle. In residue testing, the primary challenge is to eliminate naturally occurring pigments, antioxidants, and nutrients from the sample matrix in order to achieve lower limits of detection and quantification of pesticides. Traditional techniques using liquid/liquid extraction employed the use of hazardous solvents, and suffer from poor recoveries for polar pesticides. Emerging out of the shortcomings of other sample clean up techniques, the QuEChERS method has proved to be extremely versatile for simultaneous analysis of multiple classes of pesticides from various matrices. The large variety of food matrices, ranging from aqueous, fiber rich, to fat/oil based foods can undergo a relatively simple cleanup using different sorbent mixtures and a single technique. The introduction of commercially available method kits, such as the roQ QuEChERS kits, now make it easy to utilize the advantages of this sample preparation format for multi-residue testing from food. In this study, 18 pesticides and 2 internal standards were extracted using the roQ AOAC 2007.1 extraction kit (p/n KS0-8911) and PSA/GCB dSPE kit (p/n KS0-8927). Due to the high pigmentation in spinach, roQ dSPE tubes containing MgSO₄, PSA and GCB were chosen. The final extracts were split in order to analyze the sample using LC/MS/MS and GC/MS.

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Experimental Conditions

Reagents and Chemicals

Pesticide standards and internal standard triphenylphosphate (TPP) were obtained from Accustandard (New Haven, CT), Ultra Scientific (N. Kingstown, RI), and Supelco (Bellefonte, PA). HPLC grade water (Milli-Q, Millipore, Billerica, MA) was used to prepare HPLC mobile phase and for sample preparation. Methanol and acetonitrile (ACN) were obtained from Honeywell Burdick & Jackson (Muskegon, MI). Toluene was obtained from Fisher Scientific (Waltham, MA). Acetic acid and formic acid were obtained from Sigma-Aldrich (St. Louis, MO). d10-parathion and d6- α -BHC were purchased from Ultra Scientific (N Kingston, RI).

Solutions and Standards

Standard pesticide mix (80 μ g/g) stock solutions were prepared in 0.1 % formic acid in acetonitrile. A QC spiking and stock solution of 40 μ g/mL was prepared by dilution from the standard pesticide mix in acetonitrile containing 0.1 % acetic acid. Calibration curve standard solutions (5, 10, 50, 100, 250, and 1000 ng/g) were prepared from 2, 10, and 40 μ g/mL solutions in acetonitrile containing 0.1 % acetic acid by serial dilution. A 2 % TPP solution in acetonitrile (1 % acetic acid) was used to determine system suitability. d10-parathion and d6- α -BHC were used as internal standards.

Sample Preparation

Spinach was chopped into 2-4 cm pieces, placed into a zip-lock bag, and stored in a -80 °C freezer for at least 24 hours prior to further processing. The spinach was first immersed in liquid nitrogen and homogenized in a blender to generate a powdery consistency.

QuEChERS Extraction

Liquid- Liquid Partitioning

15 g of pretreated sample was weighed in a 50 mL centrifuge tube (provided in the roQ extraction kit). Two sets of QC samples were spiked at 80 ng/g and 200 ng/g using 30 μ L and 75 μ L of stock solution (40 μ g/mL) respectively. An aliquot of 15 mL of 1 % acetic acid in ACN was added to the samples and 75 μ L of d10-parathion and d6- α -BHC were added as internal standards.

A roQ^T salt packet containing a blend of 6.0 g MgSO₄ and 1.5 g NaOAc provided in roQ extraction kits (p/n KS0-8911) was dispensed into each tube. The tubes were first shaken by hand for 1 minute and then centrifuged at 3500 rpm for 2 minutes. An aliquot of 8 mL of supernatant was transferred into a roQ QuEChERS dSPE tube containing 1.2 g MgSO₄, 0.4 g PSA and 0.4 g GCB (p/n KS0-8927).

roQ dSPE Cleanup

roQ QuEChERS dSPE tubes were sealed carefully and shaken by hand for 30 seconds and then centrifuged at 3500 rpm for 1 minute. Supernatant (250 µL) was transferred into a Verex[™] vial for LC/MS/MS solvent exchange and 2 mL of supernatant was transferred into 15 mL centrifuge tubes for GC/MS solvent exchange (**Figure 1**).

QuEChERS Procedure Outline

Figure 1.

Flow chart summary for AOAC Official Method 2007.01 for pesticide residues in foods. The final extracts were split for LC/MS/MS and GC/MS analyses.



LC/MS/MS Sample Preparation

Appropriate standard solutions were added to the samples. Extracts were evaporated to dryness under a slow stream of nitrogen and reconstituted in 200 μ L of 5 mM formic acid in methanol. After sonicating and vortexing, 800 μ L of 5 mM formic acid in deionized water was added. The samples were centrifuged prior to transferring into Verex vials with low volume inserts.

GC/MS Sample Preparation

500 µL of toluene was added to each sample in 15 mL centrifuge tubes. Samples were evaporated under a slow stream of nitrogen at 50 °C until approximately 0.1 mL of volume was left. The appropriate standard solutions and toluene were added to reach the 0.5 mL mark. After adding $MgSO_4$ to each sample to remove excess water, the samples were centrifuged at 3500 rpm for 1 minute. Samples were then transferred to Verex amber autosampler vials containing inserts for GC/MS analysis.

Chromatographic Conditions

LC/MS/MS was performed using a Luna[®] 3 µm C18(2) 150 x 3.0 mm HPLC column (p/n 00F-4251-Y0) on an Agilent[®] 1200 LC system (Agilent Technologies, Palo Alto, CA, USA) with an upper pressure limit of 400 bar, equipped with a binary pump, autosampler and interfaced with an API 4000[™] triple quadrupole mass

Table 1.

LC/MS/MS MRM Transitions and Parameters



	MRM	Pair					
Analyte	Q1	Q3	Dwell Time (sec)	DP (V)	CE (V)	CXP (V)	t (mín)
Atrazine	216	173	50	66	25	18	15.4
d10-Parathion	302	238	50	51	23	8	18.2
Imazalil	297	159	50	66	29	16	10.8
Imidacloprid	256	209	50	46	21	20	8.9
Kresoxim-methyl	314	222	50	41	17	8	18.3
Linuron	249	160	50	56	35	1 06	16.8
Tebuconazole	308	70	50	36	35	6	18.5
Thiabendazole	202	175	50	51	35	18	6.45
Triphenylphosphate	327	77	50	106	47	8	18.4

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Figure 2.

MRM chromatogram of spinach extract spiked at 200 ng/g.



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Table 2. GC/MS General Information

Analyte	Base Peak	MS/MS Transition	RT
Atrazine	200.0	173, 217,202	8.181
Azoxystrobin	344.0	372, 388, 403	22.201
Bifenthrine	181.0	165,166, 182	15.078
Carbaryl	144.0	115, 116, 201	9.751
Chlorothalonil	265.9	264, 268,194	9.017
Chlorpyrifos	97.0	197, 199, 314, 316	10.545
d ₁₀ -Parathion	301.0	156, 187, 237, 269	10.494
$d_6^-\alpha$ -HCH	224.0	222, 226, 185, 189	7.766
Endosulfan Sulfate	272.0	274, 387, 229, 239	13.802
Ethion	97.0	153,231, 125	13.155
Kresoxim-methyl	116.0	131, 206, 89	12.514
L-Cyhalothrin	181.0	208, 197, 449	15.672
o,p-DDD	235.0	237, 165, 199	12.508
o-phenylphenol	170.0	169, 141, 115	6.023
Permethrins	183.0	163, 165, 184	16.756
Tebuconazole	250.0	163, 125	14.045
Triphenylphosphate	325.0	163, 125	14.045

Figure 3.

GC/MS of spinach extract spiked at 200 ng/g.



- 16. Ethion
- 17. Endosulfan Sulfate

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spectrometer (AB SCIEX, Framingham, MA, USA). The ionization source was electrospray ionization (ESI) analyzed in positive ion mode (Table 1 and Figure 2).

GC/MS analysis was performed using a Zebron[™] ZB-5MSi Guardian[™], 30 m x 0.25 mm x 0.25 µm GC column (p/n 7HG-G018-11-GGA) on an Agilent® 6890N with a 5973 mass spectrometer (Table 2 and Figure 3).

Results and Discussion

The quick, easy, cheap, effective, rugged and safe (QuEChERS) method offers a relatively simple solution for the determination of a wide range and extensive list of pesticide compounds from many different matrices. To add method consistency and throughput, roQ[™] QuEChERS kits feature pre-weighed salt packets and pre-filled dispersive SPE sorbents in centrifuge tubes to increase throughput and improve consistency. According to the AOAC 2007.01 method, acceptable recoveries in pesticide residue analysis should be between 70-120 %, with RSD <15 %. Results for extraction from spinach using Phenomenex roQ QuEChERS PSA/ GCB dSPE kits are within acceptable AOAC criteria (Figure 6).

Figure 4.

Spinach extracts after liquid partitioning step with 1 % acetic acid in acetonitrile and magnesium sulfate. The organic phase was heavily pigmented in dark green.



Figure 5.

Spinach extracts after dSPE cleanup. GCB removed a majority of the pigment from the sample matrix and the extracts were clear with a light green tint.



Fresh spinach is a common leafy vegetable in our diet because it is available year round. It is an excellent source of vitamins and minerals. The naturally occurring dark green color, along with the nutrients in spinach makes sample matrix clean up a very challenging task. Despite this challenge, using the roQ PSA/GCB dSPE kit, the final extracts were visibly clear after the dSPE step (Figure 4 & 5) indicating the majority of the pigment was removed by GCB. However, the binding to GCB is highly non-specific and a few analytes, in particular, pesticides with planar structures like chlorothalonil and thiabendazole strongly adsorb onto GCB.

Figure 6

LC/MS/MS recovery data of samples spiked at 80 ng/g and 200 ng/g.



Figure 7. GC/MS recovery data of samples spiked at 80 ng/g and 200 ng/g.



GC/MS Recovery Data

Table 3.

Average absolute recoveries of pesticides in two sets of five duplicated samples fortified at 80 ng/g and 200 ng/g levels.

		80 ng/g				200 ng/g			
Analyte	LC/ MS/MS Recovery (%)	RSD (%)	GC/MS Recovery (%)	RSD (%)	LC/ MS/MS Recovery (%)	RSD (%)	GC/MS Recovery (%)	RSD (%)	
Atrazine	76	3	89	3	72	6	88	3	
Azoxystrobin	n/a	-	111	6	n/a	-	118	10	
Bifenthrine	n/a	-	87	2	n/a	-	93	5	
Carbaryl	n/a	-	105	8	n/a	-	94	17	
Chlorothalonil	n/a	-	30	7	n/a	-	24	43	
Chlorpyrifos	n/a	-	75	6	n/a	-	71	9	
Endosulfan Sulfate	n/a	-	111	6	n/a	-	109	12	
Ethion	n/a	-	100	3	n/a	-	102	6	
Imazalil	70	5	n/a	-	75	2	n/a	-	
Imidacloprid	93	7	n/a	-	90	2	n/a	-	
Kresoxim-methyl	82	2	95	4	87	6	96	7	
L-Cyhalothrin	n/a	-	110	10	n/a	-	105	17	
Linuron	77	4	n/a	-	78	10	n/a	-	
o,p-DDD	n/a	-	98	3	n/a	-	97	6	
o-phenylphenol	n/a	-	92	5	n/a	-	75	15	
Permethrins	n/a	-	87	3	n/a	-	92	7	
Tebuconazole	80	3	88	2	76	7	91	4	
Thiabendazole	10	18	n/a	-	10	36	n/a	-	

Previous reports have identified affinity of planar molecules for GCB, which explains the lower recoveries for pesticides with a planar structure, such as cyprodinil, pymetrozine & thiabendazole.⁷⁻⁹ Indeed, this was observed in the results (shown in **Figure 6** & **7**). This can be overcome by using different extraction solvent mixtures or addition of toluene in the liquid partitioning step to inhibit binding of planar pesticides to GCB. ⁹⁻¹² Nonetheless, GCB is extremely effective in removing difficult matrix interferences and can be used without method modification if target analytes do not include pesticides with a planar structure.

After sample cleanup using the QuEChERS technique, a Luna[®] 3µm C18(2) HPLC column was used for the LC/MS/MS analysis. The original AOAC method suggested 25 % ACN in 5 mM formic acid as an injection solvent (**Figure 8a**). However, early eluting peaks were distorted with this injection solvent. When the solvent strength was reduced to 25 % methanol in 5 mM formic acid, the peak splitting issue was eliminated (**Figure 8b**). The Luna 3µm

Figure 8.

(a) Early eluting peaks were distorted when injection solvent was 25% ACN in 5 mm formic acid, (b) Peak splitting issue was resolved when injection solvent strength was reduced to 25% methanol in 5 mm formic acid.



C18(2) HPLC column provided separation for a wide range of pesticides with excellent resolution. The pesticide mix consisted of pesticides with a large variety of properties, including polars and semi-polars such as methamidophos and kresoxim-methyl, respectively. Retention times ranged from 3.4 to 21.3 minutes. The Luna column proved to be very reproducible and demonstrated ruggedness when faced with a difficult sample matrix such as spinach.

Although co-eluting matrix interferences can be an issue in GC/ MS analysis, compound specific matrix effects do not apply. GC/ MS analysis demonstrates high sensitivity and reproducibility, especially for apolar and non-polar pesticides. Baseline resolution was achieved for all 21 pesticides in the mix and 2 internal standards.

Conclusion

Phenomenex roQ[™] QuEChERS extraction and PSA/GCB dSPE kits successfully extracted 18 pesticides of different classes from spinach while providing benefits such as effective removal of pigment and other matrix interferences. This method produced acceptable recoveries and reproducibilities per the official AOAC 2007.01 method. The roQ QuEChERS PSA/GCB dSPE kit can be used for other heavily pigmented matrices using this method if analytes of interest do not include planar pesticides. This method with modification, i.e. addition of toluene in the dispersive step, can be used if the list of analytes includes planar pesticides.

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Ordering Information

roQ Extraction Kits

Extraction Kits contain fifty easy-pour salt packets and fifty 50 mL stand-alone centrifuge tubes

Description	Unit	Part No.
EN 15662 Method Extraction Kits		
4.0 g MgSO ₄ , 1.0 g NACl, 1.0 g SCTD, 0.5 g SCDS	50/PK	KS0-8909
AOAC 2007.01 Method Extraction Kits		
6.0_{g} MgSO ₄ , 1.5 g NaOAC	50/PK	KS0-8911
Original Non-buffered Method Extraction Kits		
4.0_{g} MgSO ₄ , 1.0 g NaCl	50/PK	KS0-8910
6.0 g MgSO ₄ , 1.5 g NaCl	50/PK	KS0-8912

roQ dSPE Kits

SPE Kits contain pre-weighed	sorbents/salts inside 2 mL or	15 mL centri	fuge tubes
Description		Linit	Dort No

Description	Unit	rait no.
2 mL dSPE Kits		
150 mg MgSO ₄ , 25 mg PSA, 25 mg C18-E	100/PK	KS0-8913
150 mg MgSO ₄ , 25 mg PSA, 2.5 mg GCB	100/PK	KS0-8914
150 mg, MgSO₄, 25 mg PSA, 7.5 mg GCB	100/PK	KS0-8915
150 mg MgSO ₄ , 25 mg PSA	100/PK	KS0-8916
150 mg MgSO ₄ , 50 mg PSA, 50 mg C18-E, 50 mg GCB	100/PK	KS0-8917
150 mg MgSO ₄ , 50 mg PSA, 50 mg C18-E	100/PK	KS0-8918
150 mg MgSO ₄ , 50 mg PSA, 50 mg GCB	100/PK	KS0-8919
150 mg MgSO ₄ , 50 mg PSA	100/PK	KS0-8919

15 mL dSPE Kits		
900 mg MgSO ₄ , 150 mg PSA, 150 mg C18-E	50/PK	KS0-8921
900 mg MgSO, 150 mg PSA, 15 mg GCB	50/PK	KS0-8922
900 mg MgSO ₄ , 150 mg PSA, 45 mg GCB	50/PK	KS0-8923
900 mg MgSO ₄ , 150 mg PSA	50/PK	KS0-8924
1200 mg MgSO ₄ , 400 mg PSA, 400 mg C18-E, 400 mg GCB	50/PK	KS0-8925
1200 mg MgSO ₄ , 400 mg PSA, 400 mg C18-E	50/PK	KS0-8926
1200 mg MgSO ₄ , 400 mg PSA, 400 mg GCB	50/PK	KS0-8927
1200 mg MgSO ₄ , 400 mg PSA	50/PK	KS0-8928

Bulk roQ QuEChERS Sorbents

Phases	10 g	100 g
C18-E	—	04G-4348
GCB (Graphitized Carbon Black)	04D-4615	04G-4615
PSA	-	04G-4610

Ordering Information

Luna® C18(2) HPLC Columns

µm Micr	robore and Minib	ore Columns	(mm)					SecurityGuard™ Cartridges (mm
hases	50 x 1.0	150 x 1	.0 30 >	c 2.0 5	50 x 2.0	100 x 2.0	150 x 2.0	4 x 2.0*
18(2)	00B-4251-A	40 00F-425	1-A0 00A-42	251-B0 00E	3-4251-B0	00D-4251-B0	00F-4251-B0	AJ0-4286
								for ID: 2.0-3.0 mn
um Norr	row Poro Column	o (mm)		SecurityGuar	rd			
µiii Nafi	TOW BUIE COlumn	5 (IIIII)	1500.0	Cartridges (III	111)			
10(0)	30 X 3.0	50 X 3.0	150 X 3.0	4 X 2.0"				
18(2)	00A-4251-10	008-4251-10	00F-4251-10	AJU-4286				
				tor ID: 2.0-3.0 n	nm			
						SecurityGuar	rd	
µm Ana	lytical Columns ((mm)				Cartridges (m	m)	
hases	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	4 x 3.0*		
18(2)	00A-4251-E0	00B-4251-E0	00C-4251-E0	00D-4251-E0	00F-4251-E0	AJ0-4287		
						for ID: 3.2-8.0 n	nm	
µm Micı	robore and Minib	ore Columns	(mm)					SecurityGuard Cartridges (mm)
hases	50 x 1.0	150 x 1.0	250 x 1.0	30 x 2.0	50 x 2.0	150 x 2.0	250 x 2.0	4 x 2.0*
18(2)	00B-4252-A0	00F-4252-A0	00G-4252-A0	00A-4252-B0	00B-4252-B0	00F-4252-B0	00G-4252-B0	AJ0-4286
								for ID: 2.0-3.0 mm
					Secu	rityGuard		
µm Narr	row Bore Column	is (mm)			Cartric	lges (mm)		
hases	30 x 3.0	50 x 3.0	150 x 3.0) 250 x 3	.0 4:	x 2.0*		
8(2)	00A-4252-Y0	0 00B-4252-Y	'0 00F-4252-	Y0 00G-4252	2-Y0 AJ0)-4286		
					for ID: 2	2.0-3.0 mm		
							0	it. Our and
µm Anal	lytical Columns ((mm)					Cartrid	ges (mm)
hases	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4	.6 150 x	4.6 250 x	4.6 4	x 3.0*
18(2)	00A-4252-E0	0 00B-4252-E	0 00C-4252-	E0 00D-4252	2-E0 00F-425	2-E0 00F-42	52-E0 AJ0)-4287

* SecurityGuard Analytical Cartridges require holder, Part No.: KJ0-4282

Zebron[™] ZB-5MSi

Zebron ZB-5MSi GC Columns								
ID(mm)	df(µm)	Temp. Limits °C	Part No.					
10-Meter								
0.18	0.18	-60 to 360/370	7CD-G018-08					
12-Meter								
0.20	0.33	-60 to 360/370	7DE-G018-14					
15-Meter								
0.25	0.25	-60 to 360/370	7EG-G018-11					
30-Meter								
0.25	0.25	-60 to 360/370	7HG-G018-11					
0.25	0.50	-60 to 360/370	7HG-G018-17					
0.25	1.00	-60 to 360/370	7HG-G018-22					
0.32	0.25	-60 to 360/370	7HM-G018-11					
0.32	0.50	-60 to 360/370	7HM-G018-17					
60-Meter								
0.25	0.25	-60 to 360/370	7KG-G018-11					

Note: If you need a 5 in. cage, simply add a (-B) after the part number, e.g., 7HG-G018-11-B. Some exceptions may apply. Agilent 6850 and some SRI and process GC systems use only 5 in. cages.



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