A NOVEL SORBENT FOR PASS-THROUGH SPE CLEANUP: A SIMPLE, QUICK, AND EFFECTIVE ALTERNATIVE FOR REMOVAL OF LIPIDS AND CHLOROPHYLL FROM QUECHERS EXTRACTS

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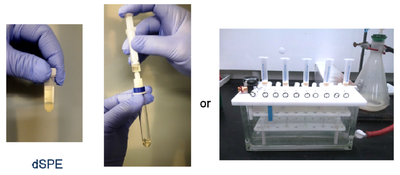
INTRODUCTION

In recent years, food safety laboratories have adopted new and simplified sample preparation methods, such as QuEChERS, to reduce analysis time and and to increase throughput. In this study, this simplified sample preparation is applied to pesticide analysis in avocado, a fruit matrix of high lipid and chlorophyll content, and in spinach, a vegetable matrix of very high chlorophyll content. In the QuEChERS extraction, significant amounts of fat, phospholipids, and chlorophyll are co-extracted along with the target pesticides. The presence of these co-extracted substances can lead to chromatographic interference, contamination of GC or LC systems, and contamination of the mass spectrometer. To avoid these complications, a cleanup step is recommended prior to the instrumental analysis. This is typically performed using cumbersome, multi-step dispersive SPE (dSPE) with mixed sorbents. In this study, Oasis PRiME HLB, a novel reversed-phase sorbent, was used for a simple, rapid, and effective pass-through cleanup to effectively remove fats, phospholipids and chlorophyll from QuEChERS extracts of avocado and spinach. Although dSPE with C18 and graphitized carbon black (GCB) can be effective for removal of fats and chlorophyll, recovery losses can occur for some pesticides, particularly those that have a planar geometry. In this study, pesticide analysis was performed using both UPLC-MS/MS and APGC-MS/MS. No loss of planar pesticides was observed and high recoveries of pesticides were achieved after the rapid and straightforward pass-through cleanup. Phospholipid removal was monitored using UPLC-MS. Fat removal was monitored using GC-MS. UPLC with UV detection was employed to monitor the effectiveness of various dSPE and pass-through SPE cleanup options for removal of chlorophyll and other pigments from the spinach extracts.

DSPE and pass-through SPE can provide effective sample cleanup in only minutes



dSPE Pass-Through SPE

INSTRUMENTAL METHODS

UPLC-MS/MS ANALYSIS

UPLC Conditions
UPLC system: ACQUITY UPLC I-Class
Column: ACQUITY UPLC BEH™ C18, 1.7µm, 100 x 2.1 mm
Mobile phase:
A: 10 mm ammonium acetate in water (pH 5.0)
B: 10 mm ammonium acetate in 99:1 methanol/water
Injection volume: 5 µL
Column temperature 45°C
Gradient: 2 % B initial, hold to 0.25 min, to 99 % B at 12.25 min, hold to 13.0 min, back to 2 % B at 13.01 min and hold to 17.0 min

MS Conditions
Mass Spectrometer: Waters Xevo TQ-S micro
Ion Mode: ESI+ (MRM mode)
Source Temperature: 150°C
Desolvation Temperature: 400°C
Desolvation Gas: 650 L/Hr (N_2)
Cone Gas: 20 L/Hr (N_2)
Collision gas: 0.18 mL/min (Ar)
Data Management: MassLynx v4.1

APGC-MS/MS ANALYSIS

APGC Conditions
GC system: Agilent 7890
Column: Restek Rxi-5ms, 30 m x 0.25mm x 0.25 µm
Flow rate: 1.0 mL/min Helium
Injection volume: 1 µL (15:1 split)
Temperature Program: 80°C initial, hold for 0.5 min, 12°C /min to 320°C and hold for 8 min

MS Conditions
Mass Spectrometer: Waters Xevo TQ-S
Ion Mode: API+ (MRM mode)
Corona: 2.8 µA
Source Temperature: 150°C
Probe Temperature: 450°C
Cone Gas: 170 L/Hr
Auxiliary Gas: 170 L/Hr
Collision gas: 0.15 mL/min (Ar)
Nebulizer: 4.0 Bar
Data Management: MassLynx v4.1

Compound	MRM	Cone (V)	Collision (eV)	RT (min)
Azoxystrobin	404.0 > 329.0	28	30	8.2
	404.0 > 372.0	28	15	
Bifenazate	301.1 > 198.0	16	20	8.8
	301.1 > 170.0	16	10	
Chlorantraniliprole	484.0 > 286.0	18	12	7.9
	484.0 > 453.0	18	17	
Etofenprox	394.3 > 177.0	26	15	11.8
	394.3 > 376.9	26	5	
Etoxazole	360.2 > 141.1	60	25	11.0
	360.2 > 57.2	50	35	
Fenpyroximat	422.2 > 366.1	32	15	11.1
	422.2 > 138.1	32	32	
Metalaxyl	280.1 > 192.1	26	17	7.6
	280.1 > 220.1	26	13	
Imidacloprid	256.1 > 175.1	34	20	4.1
	256.1 > 209.1	34	15	
Methomyl	163.0 > 88.0	26	10	3.2
	163.0 > 106.0	26	10	
Methoxyfenozide	369.1 > 149.1	34	18	8.6
	369.1 > 149.1	34	8	
Novaluron	493.0 > 341.0	30	35	10.3
	493.0 > 158.9	30	20	
Pyraclostrobin	388.1 > 163.0	31	25	9.7
	388.1 > 193.9	31	12	
Spirodiclofen	411.1 > 71.2	31	13	11.2
	411.1 > 313.0	31	13	
Spirotetramat	374.2 > 216.2	40	31	8.9
	374.2 > 302.2	40	23	
Thiabendazole	202.0 > 175.0	51	25	5.4
	202.0 > 131.0	51	30	
Thiamethoxam	292.0 > 211.2	28	22	3.4
	292.0 > 211.2	28	12	

MRM transitions (primary transition first), instrument parameters, and observed retention times (RT) for UPLC-MS

Compound	MRM	Cone (V)	Collision (eV)	RT (min)
Azoxystrobin	403.0 > 344.2	20	12	20.9
	403.0 > 329.1	20	32	
Carfentrazone-Ethyl	410.9 > 312.2	20	20	16.1
	410.9 > 346.2	20	10	
Chlorothalonil	265.9 > 170.0	20	27	12.0
	265.9 > 230.9	20	27	
Cypermethrin	163.1 > 127.0	20	10	19.1*
	163.1 > 91.0	20	10	
λ-Cyhalothrin	449.0 > 181.2	20	20	17.8*
	449.0 > 397.3	20	14	
Cyprodinil	225.1 > 210.1	20	20	13.9
	225.1 > 93.1	20	32	
Dichlorvos	184.9 > 93.0	20	20	6.1
	220.9 > 109.0	20	15	
Fenpropathrin	349.1 > 265.2	20	10	17.1
	349.1 > 210.2	20	20	
Fludioxonil	248.0 > 127.1	20	25	14.9
	248.0 > 182.1	20	20	
Folpet	259.9 > 130.0	20	13	14.3
	294.9 > 259.9	20	10	
Malathion	173.1 > 127.1	20	6	13.2
	173.1 > 99.0	20	10	
Metalaxyl	206.1 > 132.1	20	20	12.8
	206.1 > 162.1	20	8	
Oxyfluorfen	361.0 > 300.1	20	10	15.1
	361.0 > 252.2	20	30	
Permethrin	183.1 > 153.0	20	15	18.6*
	183.1 > 168.0	20	15	
Pyriproxifen	136.1 > 78.0	20	20	17.6
	136.1 > 96.0	20	20	
Simazine	201.1 > 173.1	20	10	11.2
	201.1 > 186.1	20	8	

MRM transitions (primary transition first), instrument parameters, and observed retention times (RT) for APGC-MS

SAMPLE PREPARATION

QuEChERS Extraction. For Spinach, a 15 g homogenized sample was weighed into a 50 mL centrifuge tube. Because avocado is so high in fat, the QuEChERS method was modified to reduce the sample size; 5 g homogenized avocado was weighed into a centrifuge tube and combined with 5 mL of water. For both commodities, 15 mL 1:99 acetic acid/acetonitrile were added and the sample was manually shaken for 1 minute. Then, QuEChERS salts (contents of DisQuE pouch for AOAC QuEChERS, pn 186006812) were added and the tube was shaken vigorously by hand for 1 minute. After centrifugation (3200 rcf for 5 minutes), portions of the supernatant were taken for cleanup (see Figure 1).

Pass-through SPE Cleanup. An Oasis PRiME HLB cartridge (3 cc, 150mg, pn 186008717) was mounted on a pre-cleaned vacuum manifold set to minimal vacuum (approx 2 psi). *No cartridge conditioning is required or was performed.* A 0.8 mL aliquot of the supernatant was passed-through the Oasis PRiME cartridge and discarded. Then a 1.5 mL portion of the supernatant was passed through the cartridge and collected. After centrifugation (1 minute at 13500 rcf), a portion of sample was transferred to an auto-sampler vial for analysis by APGC-MS. Another portion of the sample (100 µL) was transferred to a separate vial and diluted with 400 µL water for UPLC-MS analysis.

dSPE Cleanup (performed on Spinach samples). Into a 2 mL centrifuge tube was weighed 150 mg anhydrous sodium sulfate, 50 mg C18 silica, 50 mg PSA (primary/secondary amine silica) and 50 mg GCB. A second 2 mL tube was prepared with the same sorbents except with 10 mg GCB. A 1 mL portion of supernatant was transferred to each tube and the tubes were shaken by hand for 1 minute. Samples were then taken for APGC-MS and UPLC-MS analysis in the same manner as the pass-through SPE samples.

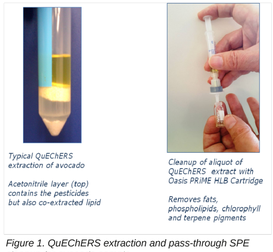


Figure 1. QuEChERS extraction and pass-through SPE cleanup for avocado sample

RECOVERY

UPLC-MS results (Figure 2) and APGC-MS results (Figure 3) are presented below for pass-through cleanup using the Oasis PRiME HLB cartridge. Avocado results are shown; spinach results were similar. Only folpet, a thermal and pH labile substance similar to captan, showed recovery losses greater than 20 % resulting from the cleanup protocol. The Oasis PRiME HLB cartridge cleanup removed greater than 95% of phospholipids, greater than 95 % of chlorophyll and greater than 80 % of fat from the avocado samples (see Figures 4 and 5). Pigment removal from spinach extracts is discussed in more detail at right.

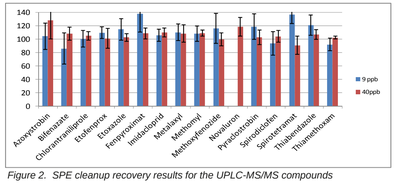


Figure 2. SPE cleanup recovery results for the UPLC-MS/MS compounds

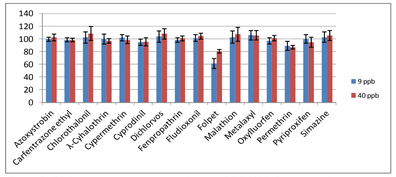


Figure 3. SPE cleanup recovery results for the APGC-MS/MS compounds

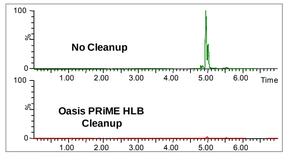


Figure 4. Effective removal of phospholipids from avocado QuEChERS extract with Oasis PRiME HLB clean-up

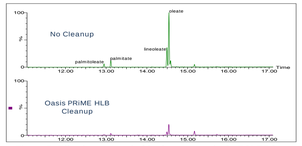
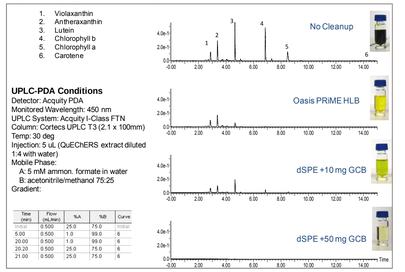


Figure 5. Effective removal of fat from avocado QuEChERS extract with Oasis PRiME HLB clean-up (determined by GC-MS as methyl esters after methanolysis/methylation)

SPINACH PIGMENT CLEANUP

UPLC with PDA provides effective method for monitoring spinach pigment cleanup



UPLC-PDA Conditions
Detector: Acquity PDA
Monitored Wavelength: 450 nm
UPLC System: Acquity I-Class FTN
Column: Cortecs UPLC T3 (2.1 x 100mm)
Temp: 30 deg
Injection: 5 uL (QuEChERS extract diluted 1:4 with water)
Mobile Phase:
A: 5 mM ammon. formate in water
B: acetonitrile/methanol 75:25
Gradient:

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	0.500	75.0	25.0	6
5.00	0.500	1.0	99.0	6
20.00	0.500	1.0	99.0	6
20.20	0.500	25.0	75.0	6
21.00	0.500	25.0	75.0	6

Figure 1. UPLC-PDA chromatograms showing removal of pigments from spinach extracts using the three cleanup protocols

APGC-MS/MS: sensitive and selective for GC pesticides

APGC Conditions
GC system: Agilent7890
Column: Restek Rxi-5ms, 30 m x 0.25mm x 0.25 µm
Flow rate: 1.0 mL/min Helium
Injection volume: 1 µL (15:1 split)
Temperature Program: 80°C initial, hold for 0.5 min, 12°C /min to 320°C and hold for 8 min

MS Conditions
Mass Spectrometer: Waters Xevo TQ-S
Ion Mode: API+ (MRM mode)
Corona: 2.8 µA
Source Temperature: 150°C
Probe Temperature: 450°C
Cone Gas: 170 L/Hr
Auxiliary Gas: 170 L/Hr
Collision gas: 0.15 mL/min (Ar)
Nebulizer: 4.0 Bar
Data Management: MassLynx v4.1

MRM Transitions
Cyprodinil: 225.1>210.1 (20 V cone, 20 eV collision)
225.1>93.1 (20 V cone, 32 eV collision)
Chlorothalonil: 265.9>170.0 (20 V cone, 27 eV collision)
265.9>230.9 (20 V cone, 27 eV collision)

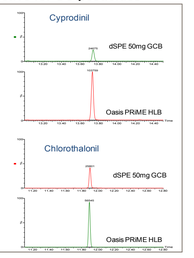


Figure 2. APGC-MS/MS ion chromatograms showing improved recovery for planar pesticides cyprodinil and chlorothalonil after cleanup with the Oasis PRiME HLB cartridge compared with dSPE cleanup with graphitized carbon

UPLC-MS/MS: sensitive and selective for LC pesticides

UPLC Conditions
UPLC system: ACQUITY UPLC I-Class
Column: ACQUITY UPLC BEH™ C18, 1.7µm, 100 x 2.1 mm
Mobile phase:
A: 10 mm ammonium acetate in water (pH 5.0)
B: 10 mm ammonium acetate in 99:1 methanol/water
Injection volume: 5 µL
Column temperature 45°C
Gradient: 2 % B initial, hold to 0.25 min, to 99 % B at 12.25 min, hold to 13.0 min, back to 2 % B at 13.01 min and hold to 17.0 min

MS Conditions
Mass Spectrometer: Waters Xevo TQ-S micro
Ion Mode: ESI+ (MRM mode)
Source Temperature: 150°C
Desolvation Temperature: 400°C
Desolvation Gas: 650 L/Hr (N_2)
Collision gas: 0.18 mL/min (Ar)
Data Management: MassLynx v4.1

MRM Transitions
Thiabendazole: 202.0>175.0 (51 V cone, 25eV collision)
202.0>131.0 (51 V cone, 30eV collision)

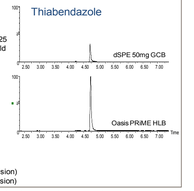


Figure 3. UPLC-MS/MS ion chromatograms showing improved recovery for planar pesticide thiabendazole after cleanup with the Oasis PRiME HLB cartridge compared with dSPE cleanup with graphitized carbon

DISCUSSION

All three cleanup methods were effective for removal of the majority of chlorophyll and carotenes from the QuEChERS extract of spinach. Pass-through cleanup with the Oasis PRiME HLB cartridge was slightly better than dSPE with 10 mg GCB for removal of chlorophyll. dSPE with 50 mg GCB per mL extract was the only cleanup that effectively remove all pigments from the QuEChERS extract. However, significant losses of planar pesticides were observed using dSPE with 50 mg GCB. In contrast, little or no recovery losses were observed for the three planar pesticides with Oasis PRiME HLB cleanup or using dSPE cleanup with 10 mg GCB per mL extract. Figure 6 shows UPLC-PDA chromatograms illustrating removal of the pigments using the three cleanup protocols. Figure 7 shows APGC-MS/MS ion chromatograms illustrating the recovery losses for cyprodinil and chlorothalonil. Figure 8 shows a UPLC-MS/MS ion chromatogram illustrating recovery loss for thiabendazole.

The Oasis PRiME HLB cartridge is available in various sizes and formats. The "vac" type cartridges are most convenient for use with vacuum/positive pressure manifold while the "plus type cartridges are suitable for use with a syringe (similar to a syringe filter) or with a vacuum/positive pressure manifold. The choice of cartridge size is made based on the volume of extract required by the analyst. Figure 9 illustrates this cartridge choice; no difference was seen in total pigment removal or pesticide recovery among the three cartridge choices.

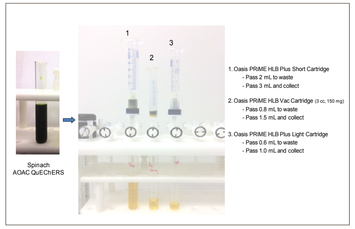


Figure 9. Oasis PRiME HLB in plus type cartridges provide identical cleanup

CONCLUSIONS

- **The Oasis PRiME pass-through cleanup was effective for removal of fats and phospholipids from the QuEChERS extract of avocado**
- **Pass-thru cleanup with an Oasis PRiME HLB cartridge effectively removed greater than 99% of chlorophyll and greater than 95% of lutein from the QuEChERS extract of spinach**
- **Pass-through cleanup with the Oasis PRiME HLB cartridge was more effective than dSPE cleanup with 10 mg GCB (per mL extract) for removal of chlorophyll and lutein from the QuEChERS extract of spinach**
- **dSPE cleanup with 50 mg GCB (per mL extract) was effective for removal of all pigments from the QuEChERS extract, but significant loss of planar pesticides was observed**

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