MULTIRESIDUE DETERMINATION OF PESTICIDES IN A FATTY MATRIX: AN ALTERNATIVE CLEANUP FOR QUECHERS EXTRACTS PRIOR TO APGC-MS/MS AND UPLC-MS/MS ANALYSIS

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INTRODUCTION

In recent years, food safety laboratories have adopted new and simplified sample preparation methods designed to reduce analysis time and related costs, as well as to increase throughput. For example, the QuEChERS methods for fruits and vegetables require only minutes for sample preparation and replace prior methods that took hours or days. In this study, this type of simplified sample preparation is applied to pesticide analysis in avocado, a fruit matrix of very high lipid content. A typical avocado contains 10 - 15 % fat and about 1 % total phospholipids. In the QuEChERS extraction, significant amounts of the fat and phospholipids are co-extracted along with the target pesticides. The presence of these co-extracted substances, particularly the phospholipids, can lead to chromatographic interference, contamination of the GC injector and column, contamination of the column and other components of the UPLC system and contamination of the mass spectrometer itself. To avoid these complications, a cleanup step is recommended prior to the instrumental analysis. This is typically performed using dispersive SPE with mixed sorbents, often with cumbersome multi-step centrifugation. In this study an Oasis PRiME HLB cartridge was used for a simple pass-thru cleanup to effectively remove fats and phospholipids. This method was applied to a number of pesticides registered for use on avocado in various world markets (see Tables 1 and 2). Other compounds are still under investigation. Updated results for this study will be presented in a poster submitted for presentation at the AOAC meeting in Dallas (September 2016).

SAMPLE PREPARATION

AOAC QuEChERS Extraction. Because avocado is so high in fat, the AOAC QuEChERS method is modified to reduce the sample size. Weigh 5 g sample into a 50 mL centrifuge tube (for a spiked sample, add the required volume of spiking standard solution). Add 5 mL water and 15 mL 99:1 acetonitrile/acetic acid. Vortex for 30 s and shake well for 2 minutes. Add QuEChERS salts (contents of DisQuE pouch for AOAC, pn 186006812). Shake the tube vigorously by hand for 1 min and centrifuge at approximately 2500 rcf for 5 min. An aliquot of the supernatant extract (top layer) is taken for analysis.

Pass-Thru SPE Cleanup Install an Oasis PRiME HLB cartridge (3 cc, 60 mg, pn 186008056) on a vacuum manifold. Set to minimal vacuum (~2 in Hg). Pass 0.4 mL of the QuEChERS extract through the cartridge to waste. Install collection vessels. Pass 0.6 mL of the QuEChERS extract and collect. For UPLC-MS analysis 100 µL of the extract is diluted to 400 µL with mobile phase A. For APGC-MS analysis 200 µL extract is taken and analyzed directly. Alternatively, a portion of the extract can be evaporated and reconstituted in toluene for splitless GC injection.

AOAC QuEChERS Extraction

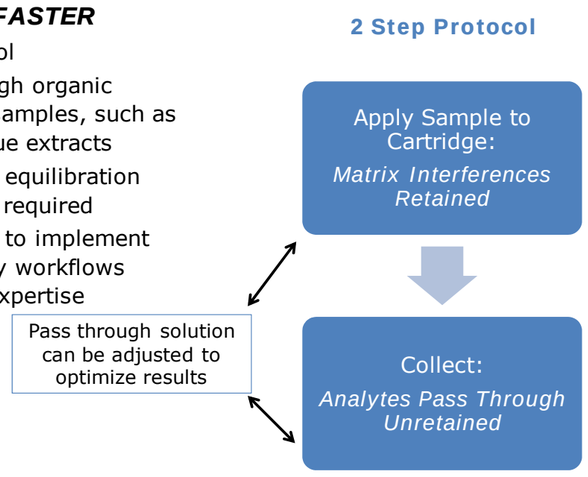
It is important to distinguish any recovery losses resulting from the SPE cleanup from losses resulting from the initial QuEChERS extraction. Therefore, the modified QuEChERS procedure was evaluated for recovery of the target compounds prior to any SPE recovery experiments. All compounds (spiked at 40 µg/kg) were recovered at greater than 80 % with the exception of etofenprox (60 %), novaluron (70 %), folpet (70 %) and pyriproxifen (75 %).

SPE cleanup recovery data (see Figures 1 and 2) were determined using blank avocado samples obtained using the modified QuEChERS protocol. Blank extracts were spiked at the 9 and 40 µg/kg (ppb) levels and were subjected to the pass-through SPE cleanup protocol. Response for each compound was compared with response obtained from identical blank sample extracts spiked after the SPE cleanup.

Oasis PRiME HLB Cartridge

SIMPLER and FASTER

- 2 Step Protocol
  - Ideal for high organic containing samples, such as food or tissue extracts
- Condition and equilibration steps are **not** required
- Fast and easy to implement into laboratory workflows without SPE expertise



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

Other instrument and calibration parameters are presented in Table 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

Other instrument and calibration parameters are presented in Table 2.

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 > 286.0	18	17	
Etofenprox	394.3 > 177.0	26	15	11.8
	394.3 > 106.9	26	43	
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 > 141.0	36	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	

Table 1. MRM transitions (primary transition first), instrument parameters, and observed retention times

Compound	MRM	Cone (V)	Collision (eV)	RT (min)
Azoxystrobin	403.0 > 344.2	20	12	20.9
	403.0 > 329.1	20	32	
CarfentrazoneEthyl	410.9 > 312.2	20	20	16.1
	410.9 > 340.2	20	10	
Chlorothalonil	265.9 > 170.0	20	27	12.0
	265.9 > 230.9	20	27	
Cypermethrin	163.1 > 127.0	20	10	19.3*
	163.1 > 127.0	20	10	
λ-Cyhalothrin	449.0 > 181.2	20	20	17.8*
	449.0 > 197.3	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	

Table 2. MRM transitions (primary transition first), instrument parameters, and observed retention times (RT) for APGC-MS compounds

RESULTS

UPLC-MS results are presented in Figure 1; APGC-MS results are presented in Figure 2. Only folpet, a thermal and pH labile substance similar to captan, showed recovery losses greater than 20 % resulting from the cleanup protocol. Figure 3 shows that the Oasis PRiME HLB cartridge cleanup removed greater than 95% of phospholipids from the avocado QuEChERS extract. Also, greater than 90 % of chlorophyll and approximately 80 % of fat was removed in the cleanup.

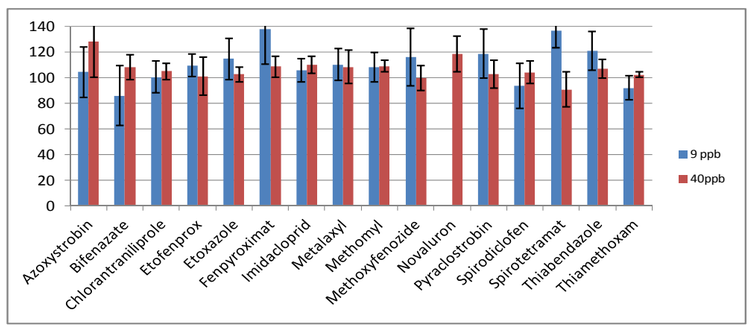


Figure 1. SPE cleanup recovery results for the UPLC-MS compounds

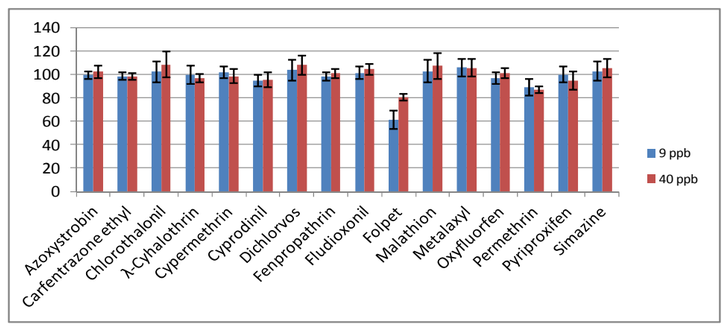


Figure 2. SPE cleanup recovery results for the APGC-MS compounds

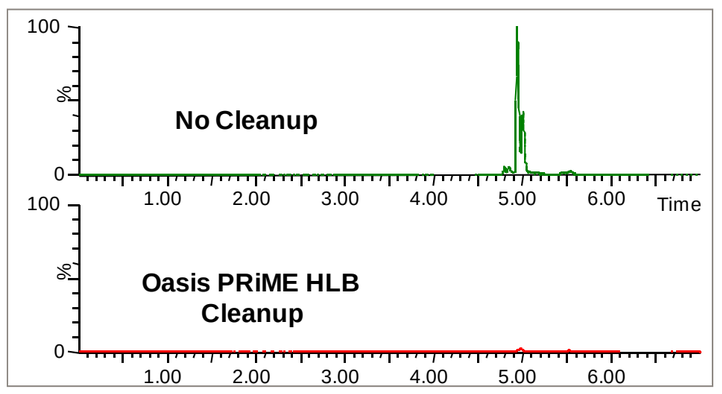


Figure 3. Effective removal of phospholipids from avocado QuEChERS extract with Oasis PRiME HLB clean-up

CONCLUSIONS

- **The Oasis PRiME pass-through cleanup was effective for removal of fats and phospholipids**
- **Cleanup was comparable to that obtained from cumbersome multi-step dispersive SPE**
- **Recoveries were comparable to those obtained from multi-step dispersive SPE**

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