Sample Cleanup Methods for Multiresidue Pesticide GC/MS/MS Analysis in Avocado

A Comparison of GPC and EMR—Lipid Methods

Application Note

Food Testing and Agriculture

Authors

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Abstract

This application note compares two sample cleanup methods after QuEChERS partitioning/extraction. The extraction of the pesticides was carried out using the Agilent Bond Elut QuEChERS extraction kit AOAC method. The two cleanup methods evaluated were Gel Permeation Chromatography (GPC) and Enhanced Matrix Removal—Lipid (EMR—Lipid). GPC is a well known method for removing matrix interferences, and outperforms traditional dispersive SPE (dSPE) with C18, which is intended for fatty samples. GPC is a very time-consuming preparation method requiring copious amounts of solvent and specialized equipment. Previously, GPC was the only acceptable option for the cleanup of high-lipid samples. Agilent Bond Elut EMR—Lipid is the next generation of sample preparation products. EMR—Lipid has exceptional lipid removal capacity, and uses simple steps such as the dSPE process without impacting analyte recovery. This work demonstrates that EMR—Lipid dSPE is a faster and simpler cleanup option for samples versus a GPC method.

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Introduction

Pesticide residue analysis in food commodities is routine for many laboratories, and the QuEChERS extraction/cleanup technique is widely used. QuEChERS was developed for general fruits and vegetables that have high water content and low fat content [1]. Removal of lipid interferences from complicated matrices is especially important for QuEChERS, where large amounts of matrix are extracted with the target analytes. [2]

The most common dispersive SPE (dSPE) used for QuEChERS cleanup is PSA/C18, which removes organic acids, sugars, lipids, and sterols. However, using this type of dispersive for lipid removal in high fat samples is not efficient [2]. This results in more instrument maintenance, which takes time and increases analysis costs. For this reason, Gel Permeation Chromatography (GPC) is the chosen method, although it takes extra time and expense.

Avocado, a difficult matrix due to its high lipid content (15 to 20 %), was selected as a representative sample for the evaluation of Enhanced Matrix Removal—Lipid (EMR—Lipid) and GPC. The comparison with other cleanup techniques was investigated in application note 5991-6097EN "Multiresidue Analysis of Pesticides in Avocado with Agilent Bond Elut EMR—Lipid by GC/MS/MS" [2].

GPC is a type of size exclusion chromatography (SEC) that separates analytes based on size. The procedure uses organic solvents and a porous hydrophobic gel (primarily a crosslinked divinylbenzene-styrene copolymer) that readily separates large molecular weight molecules from the smaller molecular weight analytes of interest. GPC cleanup is recommended for the removal of lipids, polymers, copolymers, proteins, natural resins, cellular components, and other high molecular weight compounds from a sample extract. [3]

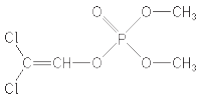
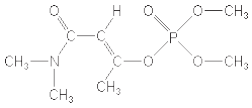
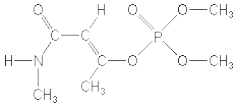
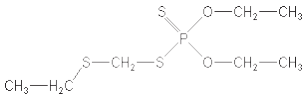
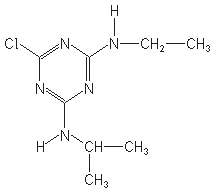
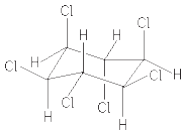
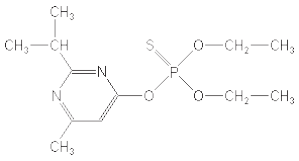
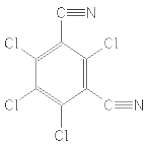
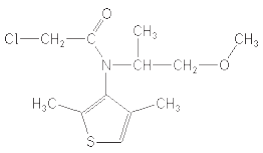
A GPC/SEC instrument consists of:

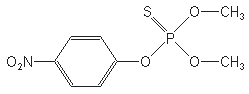
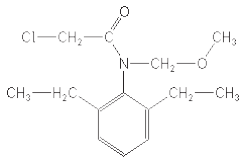
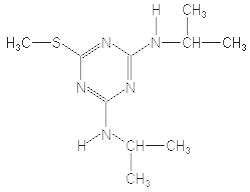
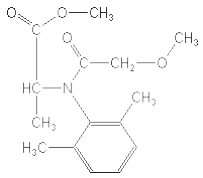
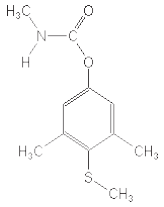
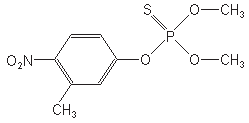
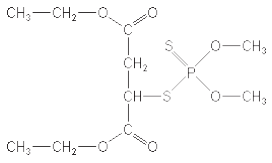
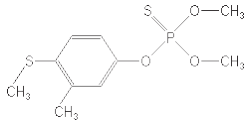
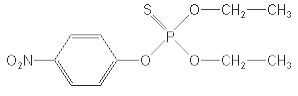
- A pump to push the solvent through the instrument
- An injection port to introduce the test sample onto the column
- A column to hold the stationary phase
- One or more detectors to detect the components as they leave the column
- Software to control the different parts of the instrument, and to calculate and display the results [4]

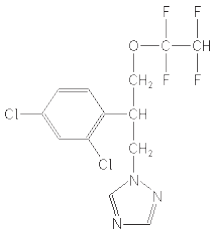
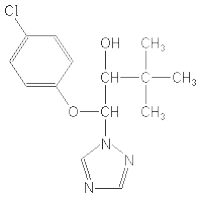
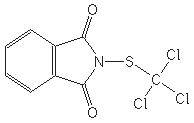
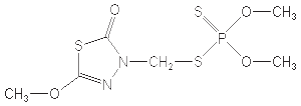
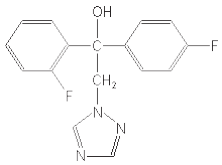
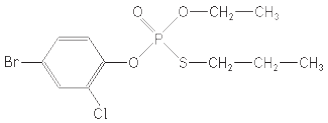
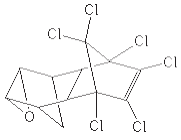
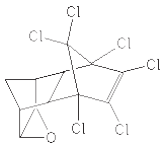
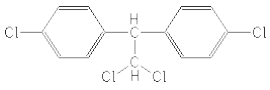
Agilent Bond Elut EMR—Lipid is a novel sorbent material that selectively removes major lipid classes from the sample extract without unwanted analyte loss.

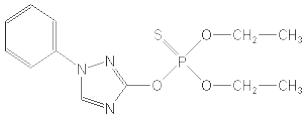
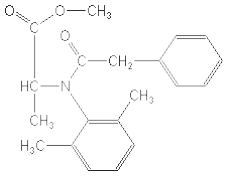
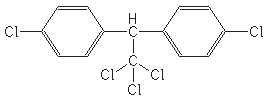
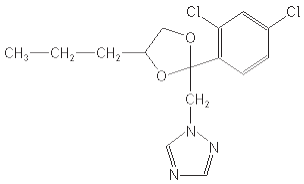
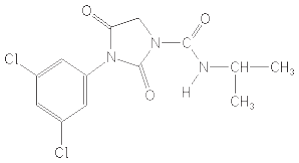
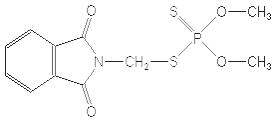
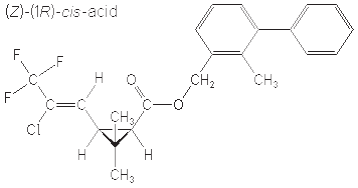
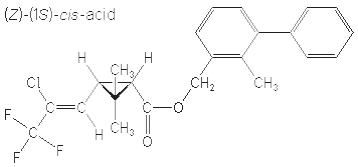
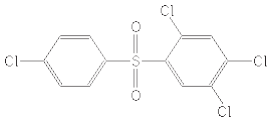
This study investigates the differences in results between these two sample cleanup methods (EMR—Lipid and GPC) in terms of recovery and reproducibility. The samples were spiked with 5 ppb, 50 ppb, and 300 ppb concentrations with six replicates each level. The evaluated analytes were a mixture of 38 pesticides from 12 different categories, listed in Table 1. The extract produced following the QuEChERS extraction/partitioning step was divided into two samples to be used for GPC and EMR—Lipid sample cleanup methods, enabling an evaluation of the differences in recovery and reproducibility of each method.

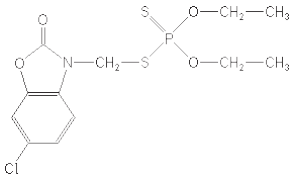
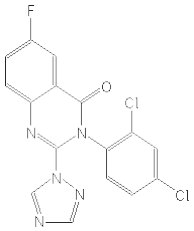
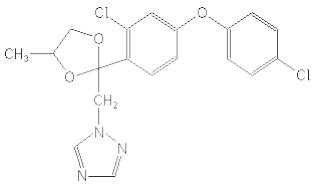
Table 1. Target Analytes, Class, Log P, Water Solubility, Molecular Formula, and Chemical Structure [5]

No.	Name	Category	Log P	Solubility in water (mg/L)	Molecular formula	Structure
1	Dichlorvos	Organophosphate	1.9	10,000	$C_4H_7Cl_2O_4P$	
2	Dicrotofos (Dicrotophos)	Organophosphate	–0.5	1,000,000	$C_8H_{16}NO_5P$	
3	Monocrotophos	Organophosphate	–0.22	818,000	$C_7H_{14}NO_5P$	
4	Phorate	Organothiophosphate	3.92	50	$C_7H_{17}O_2PS_3$	
5	Atrazine	Triazine	2.5	30	$C_8H_{14}ClN_5$	
6	BHC-*gamma* (Lindane, *gamma* HCH)	Organochlorine	3.5	8.52	$C_6H_6Cl_6$	
7	Diazinon	Organothiophosphate	3.3	40	$C_{12}H_{21}N_2O_3PS$	
8	Chlorothalonil	Chloronitrile	2.92	0.6	$C_8Cl_4N_2$	
9	Dimethenamid	Amide	2.15	1,200	$C_{12}H_{18}ClNO_2S$	

No.	Name	Category	Log P	Solubility in water (mg/L)	Molecular formula	Structure
10	Parathion-methyl	Organophosphate	3	55	$C_8H_{10}NO_5PS$	
11	Alachlor	Amide	3.09	240	$C_{14}H_{20}ClNO_2$	
12	Prometryn	Triazine	3.1	33	$C_{10}H_{19}N_5S$	
13	Metalaxyl	Anilide	1.75	7100	$C_{15}H_{21}NO_4$	
14	Methiocarb	Carbamate	3.08	27	$C_{11}H_{15}NO_2S$	
15	Fenitrothion	Phenyl Organothiophosphate	3.43	1	$C_9H_{12}NO_5PS$	
16	Malathion	Phenyl Organothiophosphate	2.75	145	$C_{10}H_{19}O_6PS_2$	
17	Fenthion	Phenyl Organothiophosphate	4.84	4.2	$C_{10}H_{15}O_3PS_2$	
18	Parathion	Phenyl Organothiophosphate	3.83	11	$C_{10}H_{14}NO_5PS$	

No.	Name	Category	Log P	Solubility in water (mg/L)	Molecular formula	Structure
19	Tetraconazole	Conazole	3.56	150	$C_{13}H_{11}Cl_2F_4N_3O$	
20	Triadimenol	Conazole	3.08	62	$C_{14}H_{18}ClN_3O_2$	
21	Folpet	Phthalimide	3.11	1	$C_9H_4Cl_3NO_2S$	
22	Methidathion	Thiadiazole Organothiophosphate	2.57	250	$C_6H_{11}N_2O_4PS_3$	
23	Flutriafol	Conazole	2.3	130	$C_{16}H_{13}F_2N_3O$	
24	Profenofos	Phenyl Organothiophosphate	4.44	20	$C_{11}H_{15}BrClO_3PS$	
25	Dieldrin	Cyclodiene	3.7	0.14	$C_{12}H_8Cl_6O$	
26	Endrin	Organochlorine	3.2	0.24	$C_{12}H_8Cl_6O$	
27	DDD-p,p'	Organochlorine	5.39		$C_{14}H_{10}Cl_4$	

No.	Name	Category	Log P	Solubility in water (mg/L)	Molecular formula	Structure
28	Triazophos	Organothiophosphate	3.55	40	$C_{12}H_{16}N_3O_3PS$	
29	Benalaxyl	Anilide	3.54	37	$C_{20}H_{23}NO_3$	
30	DDT-p,p′	Organochlorine	6.91	0.006	$C_{14}H_9Cl_5$	
31	Propiconazole I	Conazole	3.72	110	$C_{15}H_{17}Cl_2N_3O_2$	
32	Iprodione	Dicarboximide	3	13	$C_{13}H_{13}Cl_2N_3O_3$	
33	Phosmet	Organothiophosphate	2.95	22	$C_{11}H_{12}NO_4PS_2$	
34	Bifenthrin	Pyrethroid	6.6	0.1	$C_{23}H_{22}ClF_3O_2$	(*Z*)-(1*R*)-*cis*-acid  (*Z*)-(1*S*)-*cis*-acid 
35	Tetradifon	Diphenyl	4.61	0.05	$C_{12}H_6Cl_4O_2S$	

No.	Name	Category	Log P	Solubility in water (mg/L)	Molecular formula	Structure
36	Phosalone	Organothiophosphate	4.01	20	$C_{12}H_{15}ClNO_4PS_2$	
37	Fluquinconazole	Conazole	3.24	1.15	$C_{16}H_8Cl_2FN_5O$	
38	Difenoconazole I	Conazole	4.25	3.3	$C_{19}H_{17}Cl_2N_3O_3$	

Materials and Methods

Acetonitrile, isooctane, and acetone were pesticide-residue grade. Standards from AccuStandard, approximately 99 % pure, were used to prepare stock solutions at 1,000 ng/µL, and working solutions that varied in concentration.

The QuEChERS extraction was performed using the Agilent QuEChERS Extraction Kit for the AOAC 2007.01 method (p/n 5982-5755CH). In this method, 15 g of homogenized avocado sample was extracted using premixed packets of 6 g of $MgSO_4$ and 1.5 g of sodium acetate. After the extraction, the extract was divided into two aliquots. For one of these aliquots, the cleanup step was performed using Agilent Bond Elut EMR—Lipid tubes (p/n 5982–1010) followed by Agilent Bond Elut Final Polish for EMR—Lipid tubes (p/n 5982–0101). For the second aliquot, the GPC technique was used for sample cleanup.

GPC cleanup was achieved using a Gilson (Middleton, WI) Automated GX-271 GPC Cleanup System equipped with a glass column model SR25 filled with polystyrene Bio-Beads S-X3 Beads (200–100 mesh, Bio-Rad, California, USA). The preparation of this column usually takes two days, and it lasts about 100 sample runs depending on the type of the sample. The elution time is checked with standards before the sample analysis. The system uses a 5 mL sample loop at a flow rate of 5 mL/min with ethyl acetate/cyclohexane (1:1) as mobile phase using TRILUTION LC software. An overfill technique was required to fill the loop completely. Therefore, 7 mL of extract was used to fill the 5-mL loop in the GPC system. The first 18 minutes (90 mL) in the run was sent to waste, and the next 24 minutes (120 mL) of elution was collected. The last 4 minutes (20 mL) cleaned the system. The collected eluate was concentrated using a rotary evaporator to a final volume of 1 mL. A gentle nitrogen flow was used to evaporate the remaining solvent, and the sample was then reconstituted with acetonitrile. Normally, ethyl acetate is the solvent used in this step for injection into the GC system, however the same solvent as in EMR—Lipid extraction was used here for comparison purposes.

For the EMR—Lipid cleaning step, 5 mL of water was added into a preweighed 1 g EMR—Lipid 15 mL centrifuge tube, which was then vortexed. Next, 5 mL of the extract was transferred to the tube, which was then shaken for 1 minute and centrifuged for 5 minutes at 5,000 rpm. An aliquot of 5 mL of supernatant was transferred to a 15 mL EMR—Lipid polish tube containing 2 g of salts (1:4 NaCl:$MgSO_4$), and vortexed for 1 minute. The tube was centrifuged for 5 minutes at 5,000 rpm, and the upper layer was transferred to an autosampler vial. Finally, 1 µL was injected into a GC/MS/MS Triple Quadrupole System for analysis.

Figure 1 shows the steps taken during this analysis.

The GC/MS/MS system was configured according to the Agilent Pesticide Analyzer 412 configuration, using a 2 × 15 m analytical column with midcolumn backflush [6].

Instrumental

GC conditions	
Column	Agilent J&W HP-5ms Ultra Inert, 15 m × 0.25 mm, 0.25 µm (p/n 19091S-431UI) 2 units
Inlet	Split/splitless
Inlet liner	Splitless, single taper, Ultra Inert liner with glass wool (p/n 5190–3167)
Carrier	Helium
Inlet flow (column 1)	1 mL/min (constant flow mode) during run, 2 psi during backflush
PUU flow (column 2)	column 1 flow + 0.2 mL/min
Inlet temperature	280 °C
Injection volume	1 µL
Purge flow to split vent	30 mL/min at 0.75 minutes
Gas saver	On (20 mL/min at 2.0 minutes)
Oven temperature	60 °C (1 minute), 40 °C/min to 170 °C (0 minutes), 10 °C/min to 310 °C (0 minutes), 16 °C/min to 280 °C (3 minutes)
Total run	20.75 minutes
Capillary flow technology	Agilent Purged Ultimate Union (p/n G3186) used for backflushing the column
Retention time locking	Chlorpyrifos-methyl locked at 9.143 minutes
GC	Agilent 7890A series (G3440A)
Autosampler	Agilent 7693A injector and sample tray
MS conditions	
Spectrometer	Agilent 7000B Triple Quadrupole GC/MS System
Mode	Electron Impact
Transfer line temperature	280 °C
Solvent delay	2.3 minutes
Source temperature	300 °C
Quadrupole temperature	Q1 and Q2 = 180 °C

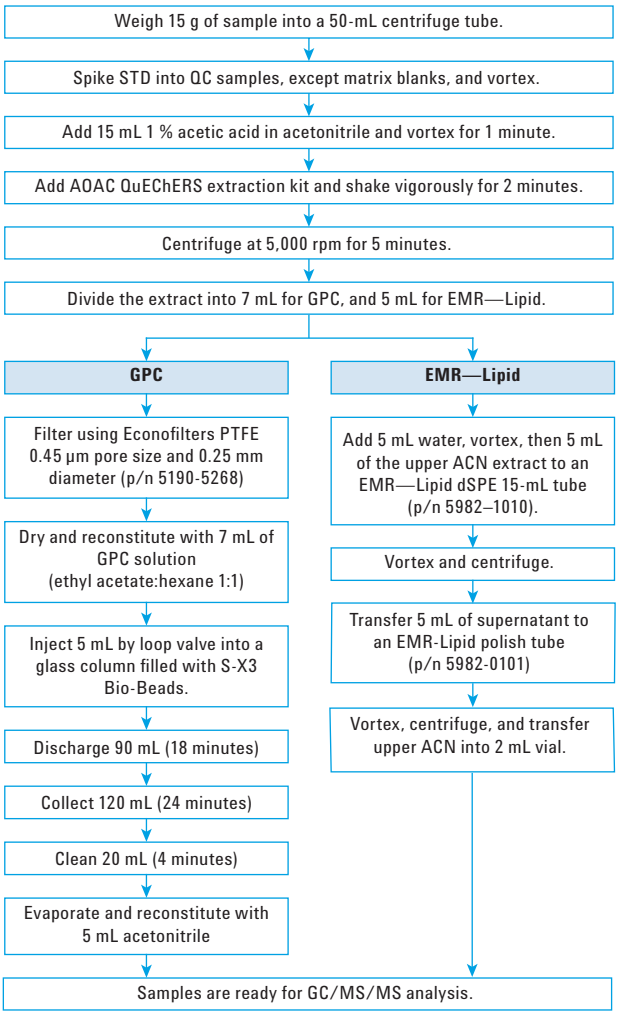


Figure 1. Sample preparation workflow showing QuEChERS extraction and GPC/EMR-Lipid cleanup.

MRMs of the compounds were selected based on the Agilent Pesticide and Environmental Pollutants MRM Database (G9250AA) and Agilent Pesticide Analysis Reference Guide [7].

Table 2. GC/MS/MS MRM Conditions and Retention Time for Pesticide Analysis

					MRMs			
	Analyte	**RT (min)**	**Quant channel**	**CE (V)**	**Qual channel**	**CE (V)**	**Qual channel**	**CE(v)**
1	Dichlorvos	4.74	184.9 → 93.0	5	144.9 → 109.0	10		
2	Dicrotophos	7.31	127.0 → 109.0	15	127.0 → 95.0	15	193.0 → 127.1	5
3	Monocrotophos	7.42	127.1 → 109.0	10	127.1 → 95.0	15	97.0 → 82.0	10
4	Phorate	7.52	121.0 → 47.0	15	128.9 → 65.0	15		
5	Atrazine	7.91	214.9 → 58.1	10	214.9 → 200.2	5	200.0 → 122.1	5
6	Lindane	8.16	216.9 → 181.0	5	181.0 → 145.0	15	218.9 → 183.1	5
7	Diazinon	8.30	137.1 → 84.0	10	137.1 → 54.0	20	199.1 → 93.0	15
8	Chlorothalonil	8.60	263.8 → 168.0	25	263.8 → 229.0	20	265.8 → 231.0	20
9	Dimethenamid	9.03	230.0 → 154.1	10	154.1 → 111.1	10	232.0 → 154.1	10
10	Parathion-methyl	9.15	262.9 → 109.0	10	125.0 → 79.0	10	125.0 → 47.0	10
11	Alachlor	9.27	188.1 → 160.2	10	188.1 → 132.1	15	160.0 → 132.1	10
12	Prometryn	9.31	226.0 → 184.2	10	199.0 → 184.1	5	241.0 → 184.2	10
13	Metalaxyl	9.34	234.0 → 146.1	20	234.0 → 174.1	10	220.0 → 192.1	5
14	Methiocarb	9.59	168.0 → 153.1	10	168.0 → 109.1	15	153.0 → 109.1	5
15	Fenitrothion	9.60	125.1 → 47.0	15	125.1 → 79.0	5	277.0 → 260.1	5
16	Malathion	9.74	126.9 → 99.0	5	157.8 → 125.0	5	172.9 → 99.0	15
17	Fenthion	9.94	124.9 → 47.0	10	124.9 → 79.0	5		
18	Parathion	9.97	138.9 → 109.0	5	138.9 → 81.0	15	290.9 → 109.0	10
19	Tetraconazole	10.07	336.0 → 217.9	20	336.0 → 203.8	30	170.9 → 136.0	10
20	Triadimenol	10.74	168.0 → 70.0	10	128.0 → 100.0	25	128.0 → 65.0	25
21	Folpet	10.85	259.8 → 130.1	15	259.8 → 232.0	5	261.8 → 130.1	15
22	Methidathion	11.00	144.9 → 85.0	5	144.9 → 58.1	15	85.0 → 58.0	5
23	Flutriafol	11.32	123.1 → 95.0	15	123.1 → 75.1	25	219.1 → 123.1	15
24	Profenofos	11.58	207.9 → 63.0	30	296.8 → 268.7	5	338.8 → 268.7	15
25	Dieldrin	11.73	262.9 → 193.0	35	262.9 → 191.0	35	277.0 → 241.0	5
26	Endrin	12.12	262.8 → 193.0	35	244.8 → 173.0	30	316.7 → 280.8	5
27	DDD-*p,p´*	12.37	234.9 → 165.1	20	234.9 → 199.1	15	236.9 → 165.2	20
28	Triazophos	12.65	161.2 → 134.2	5	161.2 → 106.1	10	161.2 → 91.0	15
29	Benalaxyl	12.87	148.0 → 77.0	35	148.0 → 105.1	20	266.0 → 148.1	5
30	DDT-*p,p´*	13.03	235.0 → 165.2	20	235.0 → 199.2	15	237.0 → 165.2	20
31	Propiconazole I	12.94	172.9 → 145.0	15	172.9 → 74.0	45	172.9 → 109.0	30
32	Iprodione	13.72	313.8 → 55.9	20	187.0 → 124.0	25	243.9 → 187.0	5
33	Phosmet	13.91	160.0 → 77.1	20	160.0 → 133.1	10	160.0 → 105.0	15
34	Bifenthrin	13.92	181.2 → 165.2	25	181.2 → 166.2	10	166.2 → 165.2	20
35	Tetradifon	14.42	158.9 → 111.0	10	226.9 → 199.0	15		
36	Phosalone	14.60	182.0 → 111.0	15	182.0 → 102.1	15	182.0 → 75.1	30
37	Fluquinconazole	15.87	340.0 → 107.8	40	340.0 → 298.0	15	108.0 → 57.0	15
38	Difenoconazole I	17.82	322.8 → 264.8	15	264.9 → 202.0	20	324.8 → 266.8	15

Results and Discussion

Two varieties were purchased from supermarkets: one was smaller and dark in color (variety 1), and the other was larger and light in color (variety 2). The third variety was collected from a tree grown in a square close to the laboratory (variety 3).

Both blank extracts of variety 1 were analyzed in GC/MS full-scan mode to see the capacity of matrix removal by each method. Figure 2 is the chromatogram overlay for the final extract after GPC and EMR—Lipid cleanup. It can be verified that the EMR—Lipid extract has lower matrix peaks compared to the GPC technique by calculating the total area before and after the cleanup step, shown in Equation 1.

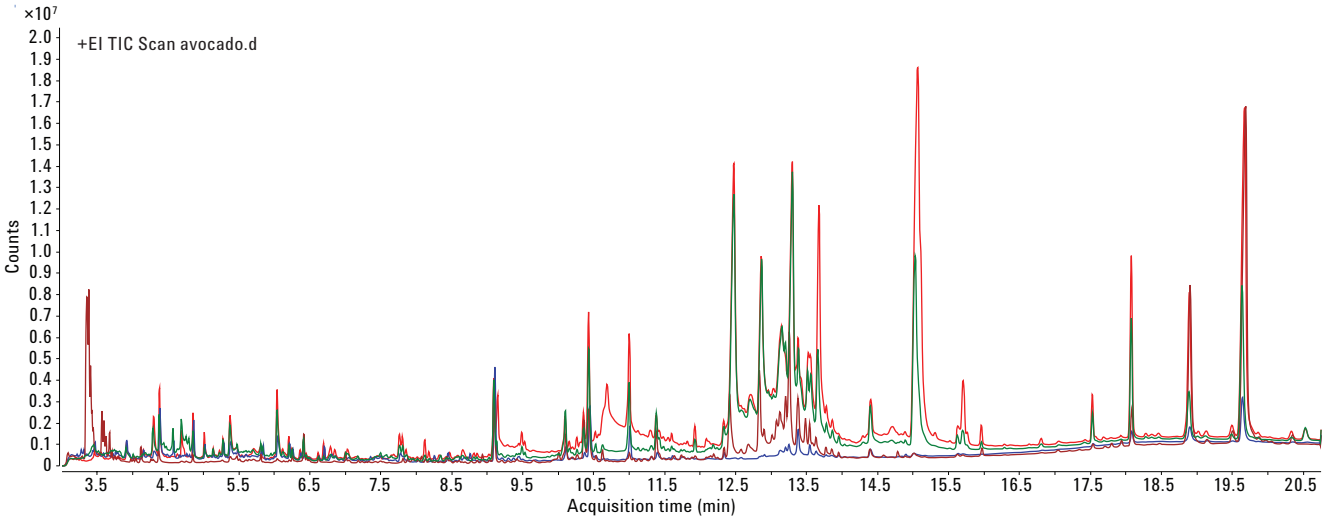


Figure 2. GC/MS full-scan chromatogram overlay of avocado (variety 1) matrix blanks prepared by a QuEChERS AOAC extraction without cleanup (red), dSPE PSA/C18 cleanup (green), GPC cleanup (brown), and EMR—Lipid cleanup (blue).

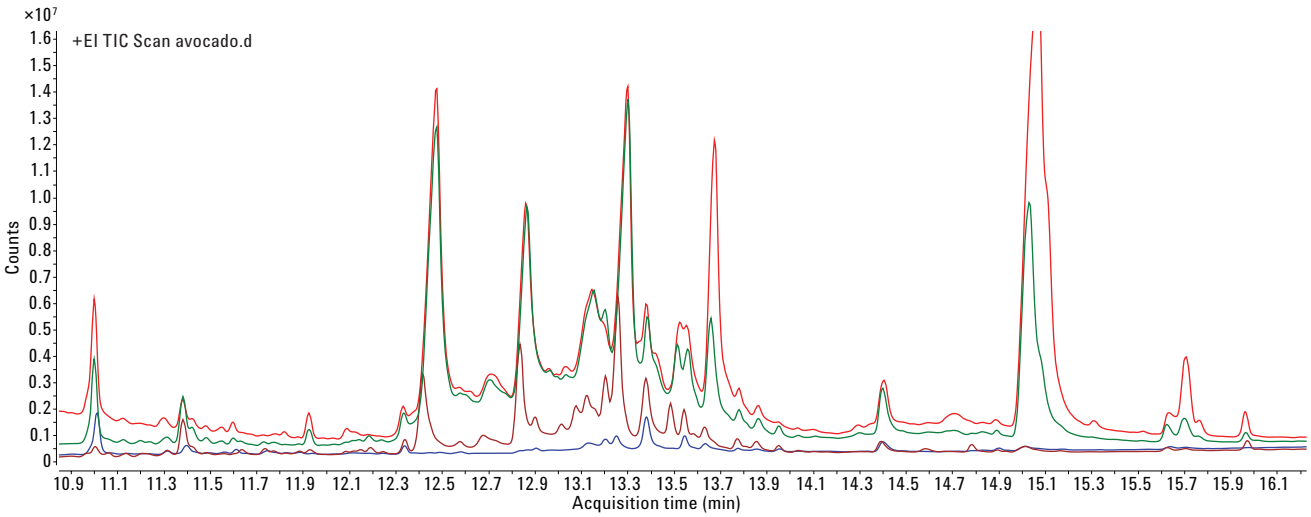


Figure 3. Zooming in on the 11 to 16 minutes region. GC/MS full-scan chromatogram overlay of avocado matrix blanks prepared by a QuEChERS AOAC extraction without cleanup (red), dSPE PSA/C18 cleanup (green), GPC cleanup (brown), and EMR—Lipid cleanup (blue).

$$\% \text{ Matrix removal} = \frac{\text{Total peak area of sample without cleanup} - \text{Total peak area of sample with EMR/GPC cleanup}}{\text{Total peak area of sample without cleanup}} \times 100$$

Equation 1.

The percentage of matrix removal for EMR—Lipid was approximately 77 %, and GPC was approximately 65 %. Figure 2 shows an overlay of the GC/MS full scan without cleanup, and with GPC, EMR—Lipid, and PSA/C18 cleanup. The PSA/C18 dSPE cleanup step was performed to show the full scan chromatogram compared with GPC and EMR—Lipid techniques. The recovery study for the PSA/C18 dSPE method was previously investigated in application note 5991-6097EN [2]. Matrix contents vary among avocado varieties, therefore, the percentage of matrix removal depends on the avocado used in the study. Calculating the percentage of matrix removal from variety 3, approximately 65 % of the matrix was removed after EMR—Lipid cleanup, and 38 % for GPC. Figure 4 shows the GC/MS full scan of different varieties of avocado extracted. The overlay of the three chromatograms demonstrates that matrix content differs significantly among varieties.

The discharge time relative to collection time in GPC can be challenging, with various varieties where the collection time must be determined to minimize analyte loss and optimize lipid removal. To avoid the loss of first-eluted analytes, some lipid is usually found in the final extract.

Among avocado varieties, the lipid content varies between 5.3 to 31.1 %, and the average is approximately 16 %. The composition of fatty acids also varies greatly depending on variety [8]. Therefore, the cleanness of the extract depends on its overall lipid content.

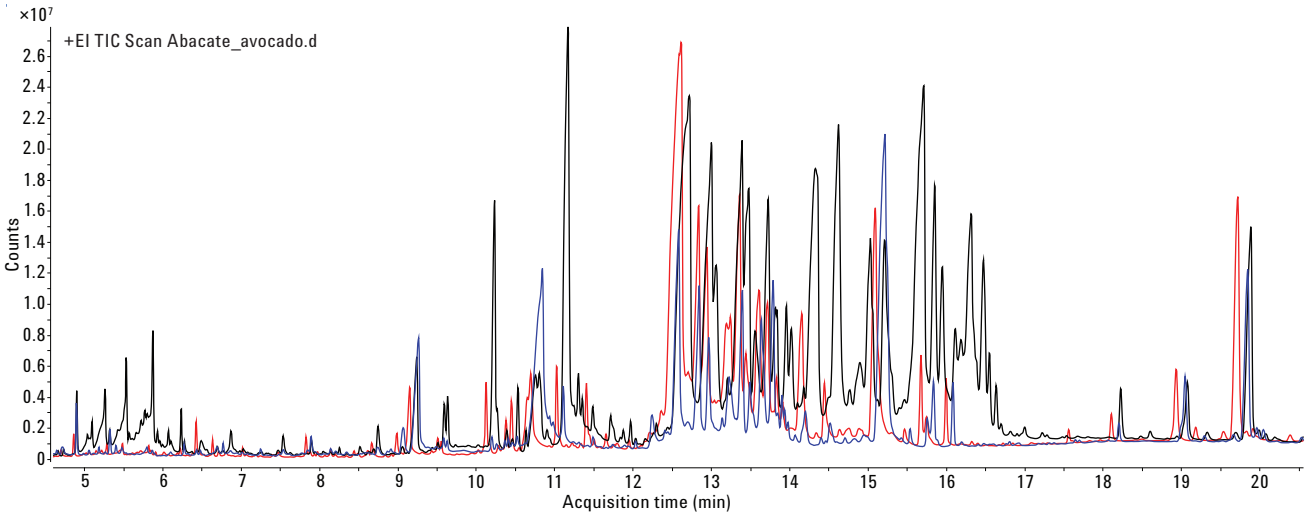


Figure 4. GC/MS full-scan chromatogram overlay of different varieties of avocado without cleanup step. Variety 1(blue), variety 2 (red), and variety 3 (black).

Performance evaluation

Avocado (variety 3) was fortified at three different levels. The recovery was calculated comparing the response area to the matrix-matched standard. The relative standard deviation (RSD) was calculated from six replicates. Table 3 shows the complete list of the results from this fortification.

Table 3. Quantitation Results for Pesticides in Avocado Spiked at 5, 50, and 300 ppb Levels

	Method accuracy and precision (ng/g QCs)											
	5 ppb				50 ppb				300 ppb			
	EMR—Lipid		GPC		EMR—Lipid		GPC		EMR—Lipid		GPC	
Analyte	**Recovery %**	**RSD**	**Recovery %**	**RSD**	**Recovery %**	**RSD**	**Recovery %**	**RSD**	**Recovery %**	**RSD**	**Recovery %**	**RSD**
Dichlorvos	90	9.7	90	12.4	91	6.2	94	5.8	107	5.3	88	16.0
Dicrotophos	–	–	–	–	74	19.6	98	9.7	92	9.1	107	9.6
Monocrotophos	–	–	–	–	77	12.3	96	9.0	89	17.9	104	9.9
Phorate	99	4.0	50	51.7	86	5.3	29	57.8	100	5.0	48	28.2
Atrazine	93	2.2	99	11.7	92	4.3	110	9.0	103	3.8	111	8.8
Lindane, *gamma* HCH	88	3.0	100	7.2	83	5.6	103	9.1	91	6.0	105	8.3
Diazinon	103	4.2	103	13.0	95	4.0	107	10.1	106	5.0	110	8.8
Chlorothalonil	57	15.8	52	20.4	80	17.0	91	18.2	85	15.2	81	28.5
Dimethenamid	95	2.9	97	8.9	95	4.5	106	10.1	102	3.8	107	8.7
Parathion-methyl	94	4.6	95	10.9	100	6.0	107	9.0	110	3.5	118	10
Alachlor	95	4.6	100	9.2	95	4.1	112	8.4	102	3.7	110	8.0
Prometryn	90	2.3	96	9.4	94	3.5	108	7.4	98	3.5	106	8.1
Metalaxyl	102	3.5	99	8.6	100	3.0	109	8.3	103	2.9	106	7.9
Methiocarb	65	17.1	33	34.5	81	18.0	87	18.8	97	12.8	97	10.4
Fenitrothion	115	2.8	120	9.6	100	3.8	109	8.5	109	4.3	119	10.2
Malathion	103	3.3	101	8.2	98	5.9	111	7.4	107	3.7	114	8.8
Fenthion	88	3.8	56	38.6	85	6.1	57	21.7	94	4.0	59	25.3
Parathion	107	3.5	111	14.0	99	3.9	108	10.9	105	3.5	119	10.7
Tetraconazole	92	2.7	87	15.6	102	2.7	107	8.3	103	2.2	100	9.0
Triadimenol	92	3.9	130	26.1	103	2.9	112	8.0	101	1.8	109	7.7
Folpet	56	20.2	102	30.8	79	17.4	99	15.1	76	23.1	88	20.9
Methidathion	81	10.7	99	8.1	90	9.5	112	6.7	100	4.1	115	8.6
Flutriafol	87	2.6	91	8.4	96	2.8	115	9.6	103	0.6	105	7.3
Profenofos	82	5.1	85	4.3	85	7.2	107	5.1	91	5.6	110	8.0
Dieldrin	82	10.5	91	7.5	77	4.3	96	8.9	80	5.9	97	8.1
Endrin	77	4.6	101	10.2	74	3.3	102	8.7	83	7.5	99	7.2
DDD-*p,p′*	75	7.7	87	9.4	76	3.3	102	7.2	75	7.2	103	9.5
Triazophos	90	3.2	101	5.8	96	7.7	113	6.8	102	2.6	116	7.8
Benalaxyl	–	–	–	–	97	6.4	86	6.7	100	2.6	108	6.4
DDT-*p,p′*	47	7.6	102	11.4	61	4.2	94	16.7	49	31.0	87	12.8
Propiconazole I	81	4.3	97	8.6	103	4.2	82	5.4	93	2.1	106	6.3
Iprodione	73	11.1	43	37.5	96	10.4	88	16.4	102	11.2	97	12.5
Phosmet	–	–	–	–	77	17.4	104	8.0	101	9.9	116	9.9
Bifenthrin	61	10.7	87	7.0	63	3.8	99	7.2	61	16.9	95	7.2
Tetradifon	73	5.5	87	7.9	81	3.1	103	6.7	79	3.9	101	6.3
Phosalone	79	5.6	82	14.8	90	8.0	106	4.5	94	4.3	113	8.1
Fluquinconazole	85	6.1	88	3.3	96	5.0	106	6.0	99	2.6	107	6.9
Difenoconazole I	80	4.4	97	10.3	88	7.0	112	7.8	99	2.7	120	8.9

Dicrotophos, monocrotophos, benalaxyl, and phosmet were not detected at the 5 ppb level. DDT-*p,p* and bifenthrin had lower recovery by EMR—Lipid than GPC. These two pesticides are highly lipophilic (high log P), and have increased solubility in ethyl acetate/cyclohexane.

Phorate and fenthion had lower recovery with GPC than with EMR—Lipid. The recoveries of those compounds were more variable than other analytes in GPC, as they tend to degrade into sulfone and sulfoxide metabolites. Losses of phorate and fenthion were found to occur in ethyl acetate solution during the GPC step, especially when fat or other protecting components were not present [9].

Chlorothalonil and methiocarb showed fluctuation in injection at the 5 ppb concentration that could be improved with the use of an internal standard.

Thirty-six compounds had recoveries between 70–120 %, and RSDs < 20 % for both techniques at 50 ppb concentration. At 5 ppb, EMR—Lipid had better results for triadimenol and iprodione. Chlorothalonil had higher RSD for GPC at 300 ppb. Figure 5 shows the recovery and RSD results for all concentrations.

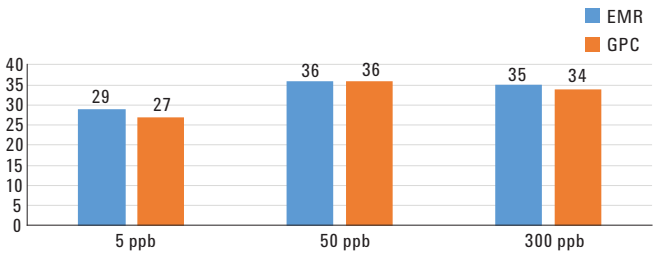


Figure 5. Recovery results for pesticide residue analysis (38) in avocado. Number of compounds with recovery between 70–120 %, and RSD < 20 %.

Saving time and solvent consumption

Handling and analyzing many samples in a short time period can be challenging, and requires a greater instrument capacity for sample preparation. Comparing the time for each method, the EMR—Lipid procedure was eight times faster than the GPC technique per sample for the analysis performed, as shown in Table 4. Moreover, several samples can be processed in batch with EMR—Lipid, while GPC requires one sample at a time in series. In addition to time spent, GPC consumes approximately 230 mL of solvent not including the conditioning and volume used for solvent exchange.

Table 4. Comparison Table of Time Spent in Both Techniques

EMR—Lipid		GPC	
Steps	**Time (min)**	**Steps**	**Time (min)**
Add 5 mL of H_2O and 5 mL of extract	2	Transfer extract to a rotary evaporator flask	1
Shake for 1 minute, and centrifuge for 5 minutes	6	Evaporate 7 mL of extract and reconstitute with GPC solvent	30
Transfer 5 mL to a Polish tube, shake for 1 minute, and centrifuge for 5 minutes	7	GPC cleaning: Discharge, collect, and clean	46
Transfer an aliquot of supernatant in a vial	1	Evaporate 120 mL of extract using rotary evaporator	40
Total	16		1 hour and 57 min

Conclusions

This study compares two cleanup techniques for samples containing approximately 20 % fat content. For routine analysis, high fat samples can be challenging and time-consuming. With traditional GPC techniques for sample cleanup of high-fat samples, there is an economic factor involving more solvent consumption and frequency of instrument maintenance. Although the GPC technique is efficient for removing the interference compounds, the ease and quick procedure of Agilent Bond Elut EMR—Lipid dSPE is a great choice for a fast, final extract cleaner, other than GPC.

Acknowledgement

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