Multiresidue Analysis of Pesticides in Avocado with Agilent Bond Elut EMR—Lipid by GC/MS/MS

Application Note

Food Testing and Agriculture

Authors

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Abstract

Agilent Bond Elut Enhanced Matrix Removal—Lipid (EMR—Lipid) is the next generation of sample preparation products, and is used in convenient, dispersive solid phase extraction (dSPE) for highly selective matrix removal without impacting analyte recovery, especially for high-fat samples. This study demonstrates the application of this novel product for the analysis of 23 GC-amenable pesticides in avocado by GC/MS/MS. The procedure involves a QuEChERS AOAC extraction followed by EMR—Lipid dSPE and polish salts. EMR—Lipid provides far superior matrix removal by weight, GC/MS full scan, and matrix effect determination when compared to C18/PSA and zirconia-based sorbents. Furthermore, less matrix is introduced into the analytical flow path. The data also demonstrate dramatically improved reproducibility for the analytes over 100 injections relative to C18/PSA and especially zirconia, which experience significant response deviations. EMR—Lipid is highly selective for lipids and does not negatively affect analyte recovery. Analyte recoveries are high and precision is outstanding. This work demonstrates that EMR—Lipid dSPE fits into a QuEChERS workflow and delivers fast, robust, and effective sample preparation with the most complete matrix removal available for multiresidue analysis of pesticides in avocado.

Agilent Technologies

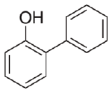
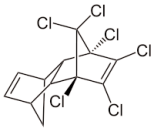
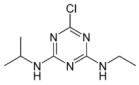
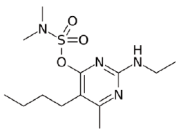
Introduction

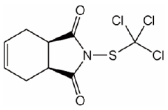
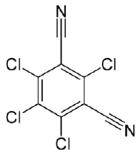
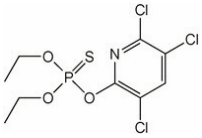
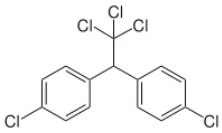
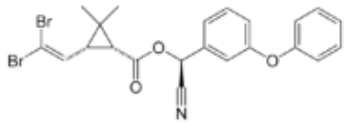
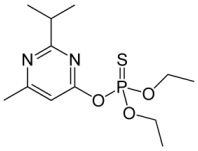
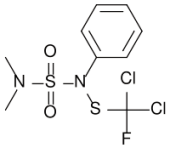
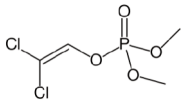
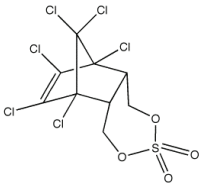
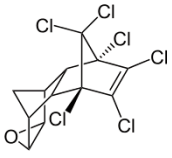
Pesticide residue analysis in food commodities is routine for many laboratories that use the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method [1,2]. This allows analysis of hundreds of pesticides at low concentrations with a single extraction. While the method has worked well for various fruits and vegetables, foods high in fat such as avocado, nuts, and foods of animal origin present new challenges [3,4]. Overcoming these challenges is a high priority for laboratories tasked with reaching the stringent validation criteria required by government agencies to ensure that food is safe for consumption.

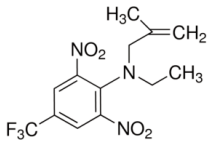
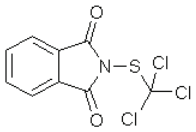
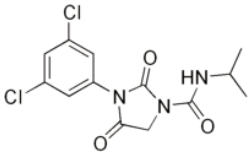
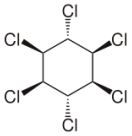
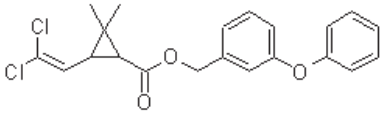
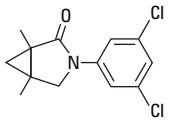
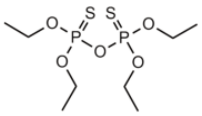
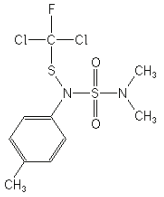
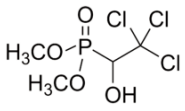
Analysis can use a combination of LC and GC to accommodate volatile, semivolatile and nonvolatile pesticides associated with many multiclass, multiresidue methods [4]. While many pesticides are amenable to both LC and GC, many are not. Each chromatographic technique has its inherent advantages and disadvantages in terms of analyte quantitation and adverse effects from coextracted matrix. Removal of these coextractives is essential to accurate quantitation in complex food matrices, requiring treatment with matrix removal sorbents such as C18, PSA, and GCB [5]. Other materials containing zirconia are commercially available, and generally improve lipid removal when compared to typical matrix removal sorbents. However, it does not target all lipid classes and can retain analytes of interest [6,7]. Samples high in lipid content may also require cleanup using solid phase extraction cartridges (SPE) [7,8,9] or gel permeation chromatography (GPC) [10], adding time and cost to an otherwise routine analysis.

Agilent Bond Elut EMR—Lipid is a novel sorbent material that selectively removes major lipid classes from the sample extract without unwanted analyte loss. Removal of lipid interferences from complicated matrices is especially important for QuEChERS, where large amounts of matrix are extracted with the target analytes. Avocado is known as a difficult matrix due to its high lipid content (15 to 20%), and was, therefore, selected as a representative sample for the evaluation of EMR—Lipid. This study investigates the sample preparation for the analysis of 23 GC-amenable pesticides in avocado using a QuEChERS AOAC extraction followed by EMR—Lipid dSPE and polishing salts. The pesticides are from 10 different classes to broaden the scope of the application (Table 1). This application note demonstrates the exceptional cleanliness that EMR—Lipid provides for complex, fatty sample such as avocado, and the high recovery and precision for 23 multiclass pesticide residues at three levels.

Table 1. Target analytes, class, log P, water solubility, and chemical structure [11].

Name	Category	Log P	Solubility in water (mg/L)	Molecular formula	Structure
2-Phenylphenol	Phenol	3.18	560	$C_{12}H_{10}O$	
Aldrin	Organochlorine	6.5	0.003	$C_{12}H_8Cl_6$	
Atrazine	Triazine	2.7	33	$C_8H_{14}ClN_5$	
Bupirimate	Pyrimidinol	2.2	22	$C_{13}H_{24}N_4O_3S$	

Name	Category	Log P	Solubility in water (mg/L)	Molecular formula	Structure
Captan	Phthalimide	2.5	5.1	$C_9H_8Cl_3NO_2S$	
Chlorothalonil	Chloronitrile	2.94	1.0	$C_8Cl_4N_2$	
Chlorpyrifos methyl	Organophosphate	4.0	2.74	$C_7H_7Cl_3NO_3PS$	
DDT	Organochlorine	6.91	0.006	$C_{14}H_9Cl_5$	
Deltamethrin	Pyrethroid	4.6	0.0002	$C_{22}H_{19}Br_2NO_3$	
Diazinon	Organophosphate	3.69	60	$C_{12}H_{21}N_2O_3PS$	
Dichlofluanid	Sulphamide	3.7	1.3	$C_9H_{11}Cl_2FN_2O_2S_2$	
Dichlorvos	Organophosphate	1.9	18,000	$C_4H_7Cl_2O_4P$	
Endosulfan sulfate	Organochlorine	3.13	0.48	$C_9H_6Cl_6O_3S$	
Endrin	Organochlorine	3.2	0.24	$C_{12}H_8Cl_6O$	

Name	Category	Log P	Solubility in water (mg/L)	Molecular formula	Structure
Ethalfluralin	Dinitroaniline	5.11	0.01	$C_{13}H_{14}F_3N_3O_4$	
Folpet	Phthalimide	3.02	0.8	$C_9H_4Cl_3NO_2S$	
Iprodione	Dicarboxamide	3.1	12.0	$C_{13}H_{13}Cl_2N_3O_3$	
Lindane	Organochlorine	3.5	8.52	$C_6H_6Cl_6$	
Permethrin	Pyrethroid	6.1	0.006	$C_{21}H_{20}Cl_2O_3$	
Procymidone	Dicarboxamide	3.3	2.46	$C_{13}H_{11}Cl_2NO_2$	
Sulfotep	Organophosphate	3.99	10	$C_8H_{20}O_5P_2S_2$	
Tolylfluanid	Sulphamide	3.9	0.9	$C_{10}H_{13}C_{l2}FN_2O_2S_2$	
Trichlorfon	Organophosphate	0.43	120,000	$C_4H_8Cl_3O_4P$	

Experimental

All regents and solvents were HPLC or analytical grade. Acetonitrile (ACN) and methanol were from Honeywell (Muskegon, MI, USA). Reagent grade acetic acid (AA), pesticide standards, and internal standard were purchased from Sigma-Aldrich, Corp. (St Louis, MO, USA).

Solution and standards

Acetic acid 1% in ACN was prepared by adding 10 mL acetic acid to 990 mL ACN. Standard and internal standard (IS) stock solutions were made in either ACN or methanol at 2.0 mg/mL. A combined working solution was prepared in ACN at 25 µg/mL, except for captan, folpet, trichlorfon, and bupirimate. Due to relatively low responses on the instrument, the concentration was made five times higher for those four compounds in the combined working solution, which was 125 µg/mL. A 25 µg/mL aliquot of combined IS working solution was prepared in ACN, including TPP, parathion ethyl d_{10}, and ^{13}C-DDT.

Equipment

Equipment and material used for sample preparation included:

- Geno/Grinder (SPEX, Metuchen, NJ, USA)
- Centra CL3R centrifuge (Thermo IEC, MA, USA)
- Eppendorf microcentrifuge (Brinkmann Instruments, Westbury, NY, USA)
- Vortexer and multitube vortexers (VWR, Radnor, PA, USA)
- Bottle top dispenser (VWR, So. Plainfield, NJ, USA)
- Eppendorf pipettes and repeater
- Agilent Bond Elut EMR—Lipid tubes (p/n 5982-1010) and Agilent Bond Elut Final Polish for Enhanced Matrix Removal—Lipid tubes (p/n 5982-0101)

Instrumentation

Analysis was completed on an Agilent 7890A GC equipped with an Agilent 7693B Autosampler and an Agilent 7000C Triple Quadrupole GC/MS system. Column backflushing was used, which is highly recommended for complex sample matrices [12]. The total run time for a sample spiked with standard was 23 minutes, with two minutes for column backflushing.

Instrument conditions

GC conditions

Autosampler:	Agilent 7693 Autosampler and sample tray 10 µL syringe (p/n G4513-80220), 1 µL injection volume Three post injection solvent A (acetonitrile) washes Three sample pumps Three post injection solvent B (isopropanol) washes
Column:	Agilent J&W DB-5ms Ultra Inert, 0.25 mm × 15 m, 0.25 µm (p/n 122-5512UI)
Carrier:	Helium, constant pressure
Gas filter:	Gas Clean carrier gas filter kit, 1/8 inch (p/n CP17974)
Inlet liner:	Agilent Ultra Inert single taper splitless liner with wool (p/n 5190-2293)
Inlet:	MMI inlet at pulsed cold splitless mode, 75 °C initially, hold for 0.02 min, then ramp to 350 °C at 750 °C/min
Injection pulse pressure:	36 psi until 0.75 min
Purge flow to split vent:	60 mL/min at 0.75 min
Inlet pressure:	17 psi during run, and 1.0 psi during backflushing
Oven:	60 °C for 2.57 min, then to 150 °C at 50 °C/min, to 200 °C at 6 °C/min, to 300 °C at 16 °C/min, hold for 3 min
Post run:	2 min at 300 °C
Capillary Flow Technology:	UltiMetal Plus Purged Ultimate Union (p/n G3182-61581) for backflushing the analytical column and inlet
Aux EPC gas:	Helium plumbed to Purged Ultimate Union
Bleed line:	0.0625 inch od × 0.010 inch id × 100 cm, 316SS tubing, on top of the oven
Aux pressure:	4 psi during run, 75 psi during backflushing
Connections:	Between inlet and Purged Ultimate Union
Restrictor:	Inert fused silica tubing, 0.65 m × 0.15 mm (p/n 160-7625-5)
Connections:	Between Purged Ultimate Union and the MSD

MSD conditions

MSD:	Agilent 7000C Triple Quadrupole GC/MS, inert, with performance electronics
Vacuum pump:	Performance turbo
Mode:	MRM
Tune file:	Atune.u
Transfer line temp:	280 °C
Source temp:	300 °C
Quad temp:	150 °C for Q1 and Q2
Solvent delay:	2.57 min
Collision gas flow:	He quench gas at 2.35 mL/min, N_2 collision gas at 1.5 mL/min
MS resolution:	MS1 and MS2 = 1.2u

The MRM parameters were easily optimized for each analyte using the Agilent Pesticides and Environmental Pollutants MRM Database (G9250AA), which contains MS/MS conditions and retention time information for over 1,070 compounds [13]. Table 2 lists the MRM transitions for the target analytes used in this study. An example of a typical GC/MS/MS chromatogram is shown in Figure 1 for the 23 pesticides under investigation.

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)**
Dichlorvos	4.70	184.9 → 93	10	109 → 79	5
Trichlorfon	5.94	110.8 → 47	30	81.8 → 47	50
2-Phenylphenol	6.39	169 → 115.1	25	170 → 141.1	25
Ethalfluralin	7.58	275.9 → 202.1	15	315.9 → 275.9	10
Sulfotep	7.83	237.8 → 145.9	10	201.8 → 145.9	10
Atrazine	8.69	214.9 → 58.1	10	214.9 → 200.2	5
Lindane	8.83	181 → 145	15	216.9 → 181	5
Chlorothalonil	9.20	263.8 → 168	25	265.8 → 231	20
Diazinon	9.22	137.1 → 54	20	199.1 → 93	20
Chlorpyriphos methyl	10.30	285.9 → 92.9	20	124.9 → 47	15
Dichlorfluanid	11.31	223.9 → 123.1	20	123 → 77	20
Aldrin	11.55	262.9 → 192.9	35	254.9 → 220	35
Parathion ethyl D_{10} (IS)	11.96	98.7 → 67	10	114.9 → 82.9	20
Tolylfluanid	12.80	136.9 → 91	20	136.9 → 65	30
Captan	12.96	151 → 79.1	15	149 → 79.1	10
Forpet	13.13	259.8 → 130.1	15	261.8 → 130.1	15
Procymidone	13.13	282.8 → 96	10	96 → 67.1	10
Bupirimate	15.44	272.9 → 193.1	15	272.9 → 108	5
Endrin	15.68	316.7 → 280.8	5	244.8 → 173	30
Endosulfan sulfate	17.44	273.9 → 238.9	15	271.9 → 237	15
^{13}C-DDT (IS)	17.69	246.5 → 177.1	15	248.5 → 177.1	15
DDT	17.69	235 → 165.2	20	237 → 165.2	20
TPP (IS)	18.20	325.9 → 169	30	325.9 → 233	27
Iprodione	18.82	313.8 → 55.9	20	187 → 124	25
Permethrin	20.68	183.1 → 153.1	15	183.1 → 153.1	15
Deltamethrin	22.51	252.9 → 93	15	181 → 152.1	25

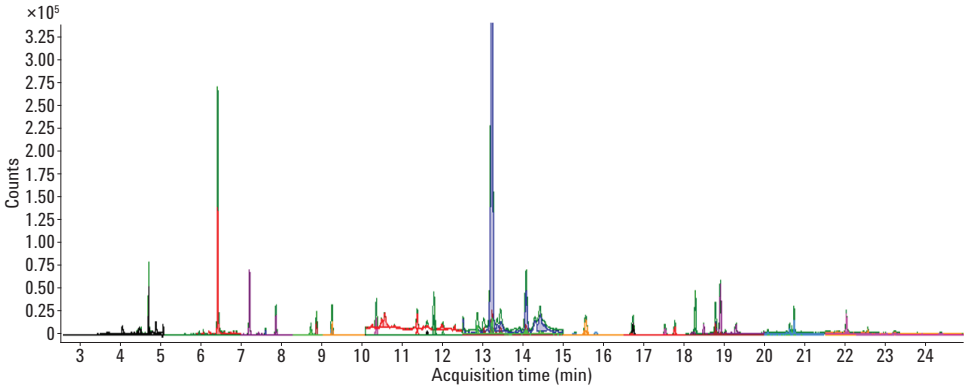


Figure 1. A typical GC triple quadrupole chromatogram (MRM) of an avocado sample fortified with a 50 ng/g pesticide standard. The sample preparation used QuEChERS followed by cleanup with Agilent Bond Elut EMR—Lipid.

Sample preparation

The final sample preparation procedure was optimized as follows:

1. Weigh 15 g (±0.1 g) homogenized avocado into 50 mL centrifuge tubes.
2. Add 15 mL of acetonitrile (1% AA) and vortex for 10 s.
3. Add AOAC extraction salt packet.
4. Mix on a mechanical shaker for 2 min.
5. Centrifuge at 5,000 rpm for 5 min.
6. Add 5 mL water to a 15 mL EMR—Lipid dSPE tube, and transfer 5 mL of supernatant to EMR—Lipid tube.
7. Vortex immediately to disperse sample, then for an extra 60 s with the entire batch on a multitube vortexer.
8. Centrifuge at 5,000 rpm for 3 min.
9. Transfer 5 mL supernatant to a 15 mL EMR—Lipid polish tube containing 2 g salts (1:4, NaCl:$MgSO_4$), and vortex for 1 min.
10. Centrifuge at 5,000 rpm for 3 min.
11. Transfer the upper ACN layer to a sample vial for GC/MS/MS injection.

The entire sample preparation workflow is shown in Figure 2.

Calibration standards and quality control samples

Prespiked QC samples were fortified with combined standard working solution at appropriate concentrations, after step 1, in replicates of six. The QC samples correspond to 5, 50, and 300 ng/g in avocado. The QC samples were 25, 250, and 1,500 ng/g for captan, folpet, trichlorfon, and bupirimate. An IS solution was also spiked into all samples except the matrix blank, corresponding to 250 ng/g in avocado.

Matrix-matched calibration standards prepared with standard and IS working solutions were added appropriately into the matrix blank samples after step 10, corresponding to 1, 5, 10, 50, 100, 200, 300, and 400 ng/g in avocado, and 250 ng/g IS. The four compounds used calibration standards at 5, 25, 50, 250, 500, 1,000, 1,500, and 2,000 ng/g.

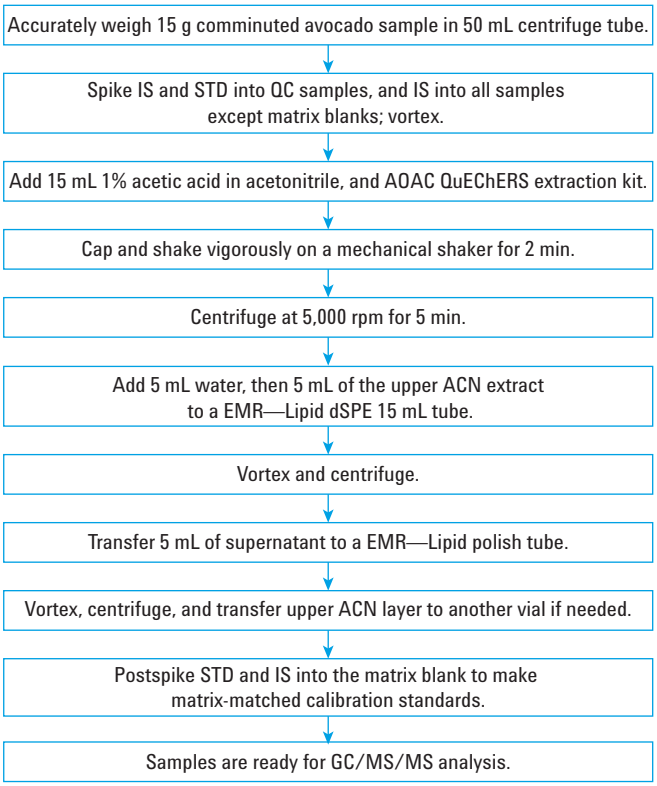


Figure 2. Sample preparation workflow showing a QuEChERS extraction with Agilent Bond Elut EMR—Lipid cleanup for the analysis of pesticides in avocado by GC/MS/MS.

Matrix cleanup assessment

The avocado extracts were applied to three different cleanup materials, fatty dSPE (C18/PSA), zirconia sorbent, and EMR—Lipid. An experiment compared the GC/MS full-scan profile of the final extract, before and after cleanup. Chromatograms were overlaid to compare the amount of matrix cleanup by chromatographic background. To quantitatively evaluate matrix cleanup efficiency, the GC/MS full-scan chromatogram was manually integrated across the entire window, and the matrix removal efficiency was then calculated according to Equation 1.

$$\% \text{ Matrix removal} = \frac{\textit{Total peak area}_{\textit{Sample without cleanup}} - \textit{Total peak area}_{\textit{Sample with cleanup}}}{\textit{Total peak area}_{\textit{Sample without cleanup}}} \times 100$$

Equation. 1

A gravimetric experiment comparing the weight of avocado coextracts after treatment with EMR—Lipid, C18/PSA, and zirconia sorbent has been published [14].

Method comparison and validation

An analyte recovery experiment compared prespiked and postspiked samples at 50 ng/g in avocado. Samples were treated using the QuEChERS AOAC extraction procedure followed by EMR—Lipid, C18/PSA, or zirconia cleanup. For EMR—Lipid cleanup, the protocol shown in Figure 2 was followed. The other materials applied the same QuEChERS extraction with a C18/PSA and zirconia sorbent cleanup. An aliquot of 1 mL crude ACN extract was then transferred to a 2 mL C18/PSA dSPE tube (p/n 5982-5122) or a 2 mL vial containing 100 mg zirconia sorbent. All samples were vortexed for one minute and centrifuged at 13,000 rpm for three minutes on a microcentrifuge. The ACN layer was then transferred into a sample vial for GC/MS/MS analysis. Matrix-matched calibration standards were prepared by postspiking the blank avocado extract with standards and internal standards. Recovery was calculated by the ratio of analyte peak areas from pre- and postspiked samples.

The EMR—Lipid method was validated in avocado at three levels in six replicates using an 8-point matrix-matched calibration curve. An internal standard (IS) was used for quantitation and data were reported as accuracy and precision.

Matrix impact on GC/MS/MS system performance

The matrix impact on GC/MS/MS system performance was investigated by evaluating the consistency for analyte response over multiple injections of avocado samples. The experiment compared the analyte response on GC/MS/MS over time by making multiple injections of avocado extracts treated with EMR—Lipid, C18/PSA, or zirconia sorbent. Each testing batch included matrix blanks and postspiked 50 ppb QC samples. The sequence injected four blanks with a QC sample on the fifth injection, and was carried out for 100 total injections. This was to determine the effect of unremoved matrix accumulation on GC/MS flow path surfaces on analyte instrument response using the different cleanup options. For each cleanup, the analyte response (peak area) was used to calculate the %RSD over the 100-injection run. To exclude the contribution of the GC flow path, Agilent Inert Flow Path consumables were used, with a new Agilent Ultra Inert liner and column for each cleanup method.

Results and Discussion

Matrix cleanup assessment

Complex matrices significantly impact GC/MS performance as matrix forms active sites on the GC flow path surface, induces matrix effects in the mass spectrometer, and introduces interferences in the final chromatogram. While GC/MS (SIM) and GC/MS/MS (MRM) show enhanced selectivity for the target ions, unremoved matrix can still cause interference and decrease performance over time. To remedy these negative effects from high-fat matrices such as avocado, more complete sample preparation cleanup methods must be applied to make samples more amenable to GC/MS analysis.

Figure 3A shows the overlaid GC/MS full-scan chromatograms for an avocado matrix blank and the chromatographic profiles obtained from EMR—Lipid, C18/PSA, and zirconia cleanup methods. The chromatogram from the sample without further cleanup (black trace) shows a high abundance of matrix interferences, which will hinder the analysis of target analytes. The chromatograms from extracts treated with C18/PSA (blue) and zirconia sorbent (green) cleanup show 36% and 55% matrix removal, respectively, as determined by Equation 1. However, the EMR—Lipid dSPE trace (red) shows near baseline removal of these interferences on the GC/MS full-scan chromatogram, corresponding to 95% matrix removal. The large amount of cleanup achieved with EMR—Lipid has obvious implications for the analysis of pesticides in avocado as there is dramatically less matrix in the sample to affect instrument performance. Furthermore, this is achieved using a simple dSPE with EMR—Lipid in a conventional QuEChERS workflow.

Figure 3B shows the overlapped GC/MS/MS MRM chromatograms for avocado samples fortified with 50 ppb of pesticide standard. Due to the improved selectivity of the MS/MS system, the matrix background is less significant than a GC/MS SIM or full-scan chromatogram. Despite the superior selectivity for analytes of interest, interference peaks are still present between 11 and 20 minutes on the chromatogram for C18/PSA (blue) and zirconia (green). These interferences affect the accurate integration for some analyte signals. The EMR—Lipid extracts show a substantially cleaner background as evident in the red trace in Figure 3B, dramatically improving the accuracy of integration.

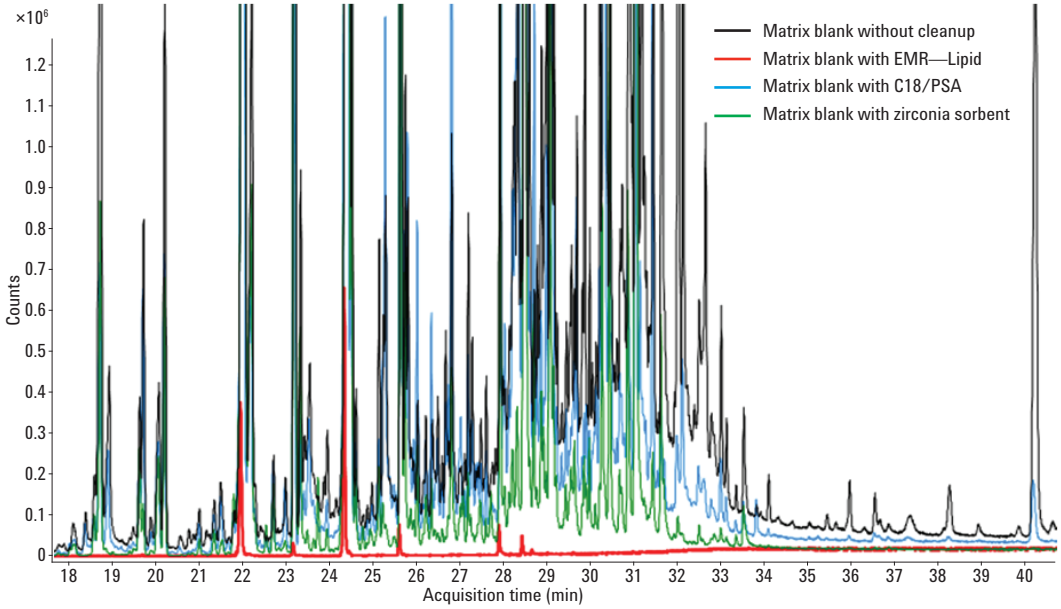


Figure 3A. GC/MS full-scan chromatogram overlay of avocado matrix blanks prepared by a QuEChERS AOAC extraction followed by dSPE using Agilent Bond Elut EMR—Lipid (red), zirconia (green), PSA/C18 (blue), or no cleanup (black).

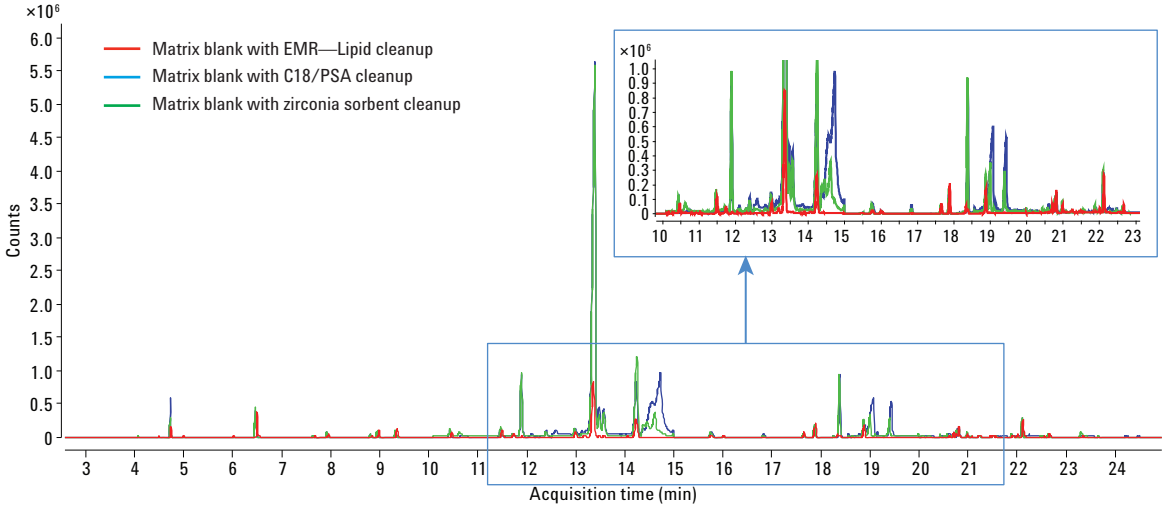


Figure 3B. GC/MS/MS MRM chromatogram overlay of an avocado sample prepared using a QuEChERS AOAC extraction followed with Agilent Bond Elut EMR—Lipid (red), C18/PSA (blue), and zirconia sorbent (green). All samples were fortified with a 50 ppb pesticide standard.

The improved matrix cleanup of EMR—Lipid and the positive effect of superior matrix removal for three example analytes are demonstrated in Figure 4. In all cases, chromatograms using EMR—Lipid cleanup show fewer interference peaks, better signal/noise, and consistent baseline integration. These improvements make data processing and review faster, and easier, and build a high degree of confidence in the analytical method.

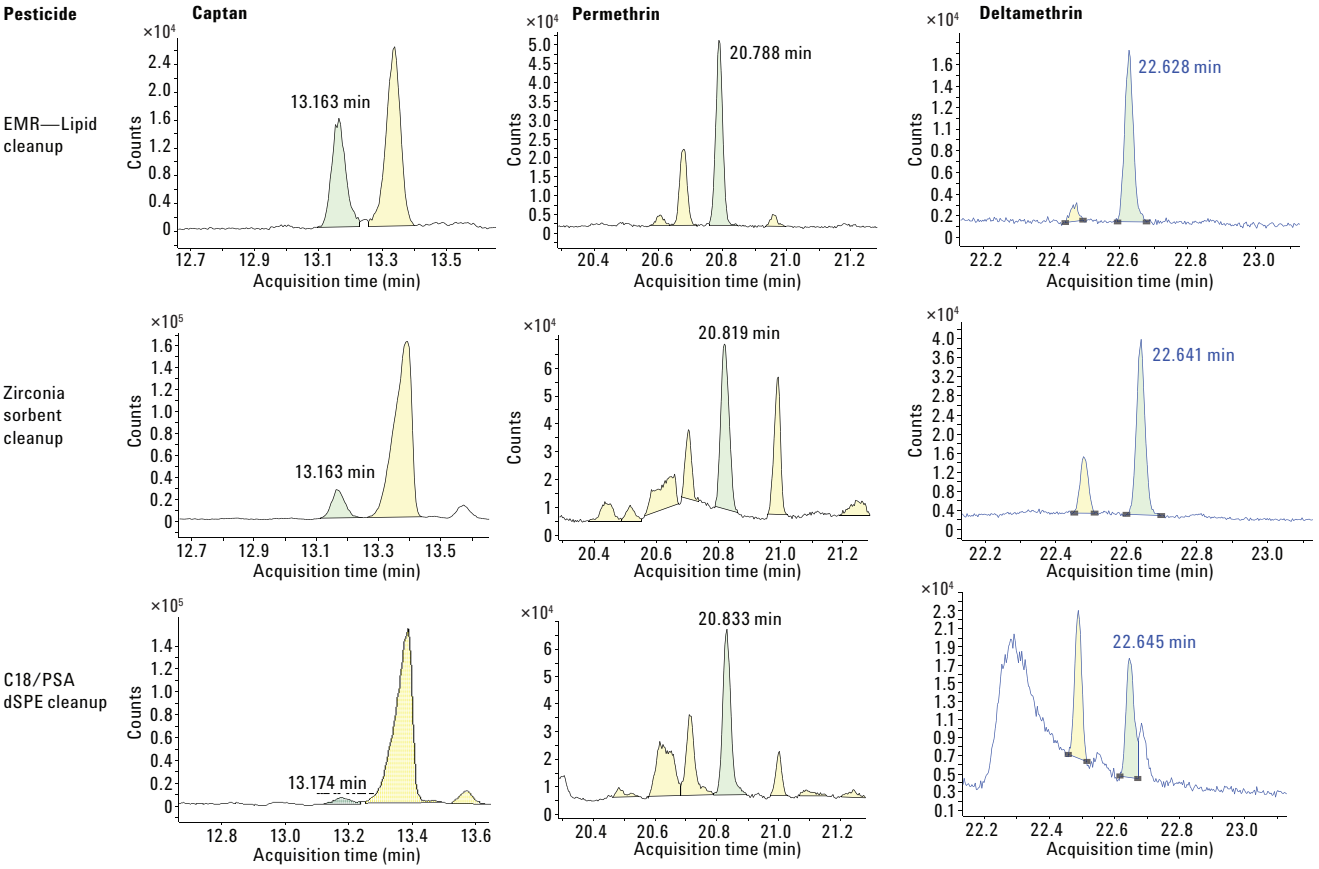


Figure 4. Chromatogram comparison for analytes of interest and the affect of matrix on peak response, peak quality, and interferences in the MRM window. Blank samples were treated with either Agilent Bond Elut EMR—Lipid, zirconia, or C18/PSA and the final sample postspiked with a 50 ppb pesticide standard.

Method comparison for analyte recovery

The optimized EMR—Lipid method was then compared with a traditional QuEChERS method using C18/PSA or zirconia sorbent. Figure 5 shows the recovery comparison for all 23 pesticides using these different cleanup materials. The results demonstrate that EMR—Lipid cleanup does not cause significant analyte retention, and thus provides comparable recovery results to C18/PSA cleanup. However, we have shown that C18/PSA and zirconia sorbents do not provide efficient matrix removal.

There are some analytes with lower absolute recoveries regardless of the cleanup method. Aldrin, endrin, and DDT had less than 60% recovery, and permethrin and deltamethrin were 63% and 75%, respectively. C18/PSA cleanup provided a slightly higher recovery than EMR—Lipid and zirconia sorbent cleanup. These pesticides are highly lipophilic (high log P) with very poor solubility in water, and are readily incorporated into high-lipid sample matrices such as avocado, making them challenging to extract with polar solvents such as acetonitrile. The use of stronger solvents may increase the extraction efficiency of these lipophilic analytes from the fatty matrix, increasing extraction efficiency and improving absolute recovery. Future work will investigate the extraction efficiency of lipophilic compounds from high-fat matrices followed by enhanced matrix removal.

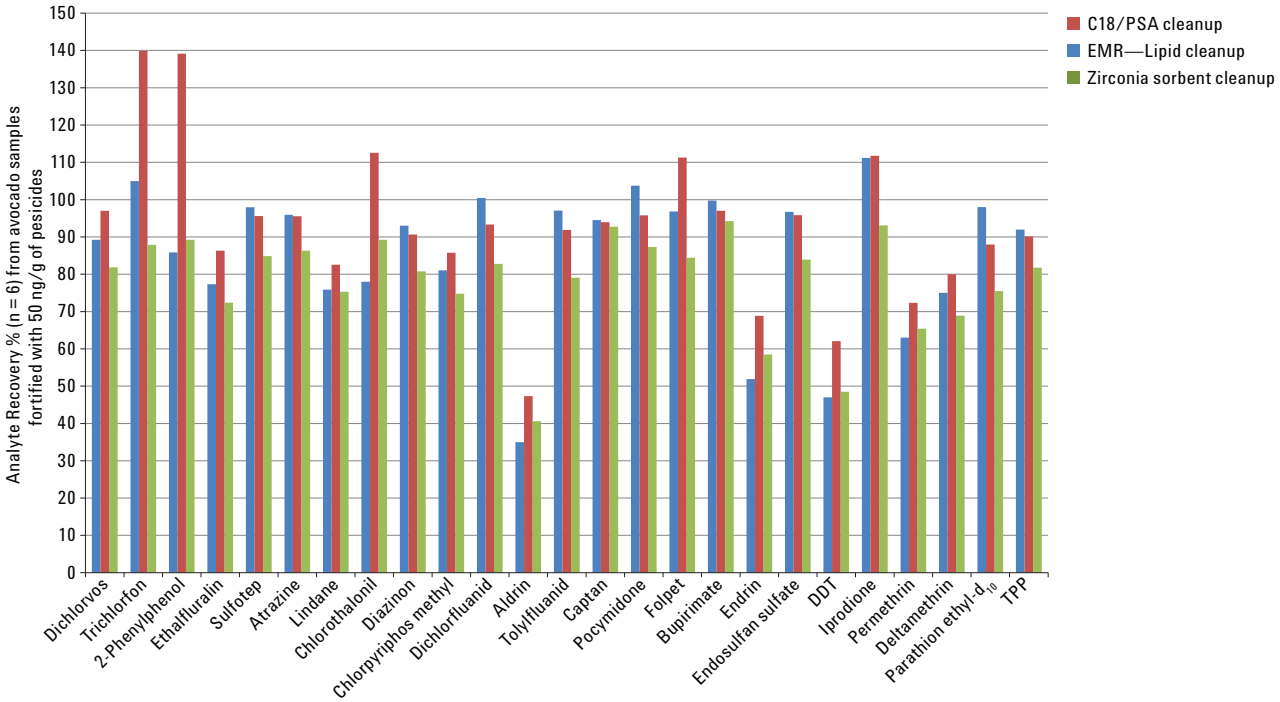


Figure 5. Recovery comparison between Agilent Bond Elut EMR—Lipid, C18/PSA, and zirconia cleanup at 50 ppb in avocado.

To correct for these compounds low in absolute recovery, a stable labeled internal standard, ^{13}C-DDT, was used to improve the accuracy of DDT, aldrin, and endrin in the final quantitation results. The use of TPP as internal standard for permethrin and deltamethrin was suitable for quantitation.

Method validation

The EMR—Lipid method was validated by running a full quantitation batch. Internal standards were used for quantitation, and results were reported as accuracy and precision. Three internal standards were used for the quantitation, namely parathion ethyl-D_{10}, ^{13}C-DDT, and TPP. The analytes with retention times before 12 minutes used parathion ethyl-D_{10} as IS, and those after 12 minutes used TPP as IS. As previously mentioned, ^{13}C-DDT was used as an IS for aldrin, endrin, and DDT to correct analyte loss due to poor extraction efficiency.

Detailed validation results are listed in Table 3. Figure 6 is a summary generated using the average accuracy and precision calculated for 18 total replicates of QCs (three levels, n = 6). Pesticide accuracy was between 70% and 120% for all but one analyte (67%), and precision was less than 20% RSD for all analytes, with 80% less than 10% RSD. Aldrin accuracy was still slightly lower than 70%, but with good precision (RSD < 6%), and is acceptable based on SANCO guidelines [15].

Table 3. Quantitation results for pesticides in avocado spiked at 5, 50, and 300 ng/g levels for six replicates.

	Calibration curve			Method accuracy and precision (ng/g QCs[1])					
				5 (25)		50 (250)		300 (1,500)	
Analyte	Regression fit/weight	R^2	Cal. range (ng/g)	Rec%	RSD	Rec%	RSD	Rec%	RSD
Dichlorvos	Linear, 1/x	0.9967	1-400	97	8.2	108	4.9	111	12.7
Trichlorfon	Linear, 1/x	0.9964	5-2000[1]	98	7.8	95	7.3	84	4.7
2-Phenylphenol	Linear, 1/x	0.9996	10-400[2]	97	14.0	104	1.7	105	5.1
Ethalfluralin	Linear, 1/x	0.9969	1-400	109	3.2	98	7.6	110	6.5
Sulfotep	Linear, 1/x	0.9958	1-400	96	5.8	76	3.9	85	9.8
Atrazine	Linear, 1/x	0.9967	1-400	91	5.0	80	2.1	76	3.9
Lindane	Linear, 1/x	0.9991	1-400	92	6.7	104	4.0	98	12.5
Chlorothalonil	Linear, 1/x	0.9944	1-400	89	13.5	103	8.6	92	19.4
Diazinon	Linear, 1/x	0.9993	1-400	102	6.8	116	5.1	108	8.9
Chlorpyrifos methyl	Linear, 1/x	0.9984	1-400	101	6.2	123	4.5	113	15.0
Dichlofluanid	Linear, 1/x	0.9989	1-400	96	10.2	85	5.1	91	4.3
Aldrin	Linear, 1/x	0.9982	1-400	76	4.8	59	2.3	65	5.1
Tolylfluanid	Linear, 1/x	0.9990	10-400	108	10.0	93	6.2	93	5.4
Captan	Linear, 1/x	0.9959	25-2000[1,2]	89	8.2	109	11.0	87	18.1
Folpet	Linear, 1/x	0.9897	5-2000[1]	76	9.5	79	9.9	87	13.2
Procymidone	Linear, 1/x	0.9977	1-400	87	5.0	76	1.9	79	7.2
Bupirimate	Linear, 1/x	0.9957	5-2000[1]	101	6.5	100	5.6	85	10.3
Endrin	Linear, 1/x	0.9967	1-400	75	10.8	88	6.7	80	13.6
Endosulfan sulfate	Linear, 1/x	0.9996	1-400	96	9.9	97	6.4	95	4.9
DDT	Linear, 1/x	0.9995	1-400	103	4.5	105	2.6	107	4.6
Iprodione	Linear, 1/x	0.9995	1-400	97	6.7	105	2.7	97	4.2
Permethrin	Linear, 1/x	0.9992	1-400	87	6.6	97	4.3	84	14.0
Deltamethrin	Linear, 1/x	0.9963	1-400	89	13.8	92	8.3	98	11.5

[1] Compounds were prepared at five times higher concentration in the combined standard working solution due to a low response. Therefore, the QC spiking and calibration standard spiking levels were five times higher than those of the other compounds.

[2] Raised LOQ due to either poor sensitivity or matrix interference peak interfered the detection of analyte at original LOQ.

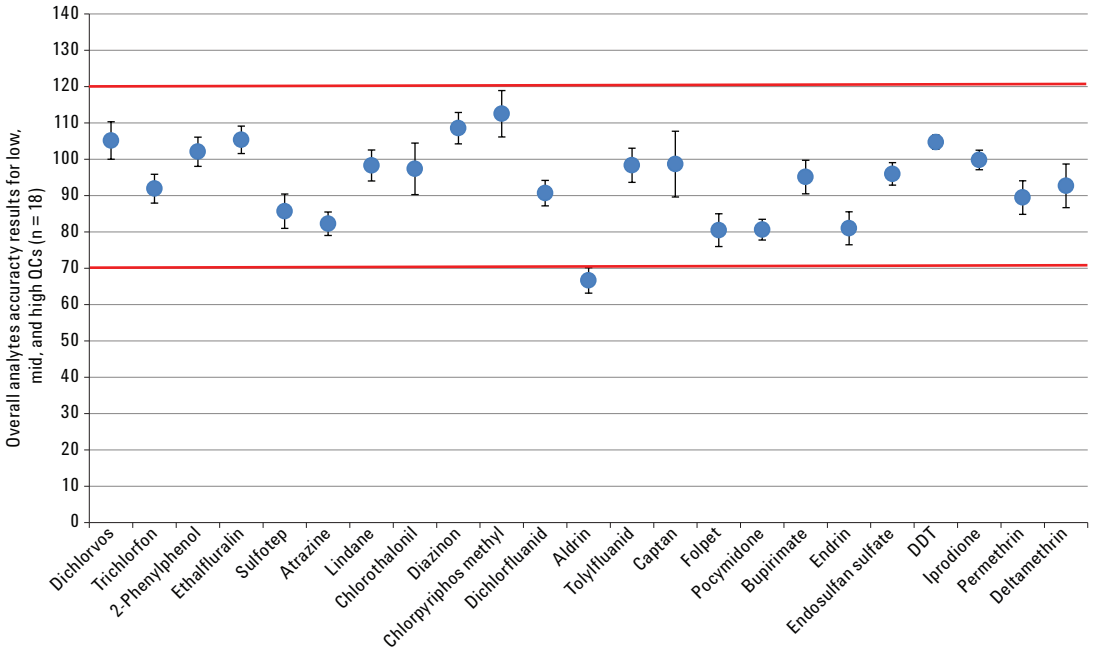


Figure 6. Quantitation results for 23 pesticides in avocado using a QuEChERS extraction with Agilent Bond Elut EMR—Lipid, dSPE. The data points represent accuracy and precision and were calculated at three levels in six replicates. Error bar = 95% CI.

Matrix impact on GC/MS/MS system performance

Matrix interferences will affect GC/MS/MS system performance over time as more samples are injected into the system. GC flow path active sites can negatively impact instrument performance. Agilent Inert Flow Path components provide the best deactivation for the entire GC flow path and significantly reduce negative interactions between analytes and active sites that result in analyte loss and chromatographic anomalies. However, if the matrix is laden with high-boiling compounds (high fat) it will accumulate on the flow path surface and generate new active sites. Over time, this can lead to analyte response variations, greatly impacting method reliability and reducing the number of injections per batch. To fix this, laboratories must perform more instrument maintenance such as liner change or column trim/change, leading to decreased laboratory productivity.

As demonstrated in the matrix cleanup assessment and gravimetric determination [14], samples that are treated with EMR—Lipid provided significantly cleaner background, showing that dramatically less matrix is being introduced into the GC/MS/MS system. The number of active sites that accumulate in the GC/MS flow path are decreased, preserving the analytical integrity of the instrument. This is demonstrated with better analyte precision (RSDs) for over 100 injections of avocado samples on the GC/MS/MS (Table 4). Samples treated with EMR—Lipid achieved RSDs <15% for 91% of the analytes, most in single digits. Two compounds, captan (RSD 29.9%) and DDT (RSD 21.6%) gave higher RSDs over the 100 injection experiment, but gave 11.1% and 6.4% RSD for the first 50 injections, respectively.

Table 4. Comparison of analyte reproducibility (RSDs) over 50 and 100 injections of avocado samples treated with Agilent Bond Elut EMR—Lipid, C18/PSA, or zirconia sorbent by GC/MS/MS. The samples were fortified at 50 ng/g. Analyte peak areas were used to calculate RSD results.

	Analyte RSD over 100 injections (n = 20)			RSD over 50 injections (n = 10)		
Pesticides	**EMR—Lipid cleanup**	**C18/PSA cleanup**	**Zirconia sorbent cleanup**	**EMR—Lipid cleanup**	**C18/PSA cleanup**	**Zirconia sorbent cleanup**
Dichlorvos	6.2	10.5	16.8	2.2	9.4	6.3
2-Phenylphenol	7.0	13.6	19.5	5.0	12.4	8.4
Ethalfluralin	12.4	18.8	32.0	5.8	10.3	7.9
Sulfotep	7.1	11.8	17.2	3.1	6.4	10.8
Atrazine	6.8	12.2	19.1	3.2	12.2	5.2
Lindane	8.5	10.8	20.0	4.6	10.9	5.1
Chlorothalonil	12.5	11.7	37.4	8.0	12.9	11.0
Diazinon	6.6	11.7	16.9	4.4	10.5	5.6
Chlorpyriphos methyl	8.4	8.9	14.9	3.8	8.6	6.6
Dichlorfluanid	11.7	9.0	25.9	5.4	9.9	5.5
Aldrin	9.8	19.3	25.7	8.6	19.3	7.1
Tolylfluanid	10.5	6.6	17.8	4.2	6.9	6.6
Captan	29.9	51.9	47.1	11.1	24.9	21.7
Procymidone	6.8	14.3	22.5	5.6	13.8	4.8
Bupirimate	6.8	10.4	20.7	7.6	11.0	6.2
Endrin	8.3	12.6	24.1	5.9	13.8	5.4
Endosulfan sulfate	8.5	12.1	22.4	5.3	12.7	6.4
DDT	21.6	22.4	42.6	6.4	12.0	11.8
Iprodione	11.0	10.7	40.0	8.2	10.9	16.3
Permethrin	6.8	11.8	18.8	5.2	11.2	8.6
Parathion ethyl-d_{10} (IS)	11.8	7.2	13.0	4.7	6.8	7.0
TPP (IS)	9.1	19.9	28.3	9.0	22.5	12.8

In comparison, C18/PSA produced RSDs <15% for 74% of analytes, and zirconia dramatically fewer, at only 9%. The zirconia-treated extract was especially problematic with 100% of the analytes above 10% RSD, 57% of which were well above 20% RSD over 100 injections. This indicates that the higher level of matrix remaining in the C18/PSA and zirconia cleanup extract is negatively affecting instrument performance, resulting in significant variability of analyte response. These results attest to the excellent matrix removal provided by EMR—Lipid, which results in less activity in the GC flow path, higher precision over multiple injections, and more samples being run before instrument maintenance.

Conclusions

A rapid, reliable, and robust method using QuEChERS AOAC extraction followed by Agilent Bond Elut EMR—Lipid cleanup was developed and validated for the analysis of 23 GC-amenable pesticides in avocado. Matrix effects were assessed and compared with traditional C18/PSA and zirconia sorbent cleanup. Results demonstrate that the EMR—Lipid provides superior chromatographic cleanliness with both GC/MS and GC/MS/MS versus C18/PSA and zirconia sorbent. Implementing EMR—Lipid cleanup facilitates the use of GC/MS for sample analysis in high-fat matrices. The recovery comparison demonstrates that EMR—Lipid cleanup produced comparable analyte recoveries relative to C18/PSA, and better recovery than zirconia sorbent. The greatest advantage of EMR—Lipid in this application was attributed to the high degree of matrix removal, providing outstanding reproducibility over 100 injections on the GC/MS/MS. The analyte responses of C18/PSA and especially zirconia-treated samples were highly variable over this 100-injection experiment. The use of EMR—Lipid as a dSPE cleanup material in a QuEChERS workflow, therefore, improves overall laboratory productivity, increases sample throughput, decreases data process and review, reduces batch reruns, and reduces instrument maintenance. Future work will examine the advantages of enhanced matrix removal for other complex, high-fat samples and target analytes.

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