Multiresidue Analysis of Pesticides in Avocado with Agilent Bond Elut Enhanced Matrix Removal-Lipid by LC/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 44 multiclass pesticides in avocado by LC/MS/MS. The procedure involves a QuEChERS AOAC extraction followed by the use of EMR—Lipid dSPE and EMR—Lipid polish salts, providing fast and effective sample cleanup. The matrix cleanup was evaluated by determining the amount of nonvolatile coextractives from an avocado extract after different dSPE cleanup, and by evaluating chromatographic matrix effects for target analytes. Compared to other matrix cleaning products, EMR—Lipid dSPE provides much more efficient matrix cleanup without impacting analyte recoveries. The optimized method delivers excellent accuracy and precision for all 44 LC-amenable pesticides in avocado by LC/MS/MS. The EMR—Lipid dSPE conveniently fits into a QuEChERS protocol, providing fast, robust, and effective sample preparation for pesticide residue analysis in high-fat avocado samples.

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Introduction

Pesticide residue analysis in food commodities is routine for many laboratories using 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 sample matrix without unwanted analyte loss. Removal of lipid interferences from complicated matrices is especially important for techniques such as QuEChERS and protein precipitation, as these methods coextract large amounts of matrix with the target analytes. This study investigates sample preparation for the analysis of 44 LC-amenable representative pesticides in avocado using a QuEChERS AOAC extraction followed by EMR—Lipid dSPE cleanup. The pesticides represent 12 different chemical classes to establish proof of concept for analytes that were not included in this application note. Table 1 lists the LC-amenable pesticides and their classes. This application note demonstrates the exceptional cleanliness that EMR—Lipid provides for complex, fatty samples such as avocado, and the high recovery and precision for 44 multiclass pesticide residues at three levels.

Table 1. LC-amenable pesticides used in this study and their associated chemical classes.

Representative pesticide	Chemical class	Pesticide group
Methamidophos	Organophosphate	Insecticide
Acephate	Organophosphate	Insecticide
Omethoate	Organophosphate	Insecticide
Dimethoate	Organophosphate	Insecticide
Malathion	Organophosphate	Insecticide
EPN	Organophosphate	Insecticide
Tepp-A	Organophosphate	Insecticide
Monocrotophos	Organophosphate	Insecticide
Mexacarbate	Carbamate	Insecticide
Carbaryl	Carbamate	Insecticide
Propoxur	Carbamate	Insecticide
Carbofuran	Carbamate	Insecticide
Methiocarb	Carbamate	Insecticide
Chlorpropham	Carbamate	Insecticide
Propham	Carbamate	Insecticide
Aminocarb	Carbamate	Insecticide
Oxamyl	Carbamate	Insecticide
Methomyl	Carbamate	Insecticide
Aldicarb	Carbamate	Insecticide
Terbuthylazine	Triazine	Algaecide
Simazine	Triazine	Herbicide
Sebuthylazine	Triazine	Herbicide
Monuron	Urea	Herbicide
Chlorotoluron	Urea	Herbicide
Diuron	Urea	Herbicide
Fluometuron	Urea	Herbicide
Isoproturon	Urea	Herbicide
Metobromuron	Urea	Herbicide
Siduron	Urea	Herbicide
Linuron	Urea	Herbicide
Neburon	Urea	Herbicide
Fenuron	Urea	Herbicide
Metoxuron	Urea	Herbicide
Carbendazim	Benzimidazole	Fungicide
Thiabendazole	Benzimidazole	Fungicide
Thiophanate methyl	Benzimidazole	Fungicide
Cyprodinil	Anilinopyrimidine	Fungicide
Imazalil	Imidazole	Fungicide
Penconazole	Triazole	Fungicide
Imidacloprid	Neonicotinoid	Insecticide
Metazachlor	Chloracetanilide	Herbicide
2,4-D Acid	Chlorophenoxy acid	Herbicide
Dichlorprop	Chlorophenoxy acid	Herbicide
Bentazon	Unclassified	Herbicide

Experimental

All reagents and solvents were HPLC or analytical grade. Acetonitrile (ACN) and methanol were from Honeywell (Muskegon, MI, USA). Reagent grade acetic acid (AA) was from Sigma-Aldrich, Corp. (St Louis, MO, USA). Pesticide standards and internal standard were from Sigma-Aldrich, Corp. and AccuStandard (New Haven, CT, USA).

Solution and standards

Acetonitrile containing 1% AA was prepared by adding 10 mL acetic acid to 990 mL ACN. Standard and internal standard (IS) stock solutions were made for some of the pesticides in either ACN or methanol at 2.0 mg/mL. The rest of the pesticide standards were from commercial mixed standard stock solutions, which were used directly to prepare the standard working solution. A combined working solution was prepared in ACN at 25 µg/mL. A 25 µg/mL aliquot of TPP IS working solution was prepared in ACN.

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 EMR-Polish tubes(p/n 5982-0101)

Instrumentation

Analysis was performed on an Agilent 1290 Infinity LC consisting:

- Agilent 1290 Infinity Quaternary Pump (G4204A)
- Agilent 1290 Infinity High Performance Autosampler (G4226A) equipped with an Agilent 1290 Infinity Thermostat (G1330B), and an Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)

The UHPLC system was coupled to an Agilent 6490 Triple Quadrupole LC/MS system equipped with an Agilent Jet Stream electrospray ionization source and iFunnel technology. Agilent MassHunter workstation software was used for data acquisition and analysis.

Instrument conditions

HPLC conditions

Column:	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 150 mm, 1.8 µm (p/n 959759-902), Agilent ZORBAX RRHD Eclipse Plus C18 UHPLC Guard, 5 × 2.1 mm, 1.8 µm (p/n 821725-902)	
Mobile phase:	A) 0.1% FA in water B) 0.1% FA in acetonitrile	
Flow rate:	0.3 mL/min	
Column temp:	35 °C	
Autosampler temp:	4 °C	
Inj vol:	3 µL	
Needle wash:	1:1:1:1 ACN:MeOH:IPA:H_2O with 0.2% FA	
Gradient:	Time (min) 0 15 15.01	%B 10 95 100
Stop time:	16 min	
Posttime:	3 min	

MS conditions

Positive/negative mode

Gas temp:	120 °C	
Gas flow:	14 L/min	
Nebulizer:	40 psi	
Sheath gas heater:	400 °C	
Sheath gas flow:	12 L/min	
Capillary:	3,000 V	
iFunnel parameters:	Positive	Negative
High-pressure RF:	100 V	90 V
Low-pressure RF:	70 V	60 V

MS MRM conditions relating to the analytes are listed in Table 2, and a typical chromatogram is shown in Figure 1.

Table 2. LC triple quadrupole MRM parameters and retention times for the pesticides used in this study.

Analyte	RT (min)	Delta RT (min)	Polarity	Precursor ion (*m/z*)	Product ion (*m/z*)	CE (v)
Methamidophos	1.83	2	Positive	142	94.1	9
Aminocarb	2.03	2	Positive	209.1	137.2	24
Acephate	2.13	2	Positive	184	143	9
Omethoate	2.54	2	Positive	214	124.9	17
Carbendazim	3.40	2	Positive	192.1	132	33
Thiabendazole	3.89	2	Positive	202	131.1	41
Mexacarbate	3.99	2	Positive	223.1	151.1	20
Oxamyl	4.24	2	Positive	237.1	72	12
Monocrotophos	4.46	2	Positive	224.1	127	10
Methomyl	4.64	2	Positive	163.1	106	4
Fenuron	6.17	2	Positive	165.1	72	20
Imidacloprid	6.43	2	Positive	256.1	209.1	13
Dimethoate	6.63	2	Positive	230	199	5
TEPP-A	7.69	2	Positive	291.1	179	20
Aldicarb	7.87	2	Positive	213.1	89.1	15
Metoxuron	7.89	2	Positive	229	46.1	12
Imazalil	7.99	2	Positive	297.1	158.9	25
Simazine	8.31	2	Positive	202.1	132	22
Monuron	8.37	2	Positive	199.1	46.1	16
Thiophanate methyl	8.95	2	Positive	343.1	151.2	4
Propoxur	9.15	2	Positive	210.1	111.1	9
Carbofuran	9.30	2	Positive	222.1	123.1	30
Chlorotoluron	9.54	2	Positive	213.1	72	20
Diuron	9.65	2	Positive	233	72.1	20
Carbaryl	9.73	2	Positive	202.1	145.1	9
Bentazone	9.73	2	Negative	239	132	15
Isoproturon	9.96	2	Positive	207.1	46.1	20
2,3-D acid	10.06	2	Negative	219	161	15
Fluometuron	10.10	2	Positive	233.1	72	16
Metobromuron	10.48	2	Positive	259	148	10
Cyprodinil	10.53	2	Positive	226.1	93.1	41
Metazachlor	10.71	2	Positive	278.1	134.2	15
Propham	10.80	2	Positive	180.1	138.1	4
Terbuthylazine	10.98	2	Positive	230.1	174.1	15
Dichlorprop	10.99	2	Negative	233	161	10
Siduron	11.26	2	Positive	233.2	137.1	12
Sebuthylazine	11.47	2	Positive	230.1	174.1	16
Methiocarb	11.47	2	Positive	226.1	169	4
Linuron	11.69	2	Positive	249	160.1	20
Chlorpropham	12.53	2	Positive	214.1	172	5
Penconazole	12.76	2	Positive	284.1	70	17
Malathion	12.85	2	Positive	331	126.9	5
Neburon	13.29	2	Positive	275.1	57.1	20
TPP (IS)	13.99	2	Positive	327.1	51.1	80
EPN	14.96	2	Positive	324.1	296.1	8

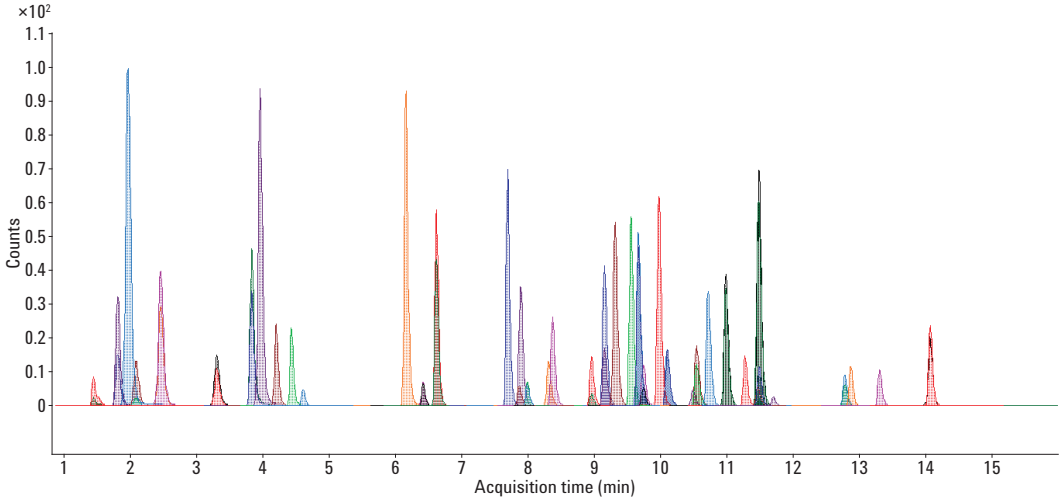

Figure 1. A typical LC/MS/MS chromatogram (MRM) of avocado sample fortified with 50 ng/g of pesticides and extracted by QuEChERS followed by cleanup with Agilent Bond Elut EMR—Lipid.

Sample preparation

The final sample preparation procedure was optimized using a QuEChERS workflow with the following steps:

1. Weigh 15 g (±0.1 g) homogenized avocado into 50 mL centrifuge tubes.
2. Add 15 mL acetonitrile (1% AA), and vortex for 10 s.
3. Add a packet of AOAC extraction salt.
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.
7. Transfer 5 mL of supernatant to EMR—Lipid dSPE tube.
8. Vortex immediately to disperse sample, then for an extra 60 s on a multitube vortexer.
9. Centrifuge at 5,000 rpm for 3 min.
10. Transfer 5 mL of supernatant to a 15 mL EMR—Lipid polish tube containing 2 g salts (1:4, NaCl:$MgSO_4$), and vortex for 1 min.
11. Centrifuge at 5,000 rpm for 3 min.
12. Combine 200 µL of upper ACN layer and 800 µL water in a 2 mL sample vial and vortex.

The sample is now ready for LC/MS/MS analysis. The entire sample preparation flow path is shown in Figure 2.

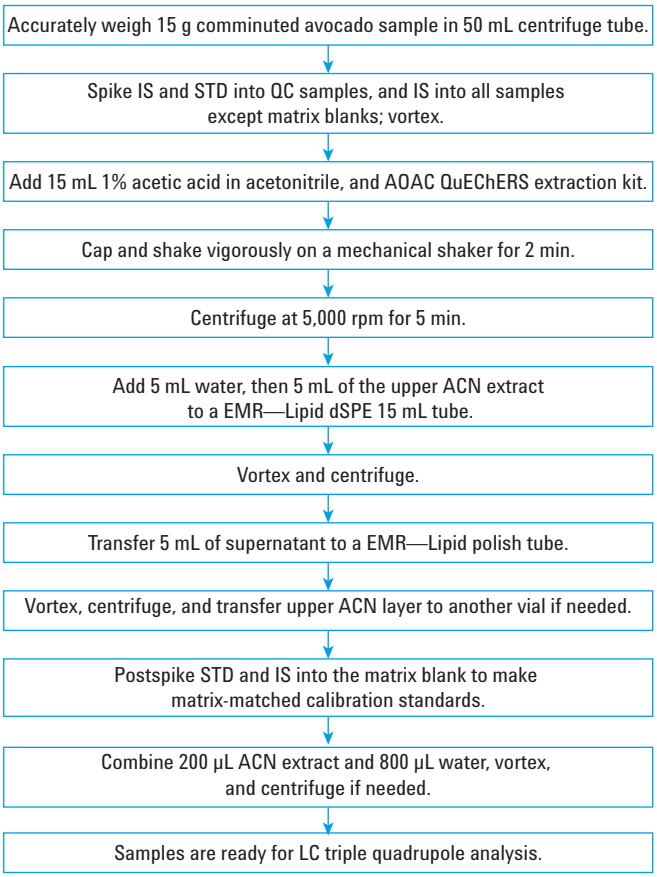

Figure 2. Sample preparation procedure using Agilent Bond Elut EMR—Lipid for the analysis of pesticides in avocado.

Calibration standards and quality control samples

Prespiked QC samples were fortified with combined standard working solution appropriately, after step 1, for six replicates. The QC samples correspond to 5, 50, and 200 ng/g in avocado. IS solution was also spiked into all the samples except the matrix blank, corresponding to 100 ng/g of TPP in avocado.

Matrix-matched calibration standards were prepared with standard and IS working solutions. Appropriate concentrations in the matrix blank samples after step 10 corresponded to 1, 5, 10, 50, 100, 150, and 200 ng/g and 100 ng/g IS (TPP). We diluted the final sample extract with water to make the sample amenable to the LC/MS/MS gradient and maintain peak shape integrity for early eluting analytes. The LC/MS/MS system provided excellent sensitivity using the final dilution as described and met the required limits of detection. If instrument sensitivity cannot meet the desired needs by sample dilution, a sample concentration step (evaporation and reconstitution), though less than ideal, should be considered

Determining amount of coextractives

The amount coextractive was determined by gravimetric measurements [2] for three different cleanup techniques: C18/PSA, zirconia sorbent, and EMR—Lipid. Samples were prepared as follows to collect data in duplicate.

1. Heat glass tubes for ~ 1 h at 110 °C to remove moisture.
2. Cool tubes to room temperature.
3. Preweigh test tubes.
4. Accurately transfer 1 mL of initial matrix blank extract (no cleanup) and the matrix blanks with various cleanups, each in duplicate.
5. Dry all samples on a CentriVap at 50 °C for 1 h, or until dry.
6. Heat the tubes for ~ 1 h at 110 °C to remove moisture.
7. Cool tubes to room temperature.
8. Reweigh the tubes.

The weight difference between after step 8 and after 3 is the amount of sample coextractive. The amount of coextractive removed by cleanup was the average weight difference of the matrix coextractives before and after cleanup.

Matrix effect assessment

Additionally, the analyte response (peak area) was compared between postspiked avocado extracts and the equivalent neat solutions. Postspiked avocado extracts were made by postspiking standard pesticide solution into the blank avocado matrix extract. The difference in response (peak area) is directly correlated to matrix effects.

Method comparison and validation

Currently, the QuEChERS method recommends fatty dSPE, which contain PSA, EC-C18, and $MgSO_4$, for the cleanup in high-fat samples such as avocado. Also, the zirconia sorbent claims to be a more efficient at lipid removal than C18/PSA dSPE. Our method comparison focused on EMR—Lipid cleanup and the other cleanup techniques. Recovery data compared pre- and postspiked samples corresponding to 50 ng/g in avocado. Extraction was carried out with the AOAC QuEChERS procedure, followed by dSPE with each cleanup protocol; EMR—Lipid, C18/PSA dSPE, and zirconia sorbent. For EMR—Lipid cleanup, the protocol shown in Figure 2 was followed. The EMR—Lipid dSPE, unlike traditional dSPE sorbents, requires extra water to activate the material, dramatically improving matrix removal performance. The supernatant from EMR-Lipid is transferred to the EMR—Lipid polish salts to phase separate the ACN/water, and remove dissolved solids. For QuEChERS with C18/PSA and zirconia cleanup, 1 mL of crude ACN extract was transferred into a 2 mL fatty dSPE tube (p/n 5982-5122), or into a 2 mL vial containing 100 mg zirconia sorbent. Samples were then vortexed for one minute and centrifuged at 13,000 rpm for three minutes on a microcentrifuge. An aliquot of 200 µL of supernatant was then transferred into a sample vial containing 800 µL water. A precipitate was generated with both the C18/PSA dSPE and zirconia sorbent cleanup protocols at this step, and samples must be filtered with a regenerated cellulose 0.45 µm filter vial before LC/MS/MS analysis. The precipitants are believed to be caused by unremoved lipids from the fatty dSPE and zirconia cleanups. This was not the case for the crude extract cleanup by EMR—Lipid, which, upon dilution, gave a clear solution with no precipitants. Filtration was, therefore, not required. It is important to make the postspiked calibrants in the corresponding matrix blanks, to prepare matrix-matched calibration 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 5, 50, and 200 ng/g levels in six replicates using a 7-point-matrix matched calibration curve. An internal standard was used for quantitation, and data were reported as accuracy and precision.

Results and Discussion

Amount of coextractives

The results of sample coextractives weight determination are shown in Table 3, clearly demonstrating that EMR—Lipid dSPE provides the best matrix cleanup efficiency by weight.

Table 3. Avocado coextractive weights from QuEChERS extraction and various cleanup materials (n = 2).

Cleanup technique	Coextractives per 1 mL ACN final extract (mg)	Matrix coextractive removal efficiency by cleanup (%)
No further cleanup	14.7	–
EMR—Lipid cleanup	4.2	71.4
Zirconia cleanup	7.0	52.4
C18/PSA cleanup	9.5	35.4

$$\text{Matrix coextractive removal efficiency (\%)} = \frac{(\textit{Amount of coextractives without cleanup} - \textit{Amount of coextractives with cleanup})}{\textit{Amount of coextractives without cleanup}} \times 100$$

Matrix effect assessment

Analyte response between postspiked matrix blanks and neat standards was compared to evaluate matrix effects. Since the majority of coextracted lipids elute late in an LC gradient (reversed phase, low to high % organic), the hydrophobic analytes are impacted to a greater extent by the sample matrix. This effect is usually known as ion suppression, which correlates to low analyte response. Because of inefficient matrix lipid removal by C18/PSA and zirconia sorbent, significantly more matrix ion suppression was observed for the late eluting compounds. Figure 3 shows three compounds as examples of the reduced ion suppression resulting from EMR—Lipid cleanup. The three pesticides are compounds with relatively high log P values; chlorpropham (log P 3.6), penconazole (log P 3.7), and EPN (log P 4.5). The higher the log P value, the more hydrophobic the compound. These pesticides show up to 80% ion suppression caused by matrix interferences, especially by lipids, which were not effectively removed using C18/PSA dSPE and zirconia sorbent. For these compounds, EMR-Lipid produced no significant matrix effects, as seen in Figure 3.

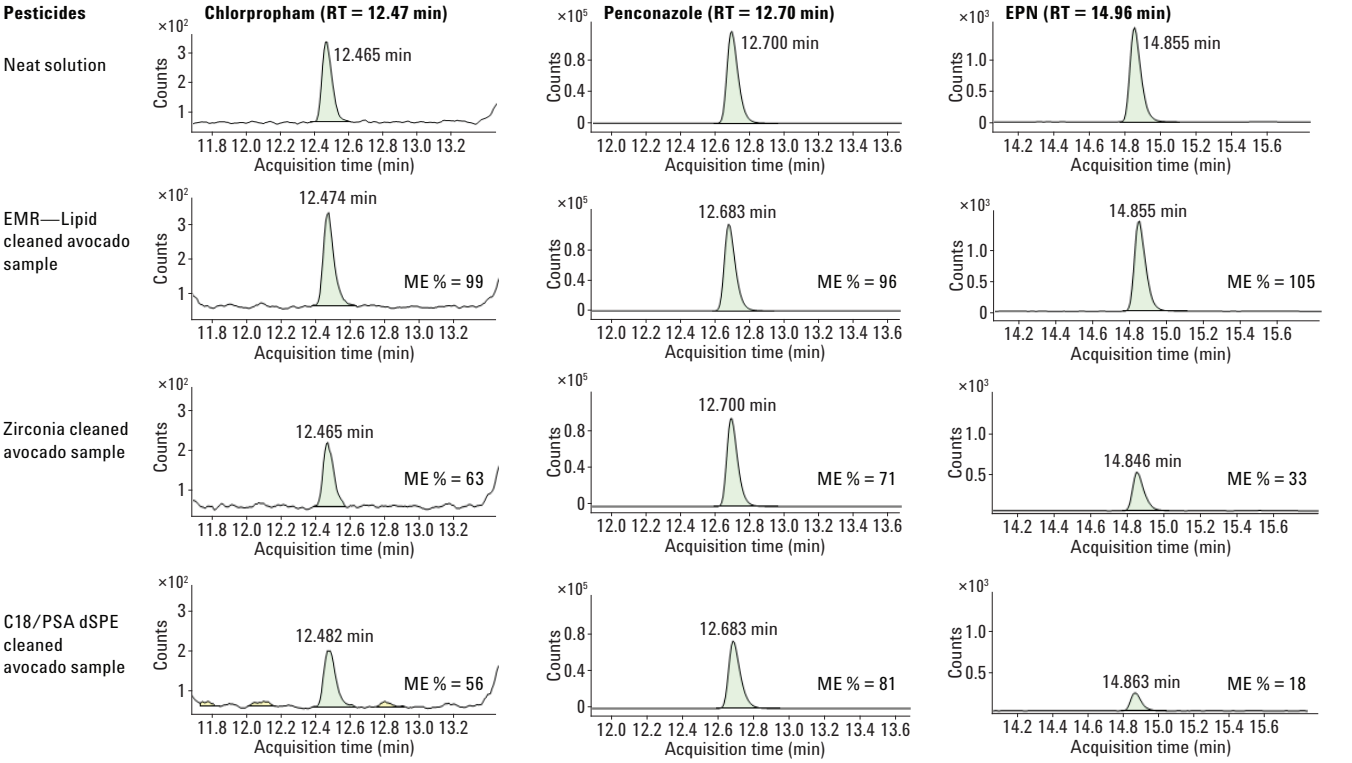

Figure 3. Matrix effect comparison for hydrophobic analytes. Matrix samples were postspiked at 50 ng/g with pesticide standard in a matrix blank.

Method comparison for analyte recovery

The optimized QuEChERS method with EMR-Lipid dSPE was then compared with C18/PSA and zirconia sorbent dSPE cleanup. Figure 4 shows the statistical recovery comparison results, and Figure 5 the selected problematic analyte comparison results.

The EMR—Lipid protocol provided overall excellent recovery and precision for most pesticides. Only two pesticides fell below the 70 to120% recovery window, namely cyprodinil (64%) and 2,4-D acid (65%), with RSD less than 10%. Therefore, they are considered as acceptable based on SANCO guidelines [11], as they meet acceptable reproducibility criteria. The recovery results for C18/PSA dSPE cleanup were good, except two acidic compounds. 2,4-D acid and dichlorprop gave very low recovery (<10%) caused by PSA. The recovery results from zirconia sorbent showed more analyte retention resulting in nine pesticide recoveries below 70%.

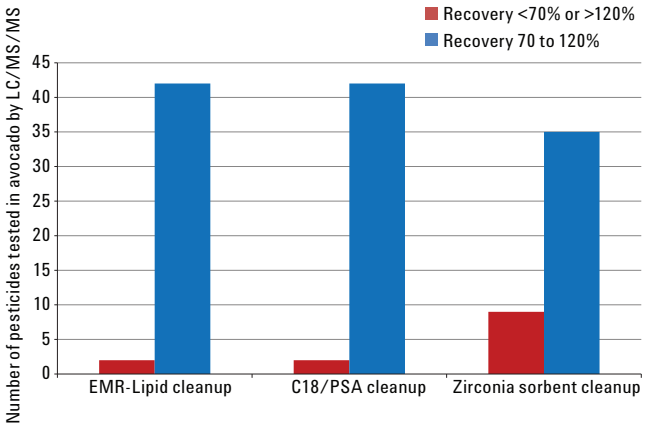

Figure 4, Statistical recovery results for the comparison of Agilent Bond Elut EMR—Lipid, C18/PSA dSPE, and zirconia sorbent.

Method validation

The EMR—Lipid protocol was validated by running a full quantitation batch. The methodology was described in the sample preparation section. An internal standard (TPP) was used for quantitation, and, therefore, the quantitation results are defined as accuracy and precision. However, the absolute recovery of IS (TPP) was above 90%, so the accuracy results correspond to absolute recovery.

Detailed validation results are listed in Table 4, and as a summarized figure (Figure 6) generated by average accuracy and precision calculated based on 18 total replicates of QC prespikes at three different levels. Accuracy results showed 95% of the 44 pesticides fell within the 70 to 120% window, except for 2,4-D acid and cyprodinil, which gave recoveries just below 70% with good RSD. The method reproducibility was exceptional with less than 10% RSD (n = 6) for 91% of the pesticides at 5 ng/g, 100% at 50 ng/g, and 98% at 200 ng/g. All other RSD values were well under 20% using the EMR—Lipid protocol. The instrumental detection limit is a likely contributor to the higher variation for these compounds above 10% RSD at the lowest spike level. The unbuffered EMR-L polish step (NaCl, $MgSO_4$) is also a potential cause of variation and so buffered polish salts will be investigated in future work.

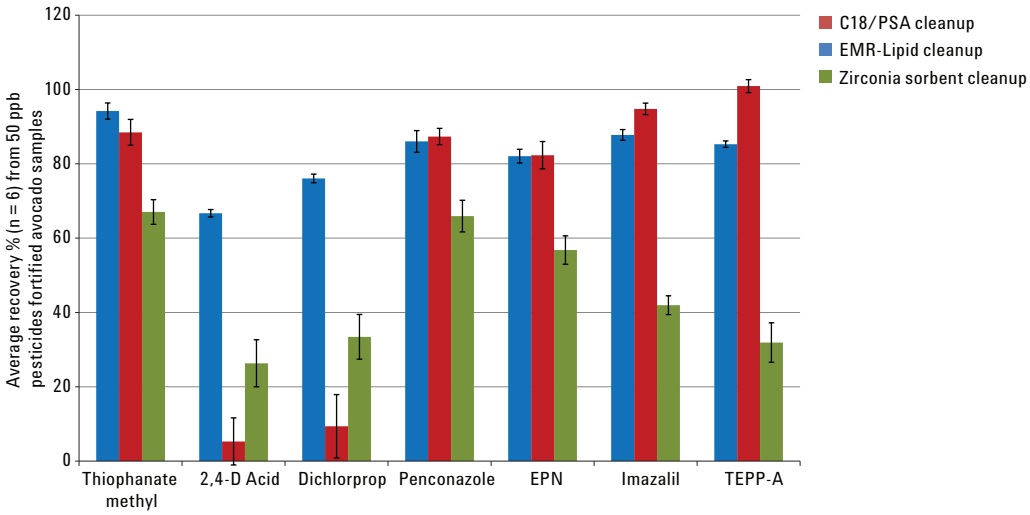

Figure 5, Recovery comparison results for Agilent Bond Elut Enhanced Matrix Removal-Lipid (blue), C18/PSA (red), and zirconia sorbent (green) dSPE cleanup.

Table 4. Validation results from EMR—Lipid protocol for 44 pesticides in avocado at 5, 50, and 200 ng/g levels (n = 6).

	Calibration curve			Method accuracy and precision					
				5 ng/g QCs		50 ng/g QCs		200 ng/g QCs	
Analyte	Regression fit/weight	R^2	Cal. range (ng/g)	Rec. %	RSD	Rec. %	RSD	Rec. %	RSD
Methamidophos	Quadratic, 1/x	0.9993	1-200	69.1	9.5	93.8	8.4	109.8	6.0
Aminocarb	Linear, 1/x	0.9990	1-200	74.6	8.4	88.0	2.7	87.0	2.0
Acephate	Linear, 1/x	0.9948	1-200	55.8	12.4	88.8	2.3	86.6	4.0
Omethoate	Linear, 1/x	0.9996	1-200	84.5	6.0	85.3	1.4	84.4	2.6
Carbendazim	Linear, 1/x	0.9995	1-200	87.1	6.3	86.2	2.2	85.4	1.2
Thiabendazole	Linear, 1/x	0.9995	1-200	49.4	24.3	76.7	1.7	79.0	2.0
Mexacarbate	Linear, 1/x	0.9993	1-200	83.6	7.8	90.4	3.3	89.0	2.1
Oxamyl	Linear, 1/x	0.9991	1-200	81.1	7.6	96.7	2.6	94.4	3.5
Monocrotophos	Linear, 1/x	0.9979	1-200	85.2	6.1	85.1	1.9	101.5	4.6
Methomyl	Linear, 1/x	0.9993	1-200	77.8	8.2	88.6	3.3	92.8	4.5
Fenuron	Linear, 1/x	0.9969	1-200	86.5	9.9	103.4	2.5	91.7	1.7
Imidacloprid	Linear, 1/x	0.9996	1-200	81.7	5.9	94.1	2.6	87.9	2.5
Dimethoate	Linear, 1/x	0.9993	1-200	83.3	8.0	99.2	3.1	94.8	2.5
TEPP-A	Linear, 1/x	0.9989	1-200	50.2	6.5	88.3	1.6	78.4	3.1
Aldicarb	Linear, 1/x	0.9989	1-200	88.6	5.6	101.2	3.5	76.2	1.9
Metoxuron	Linear, 1/x	0.9987	1-200	102.0	5.4	105.8	2.5	89.9	2.6
Imazalil	Linear, 1/x	0.9988	1-200	81.4	6.9	86.2	2.0	82.5	2.7
Simazine	Linear, 1/x	0.9984	1-200	91.8	5.4	93.8	1.9	85.4	1.6
Monuron	Linear, 1/x	0.9990	1-200	82.5	9.9	96.0	3.7	88.4	1.8
Thiophanate methyl	Linear, 1/x	0.9977	1-200	89.4	10.8	104.6	5.5	86.0	7.1
Propoxur	Linear, 1/x	0.9993	1-200	84.7	8.1	97.6	1.4	94.5	2.2
Carbofuran	Linear, 1/x	0.9993	1-200	88.3	8.5	98.9	5.1	97.2	2.4
Chlorotoluron	Linear, 1/x	0.9990	1-200	96.3	5.0	97.9	3.1	89.9	2.0
Diuron	Linear, 1/x	0.9995	1-200	86.6	6.7	98.7	2.8	97.5	3.5
Carbaryl	Linear, 1/x	0.9991	1-200	80.7	7.4	101.1	3.2	90.5	2.1
Bentazone	Quadratic, 1/x	0.9993	1-200	111.2	5.5	102.3	4.7	97.4	7.9
Isoproturon	Linear, 1/x	0.9993	1-200	98.7	4.1	98.9	2.3	92.1	2.6
2,3-D acid	Linear, 1/x	0.9985	1-200	64.3	7.6	65.4	5.1	65.6	2.6
Fluometuron	Linear, 1/x	0.9975	1-200	86.2	5.7	87.8	3.9	88.0	3.0
Metobromuron	Linear, 1/x	0.9977	1-200	96.0	6.6	100.3	4.6	92.4	4.5
Cyprodinil	Linear, 1/x	0.9986	1-200	60.3	8.3	67.0	2.6	65.5	3.6
Metazachlor	Linear, 1/x	0.9992	1-200	99.8	5.7	99.4	3.4	94.3	2.8
Propham	Linear, 1/x	0.9985	1-200	85.8	9.7	89.3	3.8	87.0	3.8
Terbuthylazine	Linear, 1/x	0.9993	1-200	90.7	6.5	91.1	2.6	85.8	2.0
Dichlorprop	Linear, 1/x	0.9992	1-200	75.6	9.7	73.3	4.6	76.9	2.3
Siduron	Linear, 1/x	0.9990	1-200	90.2	8.6	92.4	3.5	91.5	2.2
Sebuthylazine	Linear, 1/x	0.9992	1-200	95.3	4.8	89.5	2.5	83.7	2.1
Methiocarb	Linear, 1/x	0.9984	1-200	77.6	8.8	94.7	3.2	86.3	1.9
Linuron	Linear, 1/x	0.9984	1-200	84.7	7.4	85.2	3.6	84.6	3.6
Chlorpropham	Linear, 1/x	0.9994	5-200	91.6	10.0	84.3	9.3	81.1	3.8
Penconazole	Linear, 1/x	0.9992	1-200	83.0	6.3	81.1	2.4	80.7	1.5
Malathion	Linear, 1/x	0.9991	1-200	76.2	7.1	100.5	2.2	100.0	1.0
Neburon	Linear, 1/x	0.9994	1-200	66.9	6.8	83.0	1.6	84.8	1.3
EPN	Linear, 1/x	0.9995	1-200	76.4	4.7	73.8	3.9	62.9	13.2

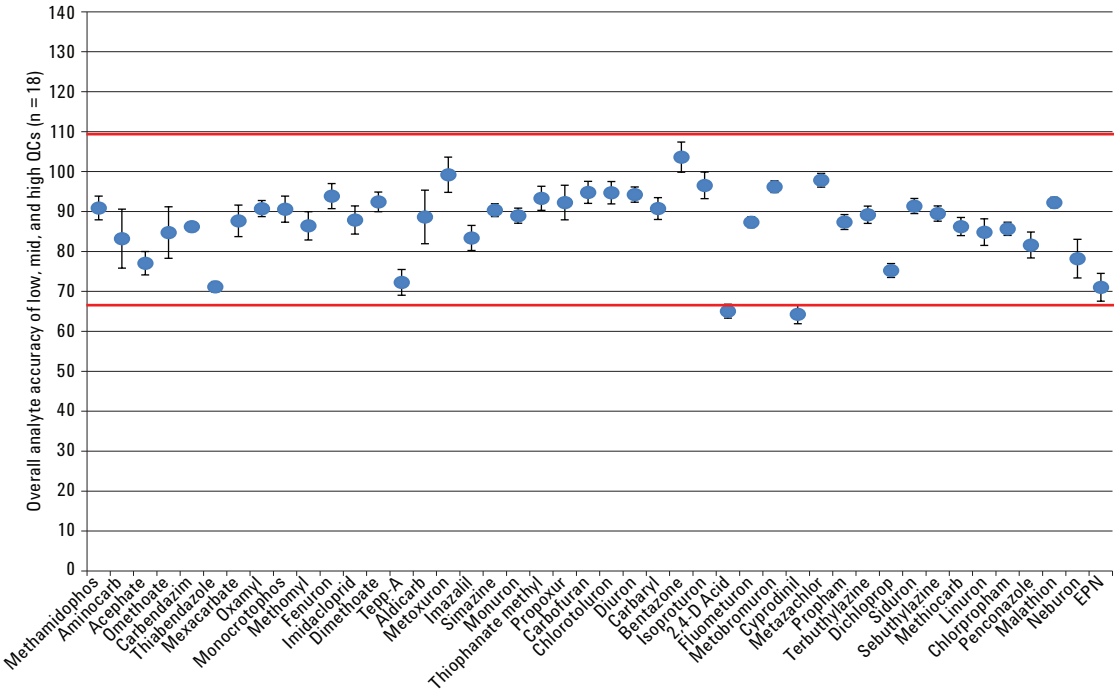

Figure 6, Quantitation results for 44 representative pesticides in avocado using the Agilent Bond Elut Enhanced Matrix Removal-Lipid workflow. The accuracy and precision data were calculated based on 18 total replicates at three different concentrations.

Conclusions

A rapid, reliable, and robust method using a QuEChERS AOAC extraction followed by Agilent Bond Elut EMR—Lipid dSPE cleanup was developed and validated for the analysis of 44 LC-amenable pesticides in avocado. Matrix effect was carefully assessed and compared with traditional C18/PSA dSPE and zirconia sorbent cleanup. Results demonstrate that EMR—Lipid provides superior matrix cleanup than C18/PSA dSPE and zirconia sorbent by weight and matrix effect. Analyte recoveries and method precision were extensively compared between the three different cleanup techniques. EMR-Lipid cleanup provides comparable analyte recoveries relative to C18/PSA dSPE with dramatically fewer coextractives. Both EMR—Lipid and fatty dSPE cleanup delivered much better recovery than zirconia sorbent, due to nonselective analyte interactions with the zirconia. The data suggest that EMR—Lipid removes most matrix, especially lipids, without significantly affecting analyte recovery.

This work demonstrates the superior cleanliness that can be achieved using EMR—Lipid as a dSPE sorbent in a QuEChERS workflow. The sorbent's high selectivity for coextracted lipids makes it ideal for the analysis of fatty samples regardless of the fat content and target analyte list. EMR—Lipid gives high recovery, precision, superior matrix removal, and ease-of-use for the quantitation of pesticides in avocado. Future work will continue to focus on multiresidue analysis in complex, high-fat samples.

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