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Analysis of 136 Pesticides in Avocado Using a Modified QuEChERS Method with LC-MS/MS and GC-MS/MS

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ABSTRACT: A simple and high-throughput screening method for the analysis of 136 pesticides in avocado (*Persea americana*) by LC-(+)-ESI-MS/MS and GC-MS/MS is presented. A modified QuEChERS sample preparation method was developed to improve the extraction recovery of highly lipophilic pesticides. Extracts from minced avocados after acetonitrile (MeCN) extraction were directly injected to LC-MS/MS, whereas other GC-amenable compounds were treated with the modified QuEChERS procedure for GC-MS/MS analysis. The average recoveries for 79 pesticides quantified by LC-MS/MS at 10, 50, and 200 ng/g fortifying levels were 86.1% or better (with maximum RSD at 9.2%), whereas GC-MS/MS analysis demonstrated 70.2% or better (RSD < 18%) for average recovery from 57 compounds at the same spike levels. The application of LC- and GC-MS/MS combined with the improved extraction procedures led to the current method, which can quantitate these pesticides even if they are present in avocados below the targeted action level by FDA. This method demonstrated the improved recovery of several challenging lipophilic pesticides in highly fat-rich avocados.

KEYWORDS: *triple quadrupole, tandem mass spectrometry, liquid chromatography, gas chromatography, pesticide residue analysis, avocado, QuEChERS*

INTRODUCTION

Anastassiades et al.[1] developed a simple extraction method for multiple residues from food samples known as QuEChERS (quick, easy, cheap, effective, rugged, and safe) to optimize extraction and cleanup procedures with an emphasis on minimizing cost, sample size, and preparation time. QuEChERS involves the extraction of pesticides from a homogenized composite sample using MeCN and salt in a centrifuge tube, followed by a solid-phase dispersive cleanup step performed in a second test tube containing sorbents to remove interfering matrix components. This method has been used successfully to screen the presence of pesticides in samples with low fat content, such as fruits and vegetables, using LC-MS/MS and GC/MS-SIM analysis.[2] The screening of more lipophilic pesticides can be difficult in samples with relatively higher fat content because lipid coextractives can adversely affect the extraction efficiency and instrument performances. Therefore, more extraction steps and cleanup procedures are usually conducted to remove most of the lipophilic portions from the sample extracts while simultaneous efforts to maintain the high recovery rates of the pesticides are exercised. Liquid−liquid extraction,[3] gel permeation chromatography (GPC),[4] and freezing technique[5] have been used for sample cleanup steps for fatty matrices. These methods require large amounts of solvents and time-consuming and labor intensive processes. Matrix solid-phase extraction (MSPD),[6] solid-phase microextraction (SPME),[7] and supercritical fluid extraction (SFE)[8] have been used with limited success.

We have previously demonstrated that the QuEChERS procedure is very practical for screening 251 pesticides in high-fat matrices including milk, salmon, fish, shrimp, almond nuts, olive oil, and avocado using LC-MS/MS instrumentation.[9,10] With a more sensitive LC-MS/MS instrument, only 1 μL of sample extract was necessary for analysis while maintaining sufficient sensitivity at 10 ng/g of fortification levels and minimizing matrix suppression effects. There was no noticeable compromise in the peak shape of chromatograms or instrument performance due to the presence of the fat residues in the samples. This method, however, was applicable only to a few classes of LC-amenable compounds such as carbamates, organophosphorus (OP), nitrogen−sulfur−oxygen (NSO), and selected organochlorine (OC) pesticides. This list did not include highly lipophilic pesticides that are incompatible with the positive electrospray ionization [(+)-ESI] mode, such as DDT, chlorothalonil, endosulfan, and hexachlorobenzene. These compounds, therefore, were analyzed by GC-MS/SIM or GC-MS/MS. The QuEChERS extraction approach combined with both LC-MS/MS and GC-MS/SIM detection has been reported by Lehotay et al.[11] to determine pesticides in milk, eggs, avocados, and animal tissues with limited success in the recovery of highly nonpolar pesticides such as hexachlorobenzene and DDE. As the fat content in samples increases, a fat layer is usually formed between the aqueous and organic layers even after centrifugation, which tends to retain the highly nonpolar pesticides, resulting in poor recovery. The study by Lehotay et al.[11] reported that recovery of hexachlorobenzene, DDE, and chlordane in avocado samples was between 25 and

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50%. Koesukwiwat et al.[12] also reported similarly low recovery of lipophilic pesticides (hexachlorobenzene, chlordane, DDT, DDE, and mirex) when the QuEChERS approach was utilized in flaxseed and peanut samples. The objective of this study was to develop an efficient pesticide screening method in avocado (*Persea americana*) using LC-MS/MS and GC-MS/MS analysis in conjunction with the improved QuEChERS extraction method for highly lipophilic pesticides to cover a wider range of pesticide classes (Table 1).

Table 1. Pesticides of Interest in the Study by Class

name	class[a]	possible analytical issue
	Fungicides	
pyrachlostrobin	strobilurin	poor GC sensitivity
chlorothalonil	OC	base sensitive
pyrimethanil	anilinopyrimidine	
imazalil	imidazole	retention time shift in fatty matrix
o-phenylphenol	phenol	poor LC-MS sensitivity
procymidone	dicarboximide	
tebuconazole	triazole	
thiabendazole	benzimidazole	poor GC peak shape
tolyfluanid	*N*-trihalo-methylthio	base sensitive
hexachlorobenzene	OC	poor extractability in QuEChERS
	Insecticides	
bifenthrin	pyrethroid	
aminocarb	carbamate	not stable in GC injector port
chlorpyrifos	pyridine OP	
chlorpyrifos-methyl	pyridine OP	poor LC-MS sensitivity
diclorvos	OP	
DDT	OC	poor LC-MS sensitivity
DDE	OC	poor LC-MS sensitivity
endosulfan	OC	poor LC-MS sensitivity
ethion	OP	
methamidophos	OP	poor peak shape on HP-5 column
acephate	OP	poor peak shape on HP-5 column
permethrin	pyrethroid	
acetamiprid	neonicotinoid	polar, poor GC analyte
	Herbicides	
prometryn	triazine	retention time shift with fatty matrix
linuron	phenylurea	GC inlet instability
trifluralin	dinitroaniline	poor LC-MS sensitivity

[a]OC, organochlorine; OP, organophosphate.

MATERIALS AND METHODS

Chemicals and Materials. Pesticide standard mixes, all ≥99% purity, were purchased from AccuStandards, Inc. (New Haven, CT, USA) consisting of 10 mixtures of analytes (total of 136 compounds) at 100 μg/mL in methanol. A composite pesticide stock solution was prepared at 10 μg/mL. This standard mix is used to fortify 3 g of avocado blank at 50 and 200 ng/g fortification levels by pipetting 15 and 60 μL into the sample before extraction. A 1 μg/mL standard mix was prepared by 1/10 dilution of 10 μg/mL standard mix, and 30 μL was pipetted to 3 g of avocado blank to obtain a 10 ng/g fortification level. Methanol, acetonitrile, and water were of HPLC grade obtained from Fisher Scientific (Pittsburgh, PA, USA), and they were used for HPLC mobile phase and extracting solvent. Formic acid was obtained as 98% solution for mass spectrometry from Fluka (Buchs, Switzerland.). Glacial acetic acid (reagent grade) was purchased from Fisher Scientific. Prepackaged 50 mL centrifuge tubes containing 6 g of magnesium sulfate ($MgSO_4$) and 1.5 g of anhydrous sodium acetate (NaOAc) were purchased from UCT, Inc. (Bristol, PA, USA). Dispersive cleanup tubes (2 mL) containing 150 mg of anhydrous $MgSO_4$, 50 mg of primary and secondary amine (PSA) sorbent, and 50 mg end-capped C-18 sorbent were also from UCT, Inc. Nitrogen and air from TriGas Generator (Parker Hannifin Co., Haverhill, MA, USA) were used for nebulizer and collision gas in LC-MS/MS. Ultrahigh-purity helium and nitrogen from NexAir (Memphis, TN, USA) were employed as the carrier gas and collision gas in GC-MS/MS. EDP 3 electronic pipetters (Rainin Instrument LLC, Oakland, CA, USA) at different capacities (0−10, 10−100, and 100−1000 μL) were used for standard fortification.

LC-MS/MS Analysis. LC-MS/MS analysis was performed using a Shimadzu HPLC system, consisting of two LC-20AD pumps, a Sil-20AC autosampler, and a CTO-20AC column oven (Shimadzu, Kyoto, Japan), coupled with a 4000 Q-TRAP mass spectrometer from AB Sciex (Foster City, CA, USA). Analyst software (version 1.4) was used for instrument control and data acquisition. An Ultra Aqueous C18 column (3 μm, 100 × 2.1 mm) and a guard column (10 × 2.1 mm) from Restek (Bellefonte, PA, USA) were used for HPLC separation at 50 °C with a sample injection volume of 1 μL. A binary mobile phase was composed of (A) 4 mM ammonium formate and 0.1% formic acid in water and (B) 4 mM ammonium formate and 0.1% formic acid in methanol. A mobile phase gradient started at 5% B (0.0−0.4 min) at a flow rate of 0.5 mL/min and went to 60% B at 5 min (curve 3) and then 95% B at 12.5 min (curve 6), was held until 14.5 min, and concluded by column equilibration at initial condition for 3 min for a total run time of 18 min. The MS determination was performed in positive electrospray mode with monitoring of the two most abundant MS/MS (precursor/product) ion transitions using scheduled mulitiple-reaction monitoring (MRM) program for 60 s for each analyte. Table 2 gives analyte-specific MS/MS conditions and LC retention time for the LC-amenable analytes. The MS source conditions were as follows: curtain gas (CUR) of 30 psi, ion spray voltage (IS) of 4500 V, collisionally activated dissociation gas (CAD) is high, nebulizer gas (GS1) of 60 psi, heater gas (GS2) of 60 psi, and source temperature (TEM) of 350 °C

GC-MS/MS Analysis. GC-MS/MS analysis was performed using an Agilent 7890A GC, coupled with a 7693 autosampler, a 7000 triple-quadrupole MS, and a computer with MassHunter software (version B.05.00412) for data acquisition and processing (Agilent Technologies, Palo Alto, CA, USA). Analytes were separated with two HP-5 ms Ultra Inert capillary columns from Agilent (0.25 mm i.d. × 15 m, 0.25 μm film thickness), connected at a back-flush union. The column head pressure was set at 12.772 psi at a constant flow rate of 1.335 mL/min, using helium as a carrier gas. The column temperature was programmed as follows: the initial temperature was 60 °C (for 1 min) and increased to 170 °C at 40 °C/min, ramped to 310 °C at 10 °C/min, then was held for 1.2 min. The total run time was 19 min. The first column was back flushed for 2.0 min at 310 °C. The injector temperature was programmed to start at 60 °C for 0.2 min and ramped to 280 °C at 600 °C/min with no hold time. The injection volume was 1.0 μL in splitless mode. The ion source and transfer line temperatures were 300 °C. Electron multiplier voltage was set to 1400 V by automatic tuning, and the multiplier voltage was 306 V above tune value. Nitrogen and helium were used as the collision gases for all MS/MS experiments, and the pressure in the collision cell was set at 1.5 and 2.25 mTorr, respectively. The optimal two ion transitions (primary and secondary transitions of a precursor to product ions) for MRM of each pesticide were determined via collision tests (Table 3). Quantitation by GC-MS/MS was based on an external standard method with peak area of the primary transition of an analyte product using the Agilent MassHunter software. Concentrations were determined by comparing the peak area in the sample to peak areas of matrix-matched standards prepared at known concentration. Identification of pesticides in fortified and incurred samples by GC-MS/MS was determined by comparing expected retention time and the ratio of the two transition (primary/secondary) results to matrix-

Table 2. Retention Time (RT) and MRM Conditions for LC-MS/MS Analysis[a]

Q1	Q3	RT (min)	analyte	DP	EP	CE	CXP
184.1	143	2.4	acephate 1	61	10	13	4
184.1	49	2.4	acephate 2	61	10	33	4
223	126	5.2	acetamiprid 1	61	10	29	12
223	99	5.2	acetamiprid 2	61	10	53	18
228.1	186.1	7	ametryn 1	71	10	21	4
228.1	96	7	ametryn 2	71	10	35	4
209.1	152	3.1	aminocarb 1	71	10	21	8
209.1	137.1	3.1	aminocarb 2	71	10	35	10
318	160.1	7.1	azinphos-methyl 1	41	10	13	10
318	132	7.1	azinphos-methyl 2	41	10	21	10
224.1	109	5.8	bendiocarb 1	61	10	27	20
224.1	167.1	5.8	bendiocarb 2	61	10	15	12
440.1	181.2	13.6	bifenthrin NH_4 1	51	10	39	14
440.1	166.1	13.6	bifenthrin NH_4 2	51	10	65	10
343	307	7.8	boscalid 1	91	10	27	4
343	140	7.8	boscalid 2	91	10	27	4
197	117.2	4.4	chlordimeform 1	81	10	41	18
197	89	4.4	chlordimeform 2	81	10	71	14
350	198	12.3	chlorpyriphos 1	56	10	25	10
350	97	12.3	chlorpyriphos 2	56	10	47	10
362.8	227	10.2	coumaphos 1	71	10	37	12
362.8	306.9	10.2	coumaphos 2	71	10	25	18
241.1	214.2	5.7	cyanazine 1	66	10	27	18
241.1	104.1	5.7	cyanazine 2	66	10	47	4
199.1	89.1	7.3	cycluron 1	50	10	21	4
199.1	89	7.3	cycluron 2	50	10	21	4
292	70	8	cyproconazole A1	66	10	39	12
292	125	8	cyproconazole A2	66	10	45	8
292.1	70.1	8.4	cyproconazole B1	66	10	39	12
292.1	125.1	8.4	cyproconazole B2	66	10	45	8
318.1	182	6.7	desmedipham 1	41	10	19	12
318.1	136	6.7	desmedipham 2	41	10	33	10
305	169.1	9.9	diazinon 1	86	10	31	10
305	153.1	9.9	diazinon 2	86	10	29	8
350	123	8.3	dichlorfluanid 1	21	10	41	10
350	224	8.3	dichlorfluanid 2	21	10	21	10
220.8	127.1	5.9	dichlorvos 1	71	10	27	22
220.8	109.1	5.9	dichlorvos 2	71	10	25	18
238.1	112.1	4.6	dicrotophos 1	66	10	19	8
238.1	193	4.6	dicrotophos 2	66	10	15	14
406.1	251.1	11.6	difenoconazole 1	81	10	37	16
408.2	253.1	11.6	difenoconazole 2	76	10	33	4
230	199	4.6	dimethoate 1	50	10	14	15
230	125	4.6	dimethoate 2	50	10	27	8
388.1	301	8.1	dimethomorph A 1	66	10	25	4
388.1	165.1	8.1	dimethomorph A 2	66	10	45	4
388.2	301.1	8.4	dimethomorph B 1	66	10	25	4
388.2	165.2	8.4	dimethomorph B 2	66	10	45	4
224.1	167	4.7	dioxacarb 1	51	10	13	10
224.1	123	4.7	dioxacarb 2	51	10	23	24
330	121.1	9.5	epoxiconazole 1	66	10	29	10
330	101.1	9.5	epoxiconazole 2	66	10	69	18
162	119	8.4	ethiolate 1	106	10	23	20
162	120.1	8.4	ethiolate 2	106	10	19	20
384.8	199.2	12	ethion 1	51	10	15	18
384.8	142.9	12	ethion 2	51	10	39	24
287.1	121.1	7.1	ethofumesate 1	81	10	23	8
287.1	259.1	7.1	ethofumesate 2	81	10	15	16
394.2	177.3	13.6	etofenprox NH_4^+ 1	46	10	21	12
394.2	107.2	13.6	etofenprox NH_4^+ 2	46	10	61	18
337	124.9	9.4	fenbuconazole 1	81	10	41	8

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Table 2. continued

Q1	Q3	RT (min)	analyte	DP	EP	CE	CXP
337	70	9.4	fenbuconazole 2	81	10	39	12
302.1	88	9.2	fenoxycarb 1	66	10	31	6
302.1	116.1	9.2	fenoxycarb 2	66	10	17	8
304	147	7.2	fenpropimorph 1	66	10	39	4
304	117	7.2	fenpropimorph 2	66	10	71	4
266	229	7.6	fludioxinil 1	41	10	23	14
266	227.1	7.6	fludioxinil 2	41	10	13	14
376	307	8.5	fluquinconazole 1	71	10	33	4
376	349	8.5	fluquinconazole 2	71	10	25	4
324.1	262.1	7.5	flutolanil 1	76	10	27	16
324.1	242.1	7.5	flutolanil 2	76	10	37	14
314.1	70	10.3	hexaconazole 1	56	10	41	12
314.1	159	10.3	hexaconazole 2	56	10	41	14
297	159	6.5	imazalil 1	66	10	33	14
297	201	6.5	imazalil 2	66	10	27	12
249.1	160	7.7	linuron 1	61	10	23	4
249.1	182.1	7.7	linuron 2	61	10	21	4
331	127.1	7.5	malathion 1	46	10	17	10
331	99.1	7.5	malathion 2	46	10	31	10
142	94	1.7	methamidophos 1	55	10	20	4
142	125	1.7	methamidophos 2	55	10	19	8
284.2	252.2	8.7	metolachlor 1	56	10	21	10
284.2	176.2	8.7	metolachlor 2	56	10	33	10
166.2	109.1	5.6	metolcarb 1	36	10	15	10
166.2	94.2	5.6	metolcarb 2	36	10	37	10
225.1	127.1	4.7	mevinphos-E 1	55	10	20	8
225.1	193.2	4.7	mevinphos-E 2	55	10	10	13
225	127	5.2	mevinphos-Z 1	55	10	20	8
225	193.1	5.2	mevinphos-Z 2	55	10	10	13
224.1	127.1	4.1	monocrotophos 1	51	10	23	12
224.1	98	4.1	monocrotophos 2	51	10	17	4
215.1	126.1	6.4	monolinuron 1	51	10	23	4
215.1	99	6.4	monolinuron 2	51	10	41	4
289	70	8.3	myclobutanil 1	71	10	37	12
289	125	8.3	myclobutanil 2	71	10	47	8
315	252.1	7.4	nuarimol 1	81	10	31	16
315	81	7.4	nuarimol 2	81	10	45	14
214	124.9	3	omethoate 1	46	10	29	4
214	182.8	3	omethoate 2	46	10	17	4
284.1	159	10.4	penconazole 1	71	10	39	10
284.1	70	10.4	penconazole 2	71	10	37	12
318	160	7.1	phosmet 1	51	10	19	10
318	133	7.1	phosmet 2	51	10	49	10
356.2	177.2	12.1	piperonyl butoxide 1	51	10	19	10
356.2	119.1	12.1	piperonyl butoxide 2	51	10	51	8
239.2	72.1	5.9	pirimicarb 1	66	10	35	12
239.2	182.1	5.9	pirimicarb 2	66	10	23	12
376	308	10.9	prochloraz 1	46	10	17	10
376	70	10.9	prochloraz 2	46	10	45	12
242.2	158.1	7.8	prometryn 1	71	10	35	4
242.2	200.1	7.8	prometryn 2	71	10	19	4
212.2	169.9	6.6	propachlor 1	66	10	23	30
212.2	93.9	6.6	propachlor 2	66	10	39	16
368.2	231.1	12.6	propargite 1	46	10	15	14
368.2	175.1	12.6	propargite 2	46	10	23	12
342.1	159	10.6	propiconazole 1	61	10	39	10
342.1	69	10.6	propiconazole 2	61	10	37	12
210.1	111	5.8	propoxur 1	39	10	19	6
210.1	168.1	5.8	propoxur 2	39	10	11	11
218.1	125	6	pyracarbolid 1	61	10	27	8
218.1	97	6	pyracarbolid 2	61	10	41	18

Table 2. continued

Q1	Q3	RT (min)	analyte	DP	EP	CE	CXP
388	194	10.5	pyraclostrobin 1	31	10	19	4
388	163	10.5	pyraclostrobin 2	31	10	29	4
365	147	13.3	pyridaben 1	46	10	31	4
365	309	13.3	pyridaben 2	46	10	19	4
200	107	7.7	pyrimethanil 1	71	10	33	4
200	82	7.7	pyrimethanil 2	71	10	35	4
308.1	162.1	12.9	quinoxyfen 1	81	10	65	10
308.1	197.1	12.9	quinoxyfen 2	81	10	45	12
226.2	170.1	6.5	secbumeton 1	50	10	35	4
226.2	100	6.5	secbumeton 2	50	10	35	4
298.2	144.2	7.9	spiroxamine 1	76	10	29	12
298.2	100.1	7.9	spiroxamine 2	76	10	47	18
323	115	8.9	sulfotep 1	46	10	39	10
323	97.1	8.9	sulfotep 2	46	10	45	10
308.2	70	9.9	tebuconazole 1	81	10	49	12
308.2	125	9.9	tebuconazole 2	81	10	51	8
334	117	12.1	tebufenpyrad 1	71	10	47	4
334	145	12.1	tebufenpyrad 2	71	10	37	4
230.3	174.2	7.7	terbutylazine 1	41	10	27	10
230.3	68	7.7	terbutylazine 2	41	10	59	10
372.1	159	8.8	tetraconazole 1	76	10	45	10
372.1	70	8.8	tetraconazole 2	76	10	47	12
202.1	175.1	4.9	thiabendazole 1	85	10	35	12
202.1	131.2	4.9	thiabendazole 2	85	10	45	8
364	237.9	9.5	tolyfluanid 1	6	10	19	10
364	137.1	9.5	tolufluanid 2	6	10	37	10
294	197.1	7.8	triadimefon 1	66	10	23	14
294	225	7.8	triadimefon 2	66	10	19	8
296.1	70	8	triadimenol 1	46	10	31	12
296.1	227.1	8	triadimenol 2	46	10	19	14
314	162	8.3	triazophos 1	56	10	25	10
314	119	8.3	triazophos 2	56	10	49	10
190	163	5.8	tricyclazole 1	81	10	33	10
190	136	5.8	tricyclazole 2	81	10	41	12
409	186	11.2	trifloxystrobin 1	31	10	23	4
409	206	11.2	trifloxystrobin 2	31	10	21	4
346.1	278.1	11.7	triflumizole 1	51	10	15	8
346.1	73	11.7	triflumizole 2	51	10	27	6
346.1	278.1	11.8	triflumizole 1	51	10	15	8
346.1	73	11.8	triflumizole 2	51	10	27	6

[a]Compound-dependent parameters: DP, declustering potential; CE, collision energy; EP, entrance potential; CXP, collision cell exit potential.

matched standards, following the criteria for identification established by the FDA and European Union.[13]

Sample Preparation Procedure. Avocados were obtained from a local market. The skin was removed, and they were cut into 3−5 cm cubes with a knife. The meat was minced by a blender/homogenizer Robot Coupe Blixer 3 (Robot Coupe USA, Jackson, MS, USA), with pulsed action until contents were uniform and had the consistency of smooth paste. The samples were weighed at 3 g each in a 50 mL centrifuge tube (Fisher Scientific) and stored in a freezer at −20 °C until use.

Sample Extraction. The frozen sample was thawed to room temperature and fortified with appropriate standard mixture to obtain standard concentrations of 10, 50, and 200 ng/g. The samples were allowed to stand for approximately 1 h. The nonfortified sample (blank) was also prepared to generate blank matrix for matrix-matched standard. Later, 5 mL of purified water and 25 mL of 1% acetic acid in MeCN were added to the sample tube. The tube was capped tightly and shaken for 10 min on a SPEX 2000 Geno grinder (SPEX Sample Prep LLC, Metuchen, NJ, USA) at 1000 strokes/min. Furthermore, 1.5 g of NaOAc and 6 g of $MgSO_4$ were added into the tube, and the mixture was shaken for another 10 min at the same speed and then centrifuged at 3500 rpm for 10 min. Approximately 1 mL of MeCN extract (top layer) was transferred into an autosampler vial, and 1 μL of the extract was injected to LC-MS/MS for LC-amenable pesticides. The pesticide concentration in the extract was quantified against standard in acetonitrile at the same concentration.

For GC-MS/MS analysis, 1 mL of MeCN extract was pipetted into a 2 mL dispersive tube containing 150 mg of anhydrous $MgSO_4$, 50 mg of PSA sorbent, and 50 mg of C18 sorbent, capped, spun for 1 min on a vortex mixer, and then centrifuged at 2000 rpm for 10 min. The sample extract was transferred into an autosampler vial and injected (1 μL) on the GC-MS/MS for GC-amenable pesticides. For quantification, the matrix-matched standard of avocado extract was prepared at 10, 50, and 200 ng/g fortifying levels equivalent by adding appropriate volumes of mixed fortification standard to the blank sample extract (after PSA/C18 dispersive cleanup).

RESULTS AND DISCUSSION

Optimization of Sample Extraction Procedure. Previously, the QuEChERS extraction technique was used to

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Table 3. GC-MS/MS Conditions for GC-Amenable Pesticides

	precursor 1	product 1	collision energy	precursor 2	product 2	collision energy	RT (min)
amitraz	293.1	162	6	293.1	132	25	14.77
benfluralin	292	160	22	292	206	12	7.29
BHC-α	219	183	7	181	145	15	7.64
BHC-β	219	183	8	217	181	7	8.03
BHC-δ	219	183	8	217	181	7	8.51
BHC-γ	219	183	8	217	181	7	8.04
bromopropylate	338.9	182.9	18	342.9	184.9	18	13.89
cadusafos	159	97	24	158	81	15	7.44
chlorothalonil	265.9	133	53	265.9	169.9	28	8.59
chlorpyrifos-methyl	285.9	93	24	285.9	208	15	9.13
cypermethrin	181	152	30	163	127	4	16.56
dacthal	298.9	164.9	54	300.9	222.9	30	10.04
DEF	202	147	2	202	113	18	11.57
dieldrin	262.9	192.9	40	262.9	190.9	38	11.70
dinitramine	261	195	23	261	241	10	8.40
endosulfan sulfate	271.9	236.9	15	271.9	116.9	48	13.00
endosulfan I	240.9	205.9	15	195	159	8	11.25
endosulfan II	195	159	8	240.9	205.9	15	12.25
endrin	262.9	192.9	40	262.9	190.9	38	12.10
EPN	157	110	14	185	110.1	25	13.92
Etridiazole	210.9	182.9	9	210.9	139.9	26	5.87
fenarimol	219	107	12	251	139	15	15.06
fenvalerate 1	167	125	12	125	89	23	17.38
fenvalerate 2	167	125	12	125	89	23	17.58
fluvalinate 1	250	55	18	250	200	24	17.55
fluvalinate 2	250	55	18	250	200	24	17.60
heptachlor epoxide	352.8	262.8	15	352.8	281.9	18	10.60
hexachlorobenzene	283.9	213.9	40	283.8	248.9	22	7.78
L-cyhalothrin	197	141	13	181	152	29	14.85
iprodione	314	56	24	314	245	10	13.68
methyl parathion	263	109	12	263	79	32	9.13
MGK-264	164	80	32	164	98	12	10.42
napropamide	271.1	72	15	271.1	128	2	11.39
o,p′-DDT	235	165	30	235	199	18	12.42
o,p′-methoxychlor	227	121	15	121	78	26	13.19
o-phenylphenol	170	115.1	45	170	141	30	6.27
oxadixyl	163	132	10	163	117	30	12.42
p,p′-DDE	246	176	35	318	246	25	11.60
p,p′-DDT	235	165	30	235	199	18	13.01
parathion	291	109	10	291	81	35	9.96
pentachloroaniline	262.9	191.9	25	264.9	193.9	28	8.91
pentachlorobenzene	249.9	214.9	21	249.9	141.9	50	6.38
permethrin-*cis*	183	153	18	183	115	30	15.62
permethrin-*trans*	183	153	18	183	115	30	15.74
phosalone	182	75	36	182	111	17	14.56
pirimiphos-methyl	290	125	24	290	233	10	9.58
procymidone	283	96	10	283	67	37	10.83
profenofos	336.9	266.9	14	336.9	188	32	11.53
pronamide	173	74	50	173	109	30	8.18
propanil	161	99	30	217	161	7	8.93
pyriproxifen	136	41.1	18	136	78.1	32	14.60
quinalphos	157	102	28	146	118	10	10.72
tetradifon	353.9	159	12	353.9	227	9	14.39
tolclofos-methyl	265	93	26	265	109	52	9.22
triallate	268	183.9	20	268	226	12	8.56
trifluralin	306	264	7	306	160	25	7.25
vinclozolin	212	172	16	187	124	22	9.10

determine the lipophilic insecticide teflubenzuron in salmon by LC-MS/MS.[14] It was noted that a visible fat layer was formed between the aqueous (bottom) and MeCN layers (top) after the extraction and centrifugation steps, due mostly to different

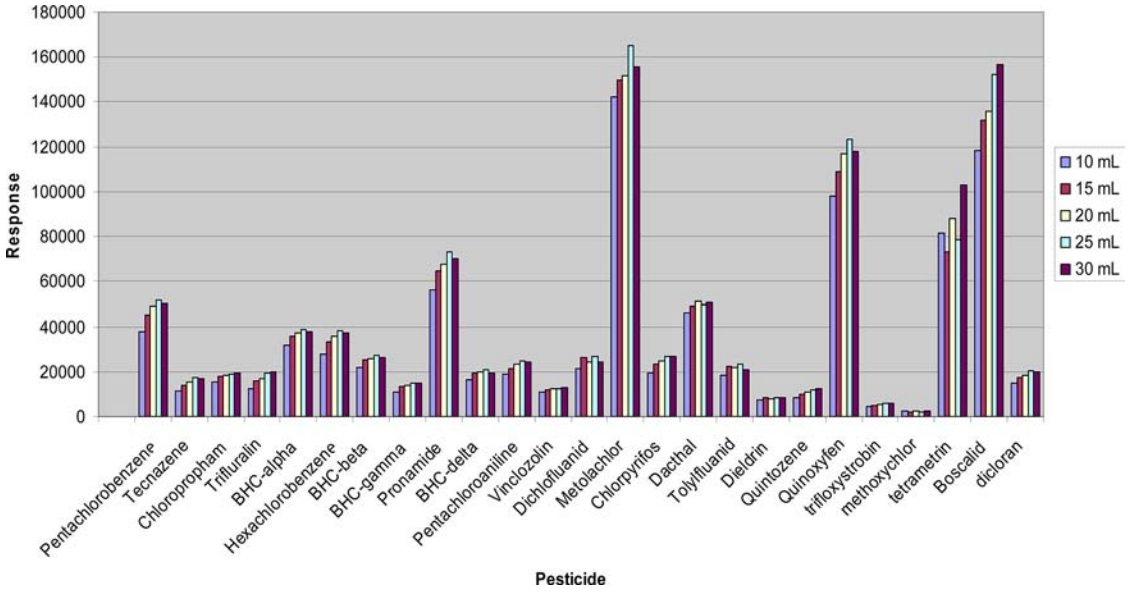

Figure 1. Response of pesticide extraction from 3 g of avocado using different amounts of MeCN and analyzed by GC-MS/MS (after matrix concentration adjustment).

miscibility between these three fractions. The middle fat layer likely plays a significant role in retaining more lipophilic pesticides, which results in poor recovery of those analytes in the MeCN layer. The extraction efficiency of pesticide residues from the sample and/or fat layer to the MeCN layer are affected by the partition coefficient of the analytes between the sample/fat layer and the extracting solvent. The sample/solvent ratio in the conventional QuEChERS extraction method is 1:1 (15 g of sample vs 15 mL of MeCN). We decided to use a modified version of AOAC Official Method 2009.01 (also called "buffered QuEChERS" method) utilizing acidic acetonitrile and NaOAC to improve recovery for base-sensitive pesticides (e.g., chlorthalonil and tolyfluanid).[15] With the increase in the ratio between solvent and sample, the extraction efficiency of the analytes was also improved.

Thus, an extraction experiment with different sample/solvent ratios was evaluated. Five avocado samples (3 g each in 50 mL plastic centrifuge tubes) were fortified with 100 μL of solution of 10 μg/mL containing 25 selected lipophilic organochlorine (OC) pesticides. Different amounts of MeCN with 1% acetic acid (10, 15, 20, 25, and 30 mL) were added to the sample to represent the solvent/sample ratios of 3.3, 5, 6.6, 8.3, and 10 to 1, respectively. Five milliliters of purified water was added to the tube, and they were shaken on the SPEX 2000 Geno Grinder at 1000 strokes/min for 10 min. A salt packet containing 6 g of $MgSO_4$ and 1.5 g of NaOAc was added to the tube followed by another 10 min shake. The samples were then centrifuged at 3000 rpm for 10 min. Two milliliters of MeCN extract was pipetted into a 15mL centrifuge tube, and the appropriate amount of MeCN was added to adjust the matrix concentration to 0.1 g sample/mL solvent. The samples were injected onto the GC-MS/MS.

The responses of the selected OC pesticides extracted from 3 g of avocado using different amounts of 1% acetic acid in MeCN are presented in Figure 1. It demonstrates that the extraction efficiency of the lipophilic OC pesticides can be significantly enhanced by decreasing the sample/solvent ratio from 3 g/10 L to 3 g/30 mL. By utilizing a sample-to-solvent ratio of 3 g/10 mL to 3 g/30 mL, the extraction yields of trifluralin, hexachlorobenzene, chlorpyrifos, *p,p′*-DDT, and dichloran were increased by 58, 38, 39, 40, and 36%, respectively. The sample/solvent ratio of 1:10 did not significantly improve the extraction; rather it seemed to dilute the sample, resulting in poor sensitivity. Therefore, the sample weight of 3 g and 25 mL of extraction solvent were selected for the method in this study as a way to improve the extraction efficiency while maintaining the sensitivity.

LC-MS/MS Analysis. In the previous work,[10] avocados were extracted with MeCN using a sample-to-solvent ratio of 1:3 (5 g of avocado mousse in 5 mL of water to 15 mL of MeCN), and it worked well with polar and moderately nonpolar pesticides. The sample extract was processed with dispersive cleanup and diluted with water at a 1:1 ratio prior to LC-MS/MS analysis. In this method, a higher sample/solvent ratio (1:8.3, with 3 g avocado in 5 mL of water to 25 mL of 1% acetic acid in MeCN) was used to improve the recovery of highly lipophilic pesticides. The concentration of sample/solvent is much lower than that from the previous method (0.12 vs 0.33 g/mL). The instrument for LC-MS/MS analysis for the study (QTRAP4000 from AB Sciex) has a sufficient sensitivity; thus, 1 μL of the final extract was more than enough to obtain adequate sensitivity and signal-to-noise (S/N) level even at the lowest fortifying level (10 ng/g). The matrix effect has been tested by comparing the responses of the standard mix (fortified at 50 ng/mL) between avocado blank extract and MeCN. Seventy of 79 compounds evaluated showed responses of analyte in the matrix within 80−110% of those from MeCN. Only three compounds were outside 80−120%, which are acceptable ranges under MS signal suppression/enhancement criteria. This modification improved overall recovery for multiresidue screening purposes while shortening the sample preparation steps by bypassing the dispersive cleanup and dilution steps. It also eliminates the need of using matrix-matched standard.

LC-MS/MS is suitable for the determination of heat-labile pesticides (carbamates) and polar pesticides (neonicotinoids and OPs) that are challenging if not impossible to analyze with GC-MS/MS. Some OP pesticides (for example, methamido-

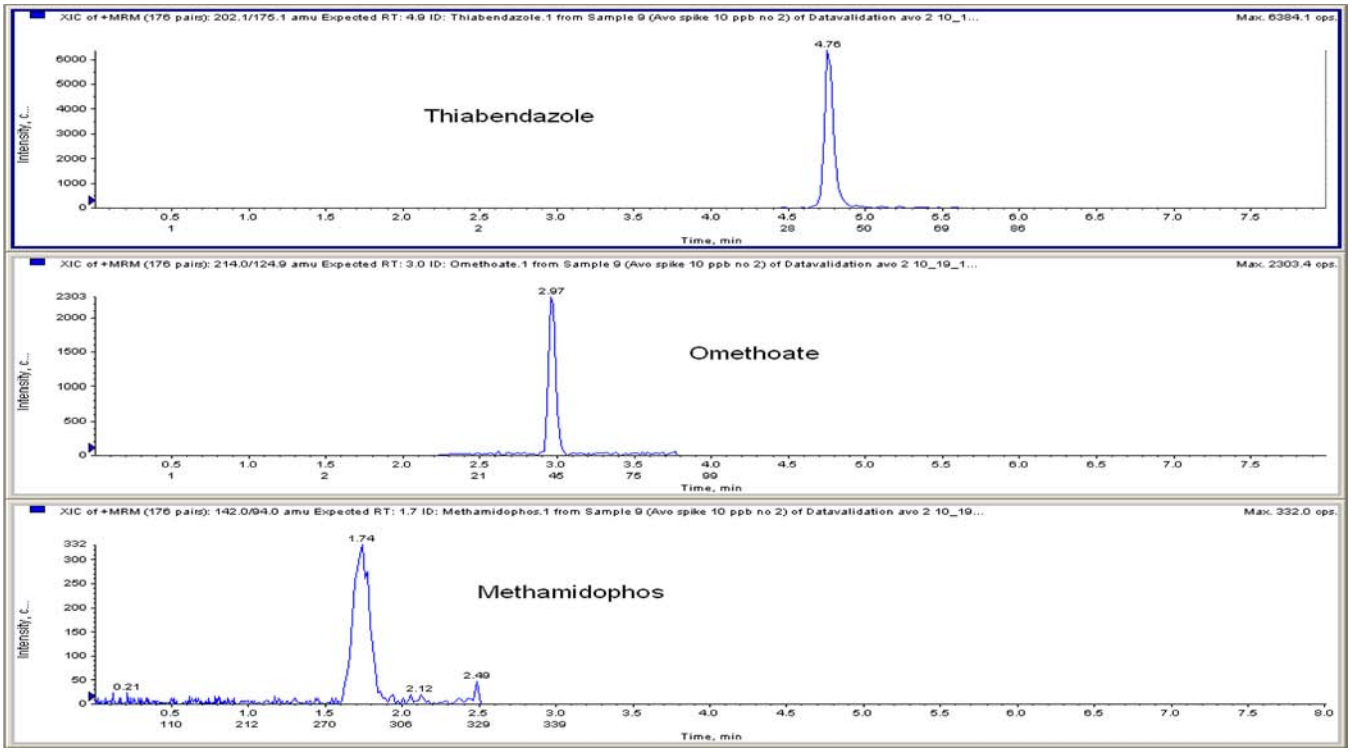

Figure 2. LC-MS/MS chromatogram (MRM) of methamidophos, omethoate, and thiabendazole spiked in blank avocado at 10 ng/g. The sample concentration is 0.12 g sample/mL solvent with 1 μL injection volume.

phos and omethoate) tended to show peak tailing via interaction with or adsorption onto active sites of the injector port or stationary phase during the GC separation, which can impede the accurate quantitation of these compounds, particularly at the trace levels. To overcome this issue, most of the OP pesticides are analyzed on a more polar GC column,[16] and therefore a separate GC injection is required. Thiabendazole (a fungicide) is another compound usually analyzed by a polar GC column.[17] The representative chromatograms of methamidophos, omethoate, and thiabendazole in avocado blank fortified at 10 ng/g levels analyzed by LC-MS/MS are shown in Figure 2. The peak shapes and sensitivity of these compounds are good enough with little or no interference. Most of the compounds analyzed by LC-MS/MS demonstrated excellent recoveries (Table 4), partly because the sample extracts were subjected to a relatively shorter extraction procedure with no sample cleanup. The chromatograms from avocado blank crude extracts have very few interference peaks when compared with those from avocado blanks spiked with 10 and 50 ng/g (Figure 3).

GC-MS/MS Analysis. LC-MS/MS analysis with electrospray ionization (ESI) interface is suitable for polar and moderately nonpolar pesticides containing labile functional groups. To screen a broad spectrum of pesticides including more lipophilic OC pesticides such as DDT, hexachlorobenzene, and dieldrin, a complementary technique such as GC-MS/SIM is required. GC-MS/SIM is a preferred technique for pesticide analysis over GC−electron capture detector (ECD) or GC−flame photometric detector (FPD), because it monitors ions with unique mass to charge ratios (m/z) for each specific target pesticides. In certain instances, these ions may not be exclusively specific only to the analytes of interests, and the ion abundances may result from coextractives in the plant matrix, which can skew the ratios among the target ions. Recently, GC-MS/MS instrumentation has been used by some pesticide laboratories for multiresidue targeted screening of pesticides in food samples.[18,19] In MS/MS, target masses are selected in the first quadrupole and fragmented in a collision chamber. Depending on the analyte, unique product ions are generated from the collision chamber, and only selected product ions are allowed to pass through the second quadrupole to be monitored and detected. The fragmentation patterns and resulting product ions are dependent on the chemical structures of the target analytes; thus, GC-MS/MS mode is more selective than GC-MS/SIM.[19] A recent study by Okihashi et al.[18] identified and confirmed the presence of about 260 pesticides in fresh produce by MS/MS with improved limits of detection (LOD, at 0.01 μg/g) over GC-element selective detection (e.g., flame photometric detection) and GC-MS/SIM.

In conventional pesticide analysis using the QuEChERS extraction method via GC-MS/SIM, the sample extracts must be concentrated (to approximately 2−4 g sample/mL solvent) to detect pesticides at the low nanogram per gram range in produce.[1] The monitoring of lipophilic pesticides at a trace level can be challenging, especially when matrices with abundant fats, such as avocado (10−20% fat content), are involved. The QuEChERS approach with MeCN extraction has already shown to be effective in minimizing coextraction of lipids from fatty foods due to low solubility of the lipids in MeCN while maintaining high recoveries of a wide range of relatively polar LC- and semipolar GC-amenable pesticides.[10] After the extraction, $MgSO_4$ and NaOAc were added to enhance the pesticide partitioning into MeCN. This is critical especially when considering the polar pesticides such as methamidophos and acephate that tend to retain in the aqueous phase.[1]

The dispersive SPE with $MgSO_4$−PSA−C18 sample cleanup technique has been used with QuEChERS extraction in

Table 4. Average Recovery and RSD of 79 Pesticides Spiked in Avocado at Three Different Concentrations via LC-MS/MS Analysis (n = 5)

	10 ng/g spike level		50 ng/g spike level		200 ng/g spike level	
	recovery (%)	RSD (%)	recovery (%)	RSD (%)	recovery (%)	RSD (%)
acephate	104.9	5.0	82.6	11.8	92.6	6.3
acetamiprid	102.7	6.7	84.6	8.9	96.4	3.9
ametryn	99.8	3.9	84.3	11.4	91.4	6.1
aminocarb	104.4	2.4	83.9	10.3	93.4	5.3
azinphos-methyl	115.0	7.3	87.7	11.1	98.3	5.6
bendiocarb	104.7	6.1	85.1	10.7	93.9	8.8
bifenthrin	121.6	7.1	105.6	14.3	85.7	6.0
boscalid	120.2	6.7	88.5	15.9	95.2	3.9
chlordimeform	102.3	9.3	86.7	12.7	91.9	4.4
chlorpyrifos	99.0	5.7	81.9	11.2	91.8	4.4
coumaphos	115.0	2.9	87.1	13.2	95.9	6.0
cyanazine	121.0	4.6	91.9	10.9	103.0	3.8
cycluron	104.0	9.2	86.7	14.3	93.9	6.0
cyproconazole A	116.6	8.3	115.2	34.6	102.5	5.1
cyproconazole B	110.8	5.9	108.3	29.9	103.4	6.8
desmedipham	112.0	3.7	87.1	11.7	95.3	4.7
diazinon	99.8	9.1	84.1	11.0	92.3	4.6
dichlorfluanid	83.2	18.8	77.2	9.3	86.8	4.0
dichlorvos	80.8	14.8	74.7	5.7	93.8	9.5
dicrotophos	103.6	3.2	84.1	11.9	92.6	5.2
difenoconazole	111.6	5.1	87.3	12.6	100.3	6.9
dimethoate	103.3	4.6	83.9	12.3	92.8	4.2
dimethomorph A	97.1	5.3	90.3	9.3	98.1	4.9
dimethomorph B	116.0	8.6	86.6	9.5	100.6	5.1
dioxacarb	97.2	4.0	83.5	12.1	92.9	5.3
epoxiconazole	107.5	4.9	86.5	11.6	98.7	6.8
ethiolate	102.0	8.3	88.8	15.8	94.0	8.7
ethion	98.3	6.5	83.3	11.4	92.4	5.5
ethofumesate	107.4	16.9	84.3	14.2	96.3	6.6
fenbuconazole	104.1	14.4	92.0	12.3	102.3	7.2
fenoxycarb	105.0	7.1	82.1	11.2	94.1	4.9
fenpropimorph	110.6	8.4	82.0	11.7	92.0	6.2
fludioxinil	118.0	13.6	83.9	16.9	102.5	8.9
fluquinconazole	146.2	7.5	90.4	18.0	97.6	5.1
flutolanil	109.0	4.9	85.8	13.7	93.3	3.5
hexaconazole	117.0	4.2	88.4	14.6	100.9	9.5
imazalil	123.4	8.6	94.5	13.9	97.7	6.3
linuron	103.2	12.4	87.4	14.2	97.1	5.3
malathion	113.0	2.3	83.1	15.9	93.3	7.7
methamidophos	102.5	2.5	81.7	11.3	94.4	6.3
metolachlor	100.1	5.9	83.3	13.3	93.5	4.6
metolcarb	108.1	8.2	84.1	11.0	90.4	3.1
mevinphos-E	99.6	14.7	83.9	9.3	91.1	4.8
mevinphos-Z	97.0	3.3	82.5	8.7	90.4	4.5
monocrotophos	105.0	4.8	85.1	11.8	93.1	5.4
monolinuron	110.4	3.1	87.0	11.6	93.0	4.5
myclobutanil	111.2	12.6	91.8	7.8	96.5	4.5
nuarimol	137.0	15.4	82.4	11.3	98.6	7.3
omethoate	101.9	2.7	83.8	10.9	95.2	6.0
penconazole	113.4	7.9	88.4	13.6	96.4	5.8
phosmet	104.8	3.1	85.4	8.3	96.0	6.4
piperonyl butoxide	106.0	4.1	83.0	10.4	91.5	6.6
pirimicarb	104.3	2.9	84.5	11.1	93.0	5.6
prochloraz	124.2	29.9	83.9	10.7	92.6	5.8
prometryn	101.0	8.5	85.6	10.4	95.7	5.4
propachlor	101.0	4.5	81.2	12.6	92.2	5.5
propargite	109.2	6.7	84.2	7.0	91.8	5.5
propiconazole	106.2	7.1	85.0	13.2	97.3	9.7

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Table 4. continued

	10 ng/g spike level		50 ng/g spike level		200 ng/g spike level	
	recovery (%)	RSD (%)	recovery (%)	RSD (%)	recovery (%)	RSD (%)
propoxur	97.0	5.1	83.8	10.3	92.4	4.2
pyracarbolid	101.3	3.2	82.9	13.4	93.0	5.7
pyraclostrobin	109.6	7.6	83.8	10.9	93.0	5.3
pyridaben	95.2	7.1	78.6	10.2	85.8	5.5
pyrimethanil	107.0	15.4	91.2	12.0	93.3	6.5
quinoxyfen	105.6	6.5	84.6	9.3	92.0	3.1
secbumeton	103.8	5.8	82.2	8.7	92.7	5.1
spiroxamine	104.6	4.3	83.4	12.6	94.5	6.1
sulfotep	108.2	7.7	84.7	11.8	91.7	5.6
tebuconazole	110.6	5.9	88.2	9.8	102.7	9.6
tebufenpyrad	106.8	10.9	81.9	11.6	95.3	5.9
terbutylazine	101.4	5.9	84.0	8.8	93.4	4.3
tetraconazole	112.4	10.7	89.4	5.6	104.0	5.9
thiabendazole	110.6	4.2	84.9	10.2	94.4	7.3
tolyfluanid	129.6	4.2	86.7	9.6	89.8	6.0
triadimefon	95.9	16.4	86.8	7.0	99.9	6.1
triadimenol	102.9	25.3	89.1	7.7	102.9	6.6
triazophos	104.3	4.5	84.0	9.1	93.3	4.2
tricyclazole	96.8	4.2	82.7	10.6	90.9	5.9
trifloxystrobin	101.5	5.7	84.0	11.1	92.1	4.7
triflumizole	103.1	3.8	82.7	10.9	90.4	5.2
av	107.1		86.1		94.8	
SD	9.9		5.8		4.0	
RSD (%)	9.2		6.7		4.2	

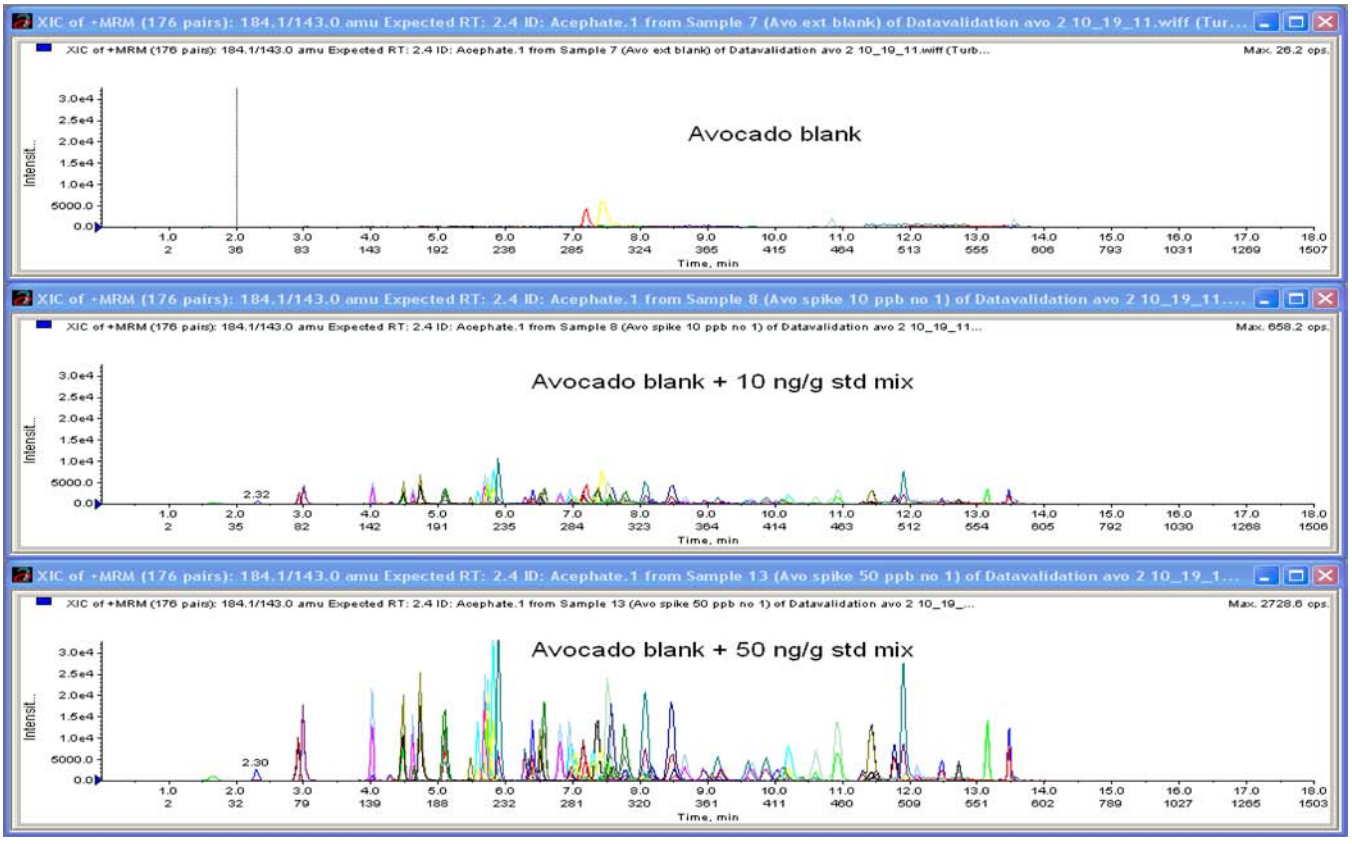

Figure 3. Reconstructed LC-MS/MS chromatogram of avocado blank, avocado blank fortified at 10 ng/g, and avocado blank spiked with 50 ng/g standard mix. The sample concentration is 0.12 g sample/mL solvent with 1 μL injection volume.

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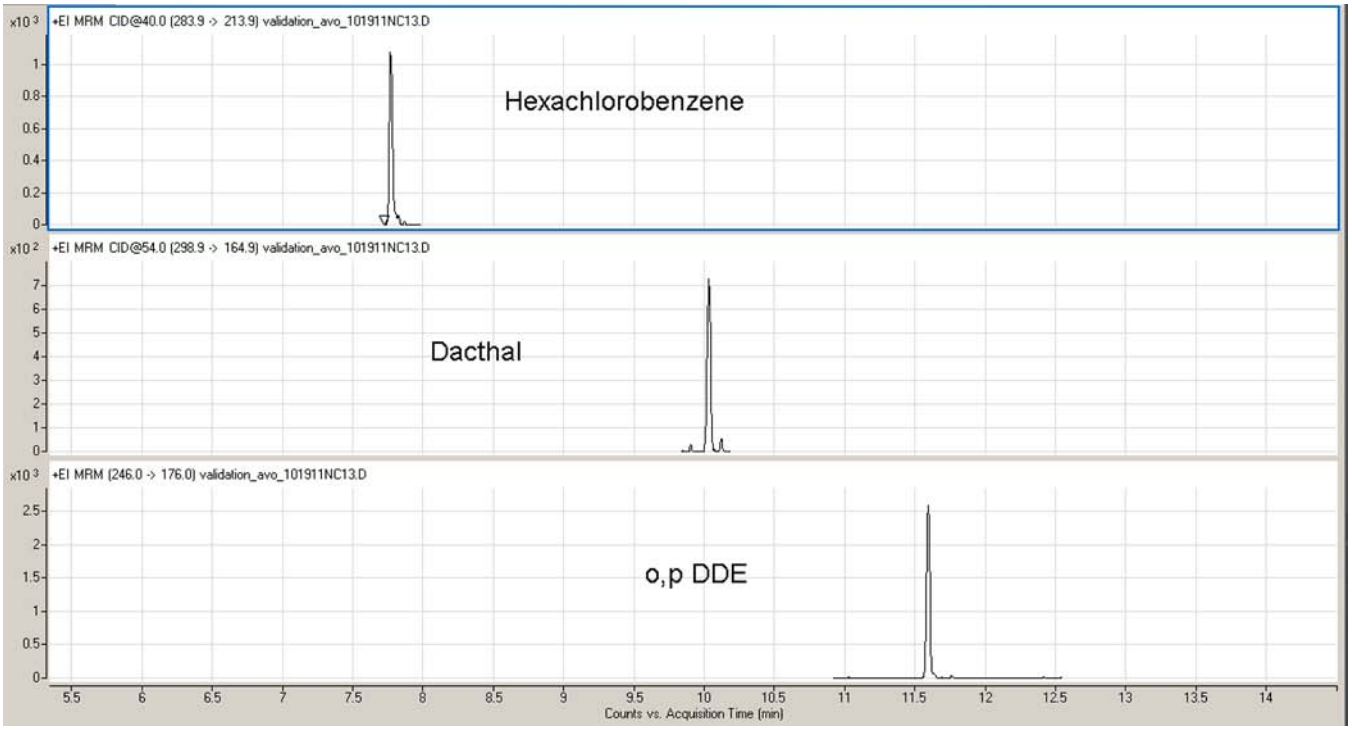

Figure 4. GC-MS/MS chromatogram (MRM) of hexachlorobenzene, dacthal, and *o,p*-DDE spiked in avocado blank at 10 ng/g. The extract concentration is 0.12 g/mL with 1 μL injection volume.

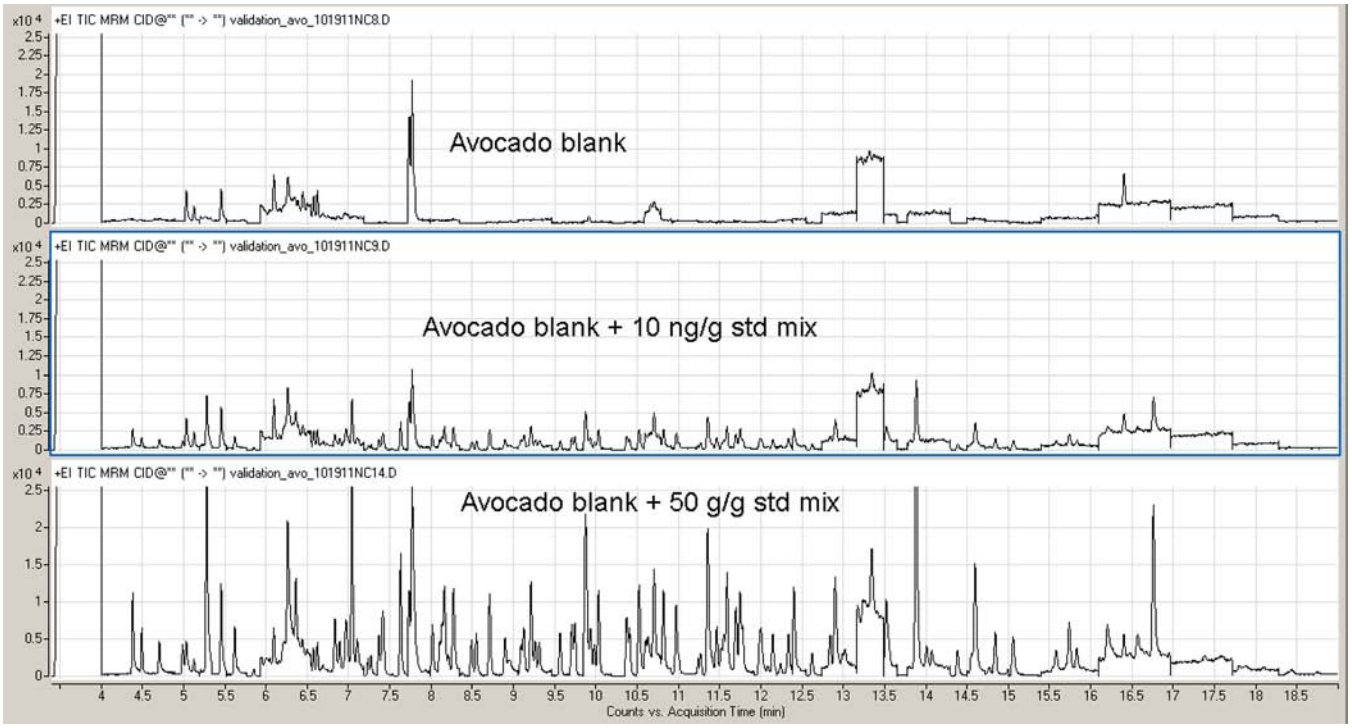

Figure 5. Reconstructed GC-MS/MS chromatograms of avocado blank and avocado blank fortified at 10 and 50 ng/g. The sample concentration is 0.12 g sample/mL solvent with 1 μL injection volume.

flaxseed.[12] The role of magnesium sulfate ($MgSO_4$) is to absorb the trace amount of water in the MeCN extract. PSA retains fatty acids from the MeCN extract with a weak anion exchange mechanism. The nonpolar sorbent C-18 retains trace amounts of lipophilic interference and/or fat residue from the extract. Graphitized carbon has not been used in the current method because it may result in a lower recovery of planar pesticides (e.g., thiabendazole and hexachlorobenzene) with MeCN without the addition of toluene.[19] The sample extract in MeCN was directly injected into GC-MS/MS after the dispersive cleanup with column back flush after each run (without solvent exchange or sample concentration steps). This

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Table 5. Average Recovery and RSD of 57 Pesticides Spiked in Avocado at Three Different Concentrations with GC-MS/MS Analysis (n = 5)

	10 ng/g spike level		50 ng/g spike level		200 ng/g spike level	
	recovery (%)	RSD (%)	recovery (%)	RSD (%)	recovery (%)	RSD (%)
amitraz	31.8	12.7	38.3	18.0	58.0	7.2
benfluralin	81.3	9.4	68.5	12.5	91.3	4.8
BHC-α	74.9	5.2	76.1	11.9	95.7	3.5
BHC-β	93.4	12.2	73.2	20.4	103.5	2.7
BHC-δ	70.5	4.8	76.5	12.0	95.4	4.1
BHC-γ	84.2	12.2	73.1	20.5	101.7	3.5
bromopropylate	60.2	15.7	69.2	13.7	97.1	5.1
cadusafos	69.8	3.4	68.8	11.4	92.0	3.1
chlorothalonil	70.4	28.2	52.0	14.2	81.9	19.4
chlorpyrifos-methyl	79.0	9.0	73.7	12.4	92.3	7.5
cypermethrin	130.7	10.99	104.2	10.3	90.2	5.9
dacthal	70.1	7.5	71.1	14.5	94.2	3.4
DEF	57.1	18.9	61.6	11.0	94.4	6.6
dieldrin	83.0	26.3	73.8	11.3	95.8	3.6
dinitramine	92.2	6.5	77.7	12.0	95.2	4.6
endosulfan sulfate	106.9	14.2	69.2	22.4	106.2	5.8
endosulfan I	91.4	31.7	72.6	16.2	92.2	11.3
endosulfan II	78.2	7.3	70.6	9.2	100.0	5.9
endrin	99.7	12.6	73.4	11.9	100.0	5.7
EPN	66.7	26.7	68.5	13.9	107.5	4.8
etofenprox	82.8	8.9	78.8	11.6	89.0	4.8
etridiazole	104.7	7.0	68.7	15.1	110.4	11.2
fenarimol	63.2	7.7	65.8	15.3	96.9	6.6
fenvalerate 1	72.2	27.7	76.9	14.3	102.9	7.7
fenvalerate 2	75.4	20.2	63.9	22.5	92.3	3.9
fluvalinate 1	58.4	31.4	65.0	17.9	99.6	5.2
fluvalinate 2	51.1	37.4	57.5	27.5	81.7	11.9
heptachlor epoxide	65.4	17.7	69.7	13.3	95.1	6.1
hexachlorobenzene	60.6	9.1	61.6	11.9	81.0	6.1
iprodione	37.0	82.8	68.7	14.1	92.7	16.9
L-cyhalothrin	66.3	13.9	75.2	9.3	98.0	6.2
methyl parathion	75.0	14.1	77.0	13.8	95.6	5.2
MGK-264	74.1	10.1	70.8	11.7	97.7	2.0
napropamide	74.4	10.2	74.7	15.4	103.7	4.9
o,p'-DDT	94.2	20.3	62.1	29.8	119.2	23.1
o,p'-methoxychlor	80.5	12.3	84.9	18.5	112.0	15.3
o-phenylphenol	105.0	17.9	76.7	11.3	83.6	5.1
oxadixyl	64.6	8.6	73.9	13.4	76.6	6.6
p,p'-DDE	61.5	7.4	67.2	14.3	89.0	4.7
parathion	58.5	14.6	66.4	13.3	94.2	4.6
pentachloroaniline	71.3	5.0	70.0	11.7	89.9	3.8
pentachlorobenzene	70.5	4.6	68.2	13.0	85.4	3.8
permethrin-*cis*	89.9	12.5	62.1	13.8	93.6	4.8
permethrin-*trans*	98.5	14.1	74.7	34.7	111.6	9.1
phosalone	74.4	15.0	75.6	11.0	108.0	8.5
pirimiphos-methyl	77.7	11.5	72.2	12.7	92.5	2.1
procymidone	76.8	5.0	75.6	11.6	98.5	13.5
profenofos	52.2	37.2	95.1	6.5	89.6	3.7
pronamide	71.3	8.6	71.7	15.7	93.2	5.2
propanil	72.4	9.0	72.2	13.8	96.1	4.7
pyriproxifen	64.8	7.4	67.9	13.4	96.1	6.4
quinalphos	79.5	15.8	67.5	13.4	91.1	5.0
tetradifon	66.3	5.9	72.1	11.3	88.4	8.5
tolclofos-methyl	81.6	3.7	75.4	10.9	94.5	3.7
triallate	70.3	4.4	67.4	17.1	92.3	4.7
trifluralin	63.9	9.2	70.8	10.4	95.5	5.7
vinclozolin	71.5	11.0	70.6	9.8	101.3	6.5

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Table 5. continued

	10 ng/g spike level		50 ng/g spike level		200 ng/g spike level	
	recovery (%)	RSD (%)	recovery (%)	RSD (%)	recovery (%)	RSD (%)
av	73.9		70.2		94.3	
SD	15.0		7.9		17.0	
RSD (%)	20.3		11.3		18.0	

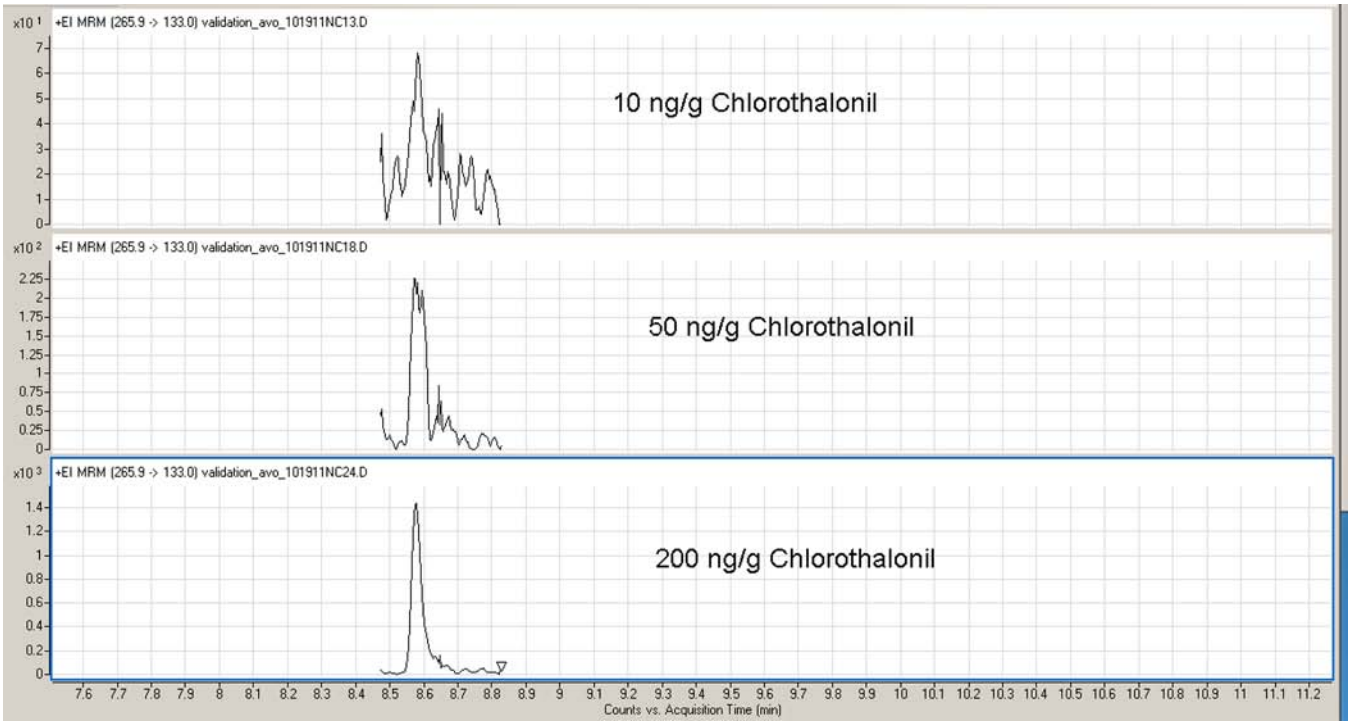

Figure 6. GC-MS/MS chromatogram (MRM) of chlorothalonil fortified in avocado blank at 10, 50, and 200 ng/g. The sample concentration is 0.12 g sample/mL solvent with 1 μL injection volume.

procedure significantly minimizes the matrix effect due to the trace amount of fatty matrix in the injector port and reduces matrix residue at the front portion of the GC column.

As expected, OC compounds exhibit good response on GC-MS/MS with minimum interference at the baseline. Figure 4 shows the chromatograms (in MRM mode) of hexachlorobenzene, dacthal, and *o*,*p*-DDE spiked in avocado blank at 10 ng/g fortification level. The peak relative response for each analyte is different depending upon the molecular structure and fragmentation. The sensitivity of the proposed GC-MS/MS is adequate to screen GC amenable pesticides at 10 ng/g fortification level using 0.12 g sample/mL solvent for extraction with minimum interference, represented in the comparison of total ion chromatograms between avocado blank and those from the blank fortified at 10 and 50 ng/g (Figure 5).

GC-MS/MS has a few drawbacks over the LC-MS/MS method due to matrix effect. It is known that matrix-matched standard is necessary for quantification in GC to correct for matrix effect in the GC injector port. It is not always possible to obtain pesticide-free sample matrices to match with the sample. During the method development, we observed a 2−3-fold increase of the signal of pesticide in avocado extract over the signal of the same pesticide in MeCN. To solve this problem, we used standards in matrix that are similar to the sample to screen the type of pesticide found and estimate the concentration from the calibration curve. To accurately determine the concentration for regulatory purposes, the standard addition method of the particular sample should be used. This will correct for the matrix effect without the need to obtain pesticide-free matrix of the same kind.

Method Validation. The proposed modified QuEChERS procedure was evaluated for the 136 pesticides listed in Tables 4 and 5. A wide range of polarity from very polar pesticides such as neonicotinoid and OP to highly lipophilic pesticides such as OC and pyrethroid were represented. These compounds were chosen to represent the wide range of challenging issues encountered routinely in the analysis of pesticides, for example, poor extractability, poor LC/MS and/or GC/MS responses, selectivity, and instability in the extraction and/or cleanup procedure. The proposed method has major advantages such as the following: (a) utilizes the simplicity of MeCN extraction/salting-out to minimize extractable lipid interference transferring from fatty matrix to the final extract; (b) saves time by eliminating the solvent evaporation step; (c) injection of diluted sample extract to LC-MS/MS to minimize matrix effect; (d) uses quick dispersive SPE to remove lipid residue from sample extract prior to GC analysis; and (e) uses GC column back flush program to maintain system integrity and reduce instrument downtime. LC-MS/MS was used not only for LC-amenable pesticides but also for some GC-amenable compounds that exhibited acceptable responses to LC-MS/MS (about 60% of the entire list). The LC-MS/MS procedure is quick (shake-and-shoot), does not need matrix-matched standard (no need for specific

blank matrix), requires minimal sample cleanup (improved recovery), and is selective and sensitive (more accuracy/precision). This method is also suitable for base-sensitive pesticides including dichlorfluanid and tolyfluanid, which tend to have stability issues when PSA is used for dispersive SPE.[1] By using the shake-and-shoot method with LC-MS/MS without using PSA, the recoveries of dichlorfluanid and tolyfluanid are at least 77% at all levels, a significant improvement.

The recoveries and RSD for all analytes quantified by the LC-MS/MS method at 10, 50, and 200 ng/g (five replicates per each level) are excellent at 107.1 ± 9.2, 86.0 ± 6.7, and 94.8 ± 4.2, respectively (Table 4). We have demonstrated that more than 200 LC-amenable pesticides in high-fat samples including avocado, olive oil, fish, milk, and almond nuts can be determined by using LC-MS/MS with acceptable results.[9,10] The proposed method confirms that MeCN extraction/salting out with LC-MS/MS method is applicable and rugged for selected pesticide analysis in avocado at the screening level of 10 ng/g and higher. No significant interference from sample matrix that may cause peak identification or quantification issue was observed.

With the majority of pesticides determined by LC-MS/MS, we use GC-MS/MS to cover the rest of the pesticides that give poor response and retention by LC-MS/MS. For the GC-MS/MS method, MeCN extraction with a salting-out procedure alone is not sufficient to eliminate lipid interference that may be harmful to the GC injector port and analytical column. A dispersive SPE cleanup technique with $MgSO_4$−PSA−C18 is suitable to trap fatty acids, water, and lipid residue remaining in MeCN without the loss of planar structure pesticides.[19] The final concentration of matrix in sample extract at 0.12 g sample/mL solvent is relatively lower than the conventional QuEChERS method with GC-MS/SIM (about 2−4 g sample/mL solvent). We rely on the more sensitive instrument of GC-MS/MS to detect low-level pesticide residued in such a diluted sample. The ability to inject diluted sample with column back flush is the key element that makes the GC-MS/MS analysis of high-fat sample a rugged method. At least 70 injections of avocado extract have been injected to the GC-MS/MS with no significant peak deterioration or sensitivity. The recovery and RSD for 57 analytes quantified by the GC-MS/MS method at 10, 50, and 200 ng/g (five replicates) are 73.9 ± 20.3, 70.2 ± 11.3, and 94.3 ± 18.0, respectively (Table 5).

The accuracy and precision of GC-MS/MS are not as good as those of the LC-MS/MS method for a few reasons. Table 5 has included some difficult compounds including amitraz[20] and L-cyhalothrin that are well-known for stability issues in solvent[2] and matrix effect in the GC injector port.[21] A few compounds such as iprodione, fenvalerate, endosulfan, and chlorothalonil have poor sensitivity at the 10 ng/g fortification level from the diluted sample extracts (0.12 g sample/mL solvent), which resulted in unreliable data at this level. Figure 6 shows the chromatograms of chlorothalonil in avocado fortified at 10, 50, and 200 ng/g, and the RSDs of chlorothalonil are 28.2, 14.1, and 19.4%, respectively. The signal/noise ratio at 10 ng/g fortification level is approximately 3:1 and considered to be semiquantitative. The signal/noise ratio at 50 ng/g fortification level is approximately 10:1 and would be considered as the LOQ level for this compound and other compounds with poor sensitivity for the GC-MS/MS method proposed here. On the contrary, for compounds that have good sensitivity such as hexachlorobenzene, dacthal, and *o,p*-DDE (Figure 4), the RSD at 10 ng/g level is <10%. To improve the LOQ of some of these compounds detected by GC-MS/MS, one may choose to concentrate the sample extract 2−5 times to approximately 0.5 g/mL but may risk contaminating the injector insert or analytical column.

A lower sample/solvent ratio has improved the recovery of very lipophilic over the previous QuEChERS method for high-fat samples.[11,12] These troublesome pesticides are hexachlorobenzene, chlorpyrifos, dieldrin, endrin, DDT, DDE, and BHC. Recovery of hexachlorobenzene has been below 50% from fatty sample using QuEChERS extraction using 1:1 or 1:2 sample/solvent ratio. After MeCN extraction and a salting-out step, for high-fat samples, a fat layer is formed between the bottom aqueous layer and the top MeCN layer. Hexachlorobenzene is very lipophilic and tends to partition between the fat layer and the MeCN layer. By increasing the sample/solvent ratio, we decrease the phase ratio of the fat layer/MeCN, hence we increase partitioning of hexachlorobenzene to the MeCN layer. In this method, average recoveries of hexachlorobenzene at 10, 50, and 200 ng/g (n = 5) are 60, 62, and 81% with RSDs of 9, 12, and 6%, respectively. Recovery of chlorpyrifos, dieldrin, endrin, DDT, and BHC are consistently >70% across the board. These compounds demonstrated poor responses by LC-MS/MS due to their instability under the positive ESI.

The average recoveries for all pesticides analyzed by both LC-MS/MS and GC-MS/MS at 10, 50, and 200 ng/g are plotted against the number of pesticides ranked by average recovery (%) (Figure 7). It shows excellent recovery at the level

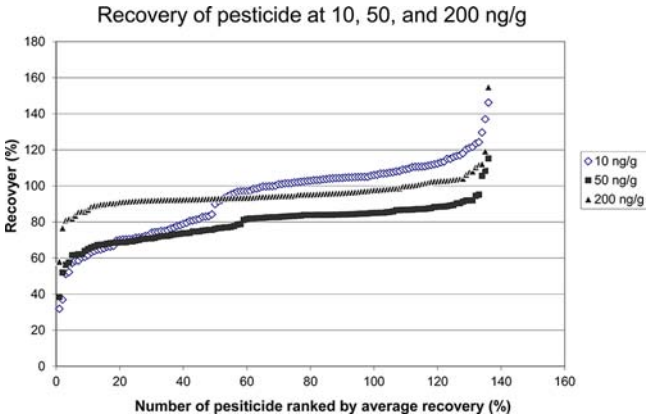

Figure 7. Average recovery of all pesticides spiked in blank avocado at 10, 50, and 200 ng/g.

of 200 ng/g fortification, where only 1 compound (amitraz) of 136 had its recovery outside the 70−120% range. At the 50 ng/g fortification level, 25 of 136 compounds have recoveries outside the 70−120% range, with a much tighter standard deviation than the 10 ng/g fortification level, which has 19 of 136 compounds have recoveries outside the 70−120% range. The compounds that falls outside the acceptable range are all analyzed by GC-MS/MS. These compounds are either not stable in solvent or the injector port or have poor sensitivity. With this drawback, we use GC-MS/MS only to determine pesticides that cannot be done by LC-MS/MS such as hexachlorobenzene and other OC compounds and analyze the rest of the pesticides by LC-MS/MS. Ultimately, the method was designed as a screening tool to cover a wide range of pesticides in a fatty matrix with a reasonable limit of quantification in a very short time. It requires minimum sample

preparation as compared with other previous methods such as EPA PAM[3] and improves recovery of very lipophilic pesticides, which were problematic with regular or buffered QuEChERS methods.[6,12] The current method will be further evaluated to cover different fatty matrix samples such as olive oil, shrimp, nuts, and fish.

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Notes

Mention of brand or firm names does not constitute an endorsement by the U.S. Food and Drug Administration above others of a similar nature.

The authors declare no competing financial interest.

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