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Journal of Regulatory Science

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Regulatory Science

Journal of Regulatory Science 01 (2015) 16–35

Analysis of Pesticides in Olive Oil 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 pesticides in olive oil is presented. A modified QuEChERS sample preparation method was developed to improve the extraction recovery of highly lipophilic pesticides. The acetonitrile extract of the olive oil was directly injected to LC-MS/MS, while other GC-amenable compounds were treated with the modified QuEChERS procedure for GC-MS/MS analysis. The method is an extension of the LIB 4517 to include olive oil. The average recoveries for 80 pesticides quantified by LC-MS/MS at 200, 500, and 1000 ng/g fortifying levels were 91% or better (RSD < 5.5%), while GC-MS/MS analysis demonstrated 81% or better (RSD < 7.2%) for average recovery from 59 compounds at the same spike levels. This method showed an improved recovery of several challenging lipophilic pesticides in olive oils.

Keywords:
QuEChERS, Pesticides Analysis, LC-MS/MS, GC-MS/MS, Olive oil

1. Introduction

Olive oil is a commodity of great economics importance for the region along the Mediterranean Basin, Spain, Greece and Italy. In order to avoid possible losses, due to insect attack, several agrochemicals (pesticides) are applied to olive groves. Too much residual pesticides in olive oil constitute an important parameter of its quality; they must be as low possible to ensure consumer protection. Garcia-Reyes et al. wrote an extensive review on analytical methods for pesticides in olive and olive oil [1] . Amvrazi and Albanis developed a liquid-liquid extraction method to detect 35 pesticides in olive oil using GC/NPD and GC/ECD [2]. A time-consuming solid-phase extraction cleanup procedure was needed to eliminate interference in the sample extract. Liquid- liquid (hexane and acetonitrile) extraction method coupled with GC/MS using has been used to determine acephate and buprofezin in olive oil [3]. This method provided high extraction yield for polar pesticides with low solubility in the fatty matrix, but it was not effective for non-polar pesticides extraction. In order to cover a wider range of pesticides in vegetable oil, Gillespie and co-workers used hexane to extract organochlorine (OC) and organophosphorus (OP) pesticides from plant oils with three types of solid phase media (Florisil, C18, and alumina) [4]. Supercritical extraction was also explored as a sample preparation strategy [5]. Tetrahydrofuran was used to extract pesticides in olive oil along with lipids [6]. Pesticides were separated from the oily matrix by gel permeable chromatography (GPC) before the determination by GC/MS and LC/MS. GPC cleaned hexane extracts of olive oil were analyzed with GC-ECD and GC/MS in order to determine 32 pesticides in virgin olive oil [7]. These methods had good sensitivity and recovery but they were time-consuming and did not cover LC amendable pesticides.

A Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method, described by Anastassiades et al. [8] and based on liquid-liquid partitioning with acetonitrile followed by a clean-up step with dispersive SPE, was recently explored for the analyses of pesticides in both olives and olive oil [9]. The extracts obtained were clean enough to be analyzed by GC-MS and/or LC-MS. However, the OC pesticides had poor recovery (below 70%) for this method. Lehotay et al. compared the QuEChERS extraction with matrix solid-phase dispersion (MSPD) technique for a wide range of pesticides in fatty food matrices and experienced low recovery of non-polar pesticides [10]. A modified QuEChERS method using higher solvent/sample ratio was developed to improve the recovery of the non-polar pesticides from olive oil [11]. GC-MS and LC-MS/MS were

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used to cover a wide range of 16 pesticides in olive oil. To obtain the detection limit of quantification of 10 ppb for GC/MS, a special injector called Direct Sample Introduction (DSI) was used. In the DSI, the sample extract (10 L) was added to a disposable microvial that was placed inside an injection liner, which was replaced in the inlet after every injection. The unvolatilized matrix contaminants were removed along with the microvial, and the system remains clean, with minimal instrument maintenance. This injector must be operated in the automatic mode and it is not widely available in most of the pesticide lab. Recently, a modified QuEChERS extraction method using high solvent/sample ratio along with GC-MS/MS and LC-MS/MS was used to determine a wide range of pesticides in avocado with good recovery of problematic non-polar pesticides [12, 13]. The objective of this study was to combine the high solvent/sample ratio QuEChERS extraction method with LC-MS/MS and GC-MS/MS to determine a wide range of pesticide classes (see Table 1) in olive oil.

2. Materials and methods

2.1. Chemicals and Materials

Pesticide standard mixtures, all 99% purity, were purchased from AccuStandards, Inc. (New Haven, CT) consisting of 10 mixtures of analytes (total of 138 compounds) at 100 μg/mL in methanol. A composite pesticide stock solution was prepared in methanol at 10 μg/mL. Methanol, acetonitrile, and water were of HPLC grade obtained from Fisher Scientific (Pittsburgh, PA) 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 (Pittsburgh, PA). Pre-packaged 50-mL centrifuge tubes containing 6 g of magnesium sulfate ($MgSO_4$) and 1.5 g anhydrous sodium acetate (NaOAc) were purchased from UCT, Inc. (Bristol, PA). Dispersive cleanup tubes (2 mL) containing 150 mg of anhydrous $MgSO_4$, 50 mg of Primary and secondary amine (PSA) sorbent and 50 mg endcapped C-18 sorbent were also from UCT, Inc. Nitrogen and air from TriGas Generator (Parker Hannifin Co., Haverhill, MA) were used for nebulizer and collision gas in LC-MS/MS. Ultra-high purity helium and nitrogen from nexAir (Memphis, TN) were employed as the carrier gas and collision gas in GC-MS/MS. EDP 3 electronic pipetters at different capacity (0-10 μL, 10-100 μL, and 100-1000 μL) were purchased from Rainin Instrument LLC (Oakland, CA) and were used for standard fortification.

2.2. Sample Preparation and Extraction Procedure

Olive oil was obtained from a local market. The samples were weighed at 0.5 g each in a 50-mL centrifuge tube (Fisher Scientific, Pittsburgh, PA) and fortified with 10, 25, and 50 μL of standard mix 10 μg/mL to obtain standard concentration of 200, 500, and 1000 ng/g, respectively. The samples were mixed for 1 minute on a vortex mixer and allowed to stand for approximately 1 hour. A non-fortified sample (blank) was also prepared and used as matrix matched standard. About 5 mL of purified water and 30 mL of 1% acetic acid in acetonitrile 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) at 1000 stroke/min. About 1.5 g of NaOAc and 6 g $MgSO_4$ were added into the tube and mixture was shaken for another 10 min at same speed then centrifuged at 3500 rpm for 10 min. Approximately 1 mL of acetonitrile 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. For quantification, a calibration standard of all pesticides was prepared in 1% acetic acid in acetonitrile at the concentration of 2, 5, 10, 25, 50 and 100 ng/mL and used to construct the calibration curve for external calibration standard method. For GC-MS/MS analysis, 1 mL of acetonitrile extract was pipetted into a 2-mL dispersive tube containing 150 mg of anhydrous $MgSO_4$, 50 mg of PSA sorbent and 50 mg C18 sorbent, capped, spun for 1 min on a vortex mixer, 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, a matrix matched standard of olive oil extract was prepared at 200, 500, and 1000 ng/g spiking levels equivalent by adding appropriate volumes of mixed fortification standard to the blank sample extract (after PSA/C18 dispersive cleanup).

2.3. LC-MS/MS Analysis

LC-MS/MS analysis was performed using a Shimadzu HPLC system. The instrument is equipped with 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). The Analyst software (version 1.4) was used for instrument control and data acquisition. An Ultra Aqueous C18 column (3 μm, 100 x 2.1 mm) and a guard column (10 x 2.1 mm) from Restek (Bellefonte, PA) were used for HPLC separation at 50 oC with 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), then 95% B at 12.5 min (curve 6), 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 a scheduled MRM program for 60 seconds for each analyte. Analyte-specific MS/MS conditions and LC retention time for the LC-amenable analytes were shown in Table 2. The MS source conditions were as follows: curtain gas (CUR) of 30 psi, ion spray voltage (IS) of 4500 volts, collisionally activated dissociation gas (CAD) is high, nebulizer gas (GS1) of 60 psi, heater gas (GS2) of 60 psi, source temperature (TEM) of 350^{o}C.

2.4. GC-MS/MS Analysis

GC-MS/MS analysis was performed using an Agilent 7890A GC, coupled with a 7000 triple- quadrupole MS and a computer

Table 1. Pesticides of interests in the study by their classes.

	Name	Class	Possible analytical issue
Fungicides	Pyrachlostrobin	Strobilurin	poor GC sensitivity
	Chlorothalonil	OC	base sensitive
	Pyrimethanil	Anilnopyrimidine	
	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-Trihalomethylthio	base sensitive
	Hexachlorobenzene	OC	poor extractability in QuEChERS
Insecticide	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
Herbicide	Prometryn	Triazine	retention time shift with fatty matrix
	Linuron	Phenylurea	GC inlet instability
	Trifluralin	Dinitroaniline	poor LC/MS sensitivity

OC = Organochlorine, OP = Organophosphate

with MassHunter software (version B.05.00412) for data acquisition and processing (Agilent Technologies, Palo Alto, CA). The GC is equipped with a 7693 autosampler and an air cool multimode inlet. The injector temperature was programmed to start at 60^{o}C for 0.2 min and ramped to 280^{o}C at 600^{o}C/min with no hold time. The injection volume was 1.0 μL in splitless mode. Analytes were separated with two HP-5ms Ultra Inert capillary columns from Agilent (15m x 0.25 mm ID, 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^{o}C (for 1 min) and increased to 170^{o}C at 40^{o}C /min, ramped to 310^{o}C at 10^{o}C/min, then held for 1.2 min. The total run time was about 19 minutes. The first column was back flushed for 2.0 min at 310^{o}C and a flow rate of 3.5 mL/min after each run. The ion source and transfer line temperatures were at 300^{o}C. Electron multiplier voltage was set to 1400V by automatic tuning and the multiplier voltage was 306V above tune value. Nitrogen and helium (at 1.5 and 2.25 mTorr, respectively) were used as the collision gases for all MS/MS experiments. 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-match 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-matched standards, followed the criteria for identification established by the FDA and European Union [14].

3. Results and discussion

3.1. Optimization of Sample Extraction Procedure

Olive oil is not very soluble in acetonitrile. Cunha et al. did an experiment by shaking olive oil with acetonitrile/water mixture and estimated that only approximately 1.8% of olive oil was partitioned into acetonitrile and only half of it remained in the acetonitrile after the dispersive SPE cleanup with PSA/C18/GCB) [11]. It is very important to minimize the amounts of fat residue in the final extract to reduce the matrix enhancement effect in the GC injector port and keep the injector port clean. The sample size of olive oil used in the sample preparation also affected the recovery of lipophilic pesticides in olive oil. They found that the recovery of p,p′ DDE decreased as the amount of oil increased. Therefore, the sample size of 0.5 g of olive oil was chosen in this proposed method to minimize the detrimental effect to the GC system. The modified version of the AOAC Official Method 2009.01 (also called, buffered QuEChERS method) utilizing acidic acetonitrile and NaOAc was se-

Table 2. Retention time (RT) and MRM conditions for LC-MS/MS analysis.

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	7.0	Ametry 1	71	10	21	4
228.1	96	7.0	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 NH4 1	51	10	39	14
440.1	166.1	13.6	Bifenthrin NH4 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	8.0	Cyproconazole A 1	66	10	39	12
292	125	8.0	Cyproconazole A 2	66	10	45	8
292.1	70.1	8.4	Cyproconazole B1	66	10	39	12
292.1	125.1	8.4	Cyproconazole B 2	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 + 1	46	10	21	12
394.2	107.2	13.6	Etofenprox NH + 2	46	10	61	18
337	124.9	9.4	Fenbuconazole 1	81	10	41	8
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	3.0	Omethoate 1	46	10	29	4
214	182.8	3.0	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	6.0	Pyracarbolid 1	61	10	27	8
218.1	97	6.0	Pyracarbolid 2	61	10	41	18
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	8.0	Triadimenol 1	46	10	31	12
296.1	227.1	8.0	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

Compound dependent parameters: DP = declustering potential, CE = collision energy, EP = entrance potential, CXP = collision cell exit potential.

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-alpha	219	183	7	181	145	15	7.64
BHC-beta	219	183	8	217	181	7	8.03
BHC-delta	219	183	8	217	181	7	8.51
BHC-gamma	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	11.7
Dinitramine	261	195	23	261	241	10	8.4
Endosulfan Sulfate	271.9	236.9	15	271.9	116.9	48	13
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	12.1
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	17.6
Heptachlor Epoxide	352.8	262.8	15	352.8	281.9	18	10.6
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	11.6
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	14.6
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	9.1

lected for the method in order to improve recovery for base sensitive pesticides (e.g. chlorothalonil and tolyfluanid) [10].

An extraction experiment with different solvent/sample ratios was evaluated. Five olive oil samples (0.5 g each in 50 μL solution of 10 μg/mL containing 26 selected lipophilic OC pesticides. Different amounts of acetonitrile with 1% acetic acid (10, 15, 20, 25, and 30 mL) were added to the sample to represent the solvent/sample ratios of 20, 30, 40, 50, and 60 to 1, respectively. Five milliliters of purified water was added to the tubes and they were shaken on the SPEX 2000 Geno Grinder at 1000 stroke/min for 10 min. A salt packet containing 6 g of MgSO4 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 acetonitrile extract was pipetted into a 15-mL centrifuge tube and the appropriate amount of acetonitrile was added to adjust the matrix concentration to 0.0167 g sample/mL solvent. The samples were injected onto the GC-MS/MS. The responses of the selected OC pesticides extracted from 0.5 g of olive oil using different amounts of 1% acetic acid in acetonitrile are presented in Figure 1. It demonstrates that the extraction efficiency of the lipophilic OC pesticides can be significantly enhanced by increasing the solvent/sample ratio from 10:1 to 60:1. The recovery of highly lipophilic pesticides increased as the amount of extraction solvent increased. Recovery of hexachlorobenzene was improved from 53 to 95% when the extraction solvent was increased from 10 to 30 mL. This pesticide was difficult to extract from fatty food using the QuEChERS approach with 4:1 solvent/sample ratio [15]. In order to maximize the recovery of lipophilic pesticides and minimize the amount of fat residue in the final sample extract, the 30 mL of acetonitrile was selected to extract pesticide from 0.5 g of olive oil sample in this method.

3.2. LC-MS/MS Analysis

In the previous work [16], olive oil samples were extracted with acetonitrile using a solvent-to- sample ratio of 3:1 (5 g olive oil in 5 mL of water to 15 mL of acetonitrile), and it worked well with polar and moderately non-polar pesticides. The sample extract was processed with dispersive cleanup and diluted with water at a 1:1 ratio prior to LC-MS/MS analysis. For the proposed method, higher solvent to sample ratio of 60:1 (0.5 g olive oil in 5 mL of water to 30 mL of 1% acetic acid in acetonitrile) was used to improve the recovery of highly lipophilic pesticides and minimize amount of fat in the sample extract. The concentration of sample/solvent was much lower than those from the previous method (0.0167 g/mL vs. 0.33 g/mL). The instrument for LC-MS/MS analysis for the study (QTRAP4000 from AB Sciex) had sufficient sensitivity, thus 1 L of the final extract was more than enough to obtain adequate sensitivity and signal-to-noise (S/N) level at the 200 ng/g fortification or even lower. The matrix effects were examined by comparing the response obtained from olive oil blank and acetonitrile samples fortified with 50 ng/ml of the standard mix. Table 4 shows that the recovery of analytes in matrix are within 91- 115% of those from acetonitrile; therefore, matrix is not the si gnificant issue in the LC-MS/MS analysis. This modification improved overall recovery for multiresidue screening purposes, while shortened the sample preparation steps by bypassing the dispersive cleanup. It also eliminates the need of using matrix-matched standard. This result suggested that the sample size may be increased to lower the limit of quantification. However, for this method, it was decided to keep the overall fat content in the sample to approximately 0.5 g.

LC-MS/MS is suitable for the determination of heat-labile pesticides (carbamate) and polar pesticides (neonicotinoids and OPs) that are challenging if not impossible to analyze with GC-

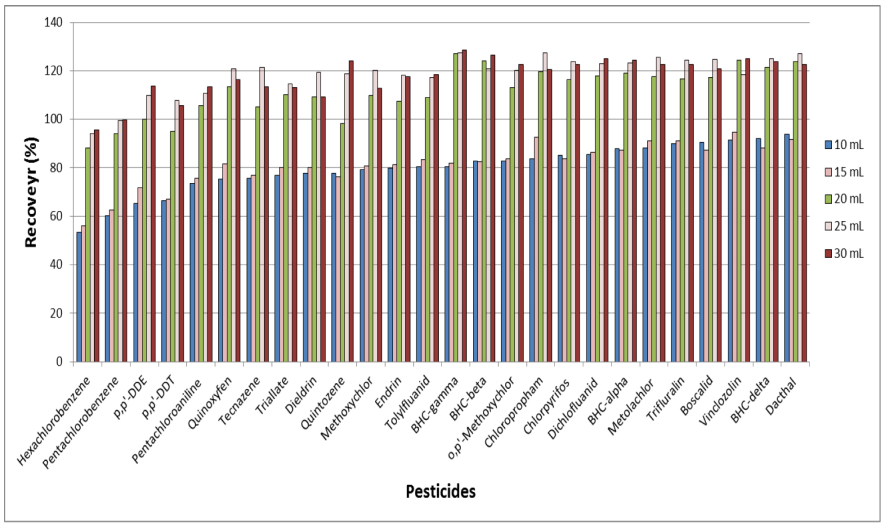

Figure 1. The response of pesticide extraction from 0.5 g of olive oil using different amount of acetonitrile and analyzed by GC-MS/MS (after matrix concentration adjustment).

MS/MS. Some of OP pesticides (for example, methamidophos, acephate , omethoate, and thiabendazole) tend to show peak tailing via interaction with or adsorption onto active sites of the injector port or stationary phase during the GC separation This can impede the accurate quantitation of these compounds particularly at the trace levels. Therefore, a separate injection using a more polar GC column is required in order to overcome this tailing problem [17, 18]. The representative chromatograms of thiabendazole, tolyfluanid, omethoate, and acephate in olive oil blank fortified at 200 ng/g levels analyzed by LC-MS/MS are shown in Figure 2 show good peak shapes and sensitivities for all compounds and revealed little or no interference. Most of the compounds analyzed by LC-MS/MS demonstrated the excellent recoveries (Table 5), partly because the sample extracts were subjected to a relatively shorter extraction procedure with no sample cleanup. The chromatogram from olive oil blank crude extract has very few interference peaks when it is compared with olive oil blanks spiked with 200 ng/g (Figure 3). Standard mix at 10 ng/mL in acetonitrile is also plotted in the Figure 3 to demonstrate the sample matrix (with minimal cleanup) did not affect the peak shapes of the analytes.

3.3. GC-MS/MS Analysis

LC-MS/MS analysis with electrospray ionization (ESI) interface is suitable for polar and moderately non-polar 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. 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 in order 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 [20] . Recent study by Okihashi et al. [18] identified and confirmed the presence of about 260 pesticides in fresh produce by MS/MS with the 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) in order to detect pesticides at the low ng/g range in produce [8].The monitoring of lipophilic pesticides at a trace level can be challenging, especially for matrices with abundant fats, such as olive oil (> 95% fat content). The QuEChERS approach with acetonitrile extraction has already shown to be effective in minimizing coextraction of lipids from fatty foods due to low solubility of the lipids in acetonitrile, while maintaining high recoveries of a wide range of relatively polar LC and semi-polar GC-amenable pesticides [14]. After the extraction, $MgSO_4$ and NaOAc are added toenhance the pesticides partitioning into acetonitrile. This is critical especially for polar pesticidessuch as methamidophos and acephate that tend to retain in the aqueous phase [8]. The dispersive SPE with $MgSO_4$-PSA-C18 sample cleanup technique is used with QuEChERS extraction in flaxseed [15]. The role of magnesium sulfate ($MgSO_4$) is to absorb the trace amount of water in the acetonitrile extract. PSA retains fatty acids from the acetonitrile extract with a weak anion exchange mechanism. The non-polar sorbent C-18 retains trace amounts of lipophilic interference and/or fat residue from the extract. Graphitized carbon is not used in the current method because it may result in a lower recovery of planar pesticides (e.g. thiabendazole and hexachlorobenzene) with acetonitrile without the addition of toluene [19]. The method presented here uses 0.5 g of olive oil with 30 mL of extracting solvent. If only 1% of olive oil is transferred to the acetonitrile extract, the fat content in the extract would be approximately 0.167 μg/μL. This small amount of oil should not have any effect on the injector port or col-

Table 4.Matrix effect experiment on LC/MS method

Analyte	Recovery (%) n = 2	RSD (%)	Analyte	Recovery (%) n = 2	RSD (%)
Acephate	98	2.53	Linuron	99	4.43
Acetamiprid	100	3.12	Malathion	105	0.13
Ametryn	99	0.57	Methamidophos	101	3.23
Aminocarb	100	0.85	Metolachlor	99	0.64
Azinphos-methyl	100	1.49	Metolcarb	91	0.86
Bendiocarb	100	2.06	Mevinphos	95	5.76
Bifenthrin NH4	101	2.94	Monocrotophos	99	2.49
Boscalid	92	1.61	Monolinuron	99	1.00
Chlordimeform	102	1.39	Myclobutanil	98	2.45
Chlorpyriphos	97	1.53	Nuarimol	99	3.63
Coumaphos	99	0.93	Omethoate	98	2.02
Cyanazine	102	2.77	Penconazole	97	1.46
Cycluron	97	1.53	Phosmet	98	2.67
CyproconazoleA	101	7.00	Piperonyl butoxide	100	1.27
CyproconazoleB	95	0.00	Pirimicarb	95	1.19
Desmedipham	96	5.74	Prochloraz	99	0.50
Diazinon	99	0.50	Prometryne	97	0.22
Dichlorfluanid	108	1.97	Propargite	100	1.27
Dichlorvos	104	3.42	Propiconazole	99	6.01
Dicrotophos	99	0.43	Propoxur	96	4.13
Difenoconazole	101	1.40	Pyracarbolid	98	2.59
Dimethoate	97	0.07	Pyraclostrobin	99	0.64
DimethomorphA	103	2.75	Pyridaben	101	1.83
DimethomorphB	106	4.00	Pyrimethanil	103	3.45
Dioxacarb	97	6.37	Quinoxyfen	98	1.51
Epoxiconazole	99	1.79	Secbumeton	101	0.14
EPTC	97	1.82	Spiroxamine	98	2.45
Ethiolate	107	2.64	Sulfotep	99	0.50
Ethion	100	0.77	Tebuconazole	101	0.21
Ethofumesate	104	1.36	Tebufenpyrad	101	0.14
Etofenprox NH4+	100	0.21	Terbutylazine	99	1.21
Fenbuconazole	100	4.97	Tetraconazole	104	2.05
Fenhexamid	106	2.01	Thiabendazole	98	2.02
Fenoxycarb	98	0.51	Tolufluanid	101	0.70
Fenpropimorph	102	3.13	Triadimefon	105	2.03
Fludioxinil	115	4.92	Triadimenol	101	2.23
Fluquinconazole	105	0.68	Triazophos	99	1.50
Flutolanil.1	102	0.70	Tricyclazole	99	1.36
Hexaconazole	97	2.47	Trifloxystrobin	98	1.59
Imazalil	101	2.00	Triflumizole	99	0.01

Table 5. Average recovery (%) and RSD (%) of 80 pesticides spiked in olive oil at three different concentrations via LC-MS/MS analysis (n = 3).

Analyte	200 ng/g spike level		500 ng/g spike level		1000 ng/g spike level	
	Recovery (%)	RSD%	Recovery (%)	RSD%	Recovery (%)	RSD%
Acephate	106	11.1	110	0.5	96	15.2
Acetamiprid	104	12.0	108	3.2	95	12.9
Ametryn	104	16.6	111	1.0	99	12.6
Aminocarb	136	10.7	128	0.4	109	13.3
Azinphos-methyl	112	15.5	115	2.6	98	14.6
Bendiocarb	106	10.9	113	2.3	102	17.2
Bifenthrin NH4	109	6.0	119	1.8	106	17.5
Boscalid	110	5.3	117	6.7	105	17.8
Chlordimeform	103	11.1	108	3.9	101	15.9
Chlorpyriphos	107	8.0	113	0.5	97	14.2
Coumaphos	108	12.2	116	1.5	101	15.0
Cyanazine	110	13.8	111	3.9	98	12.8
Cycluron	105	11.8	112	2.2	101	13.9
Cyproconazole A	122	9.0	115	5.0	101	18.2
Cyproconazole B	118	4.3	117	2.8	102	11.5
Desmedipham	109	8.8	115	1.3	100	15.2
Diazinon	109	10.4	113	1.5	96	12.7
Dichlorfluanid	107	11.3	112	5.9	98	14.7
Dichlorvos	109	9.0	115	4.6	94	13.3
Dicrotophos	111	12.8	112	0.9	99	14.6
Difenoconazole	110	14.4	115	1.8	101	13.7
Dimethoate	105	14.1	110	1.6	99	14.1
DimethomorphA	91	8.2	117	0.5	102	7.3
DimethomorphB	100	11.1	114	4.0	101	12.7
Dioxacarb	104	8.3	109	1.8	97	13.5
Epoxiconazole	111	9.1	116	2.0	101	13.3
EPTC	98	1.8	107	11.6	92	9.6
Ethiolate	107	8.5	121	3.3	99	10.3
Ethion	109	10.8	113	0.5	98	13.9
Ethofumesate	110	3.7	110	2.9	96	9.5
Etofenprox NH4+	106	12.2	110	0.5	93	12.1
Fenbuconazole	105	6.1	111	5.4	98	13.1
Fenoxycarb	109	11.1	115	3.9	100	13.7
Fenpropimorph	108	10.4	112	4.4	98	11.3
Fludioxinil	113	1.4	125	14.1	115	5.6
Fluquinconazole	95	15.4	115	3.1	105	13.3
Fenhexamid	97	8.7	106	7.7	104	8.4
Flutolanil	106	11.7	114	2.2	98	12.4
Hexaconazole	110	4.0	114	1.0	101	14.7

Imazalil	120	10.3	125	6.2	109	9.6
Linuron	113	7.9	121	9.8	104	14.6
Malathion	104	13.7	113	4.6	98	12.7
Methamidophos	114	9.7	118	1.0	107	13.2
Metolachlor	105	10.9	114	1.8	101	12.8
Metolcarb	108	10.5	110	2.9	95	14.5
Mevinphos	101	11.9	106	1.1	96	11.7
Monocrotophos	106	12.5	111	0.5	100	12.8
Monolinuron	108	8.8	111	2.3	96	15.2
Myclobutanil	106	9.2	112	4.2	102	18.1
Nuarimol	97	5.0	109	2.9	102	19.2
Omethoate	105	12.0	112	1.0	102	12.6
Penconazole	109	8.8	111	2.7	98	12.2
Phosmet	111	7.9	111	1.8	96	9.7
Piperonyl butoxide	108	11.3	115	0.9	98	13.8
Pirimicarb	107	10.4	112	1.4	99	10.8
Prochloraz	109	9.2	114	1.0	101	12.5
Prometryne	111	11.4	116	1.8	100	12.7
Propargite	110	14.0	116	0.9	98	12.6
Propiconazole	110	6.4	115	1.3	99	10.7
Propoxur	109	11.8	113	1.3	97	11.3
Pyracarbolid	108	7.3	115	1.8	99	9.7
Pyraclostrobin	110	9.7	113	2.9	99	13.1
Pyridaben	109	7.4	117	1.3	99	14.0
Pyrimethanil	97	12.2	115	4.6	105	16.9
Quinoxyfen	100	13.4	107	2.5	93	11.5
Secbumeton	106	11.5	114	2.2	102	13.7
Spiroxamine	113	12.4	119	2.1	103	13.0
Sulfotep	106	11.2	112	3.2	98	12.8
Tebuconazole	109	10.3	115	2.2	101	13.0
Tebufenpyrad	104	14.9	115	2.6	98	10.9
Terbutylazine	112	10.2	107	3.2	97	9.3
Tetraconazole	114	4.9	118	3.0	100	13.4
Thiabendazole	108	11.9	113	1.3	99	13.7
Tolufluanid	112	11.0	118	2.5	103	12.1
Triadimefon	112	8.9	122	5.3	105	15.1
Triadimenol	107	10.5	117	7.7	99	12.3
Triazophos	110	13.7	114	3.8	103	14.4
Tricyclazole	106	8.9	115	1.3	101	13.1
Trifloxystrobin	111	13.4	116	1.3	99	11.4
Triflumizole	110	11.0	118	0.5	101	13.9
Average	108		114		100	
Std deviation	5.97		4.15		3.81	
RSD (%)	5.53		3.64		3.81	

Table 6. Average recovery (%) and RSD (%) of 59 pesticides spiked in olive oil at three different concentrations with GC-MS/MS analysis (n = 3).

Analyte	200 ng/g spike level		500 ng/g spike level		1000 ng/g spike level	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Amitraz	87	19.7	82	5.2	81	13.6
BHC-alpha	114	12.3	123	1.7	110	14.0
BHC-beta	103	16.8	120	2.1	113	12.3
BHC-delta	112	14.3	119	4.5	109	15.5
BHC-gamma	103	16.8	118	3.0	113	12.2
Bromopropylate	101	19.8	109	8.5	103	16.7
Bupirimate	114	11.5	113	3.5	106	12.8
Cadusafos	109	8.8	116	1.1	107	12.5
Chlordane-cis	107	13.8	113	3.2	104	13.3
Chlordane-trans	105	16.0	112	4.6	105	14.2
Chlorofenvinphos	105	11.0	114	3.5	106	15.7
Chloropropham	108	14.6	115	1.2	106	17.7
Chlorothalonil	108	15.6	116	2.8	115	15.7
Chlorpyrifos	110	9.0	114	1.3	106	15.6
cis-Permethrin	95	11.6	105	4.1	105	10.7
Cyprodinil Results	108	14.9	112	2.1	108	14.5
Dacthal	106	13.3	118	1.2	110	13.1
DEF Results	101	13.9	97	6.2	97	16.1
Dichlobenil	118	2.5	122	0.9	116	7.0
Dieldrin	100	27.4	107	7.2	104	15.9
Dinitramine	118	15.6	117	3.9	107	9.0
Endosulfan sulfate	108	8.8	113	1.3	111	9.1
Endosulfan-I	107	27.9	123	5.6	105	15.5
Endosulfan-II	109	13.6	114	1.9	106	15.2
Endrin	113	3.9	116	2.6	107	14.2
EPN	112	10.0	105	6.5	107	20.5
Ethoxyquin	107	13.3	116	0.9	106	13.5
Etofenprox	105	12.1	105	2.7	98	18.8
Etridiazole	108	13.9	117	2.2	113	17.1
Fenarimol	109	11.4	108	4.7	104	15.4
Fenthion	111	11.4	117	1.2	113	12.4
Fenvalerate 1	124	8.2	103	3.9	102	20.9
Fenvalerate 2	110	12.2	98	3.1	93	18.6
Fluvalinate 1	113	26.2	109	12.7	118	24.2
Fluvalinate 2	123	9.5	107	9.3	102	24.9
Heptachlor Epoxide	112	5.4	119	5.7	109	12.1
Hexachlorobenzene	88	11.7	89	2.0	85	12.7
L-Cyhalothrin	115	19.6	113	3.5	105	17.1
Methidathion	107	12.3	107	4.3	101	13.9
Methyl Parathion	112	9.1	110	3.6	106	14.1
MGK-264	112	20.5	119	2.0	111	13.5

Myclobutanil	107	8.4	105	5.1	103	12.5
Napropamide	111	11.8	114	1.2	110	12.7
o,p'-DDT	97	16.3	108	6.2	115	25.4
o,p'-Methoxychlor	107	14.6	111	1.0	114	20.0
o-phenylphenol	107	9.7	107	3.2	104	5.3
Oxadixyl	119	8.4	116	3.4	103	14.4
p,p'-DDE	99	16.7	107	2.4	97	13.4
Parathion	112	14.4	113	3.4	104	15.9
Pentachloroaniline	100	4.5	108	3.9	101	13.5
Pentachlorobenzene	98	14.5	103	1.4	91	15.1
Phosalone Results	101	23.6	97	7.6	100	15.9
Pirimiphos-methyl	108	10.1	118	2.2	112	16.2
Procymidone	121	17.8	124	2.0	116	14.6
Profenofos	109	16.5	113	1.8	110	14.5
Pronamide	116	14.8	119	1.7	108	13.9
Propanil	103	10.6	110	3.3	108	15.1
Prothiophos-Tok	102	14.8	106	5.4	97	11.7
Pyriproxifen	108	13.8	112	1.6	103	13.5
Quinalphos	114	6.1	109	1.7	107	13.3
Tecnazene	108	8.1	113	3.4	103	10.9
Terbuthylazine	115	11.7	122	4.6	110	12.2
Tetradifon	104	8.1	110	5.5	107	17.1
THPI Results	107	1.4	99	10.4	98	10.4
Tolclofos-methyl	111	10.1	115	0.7	108	15.9
Tolylfluanid	107	21.0	116	5.3	110	19.3
trans-Permethrin	92	11.6	98	5.7	95	13.2
Triallate	106	12.3	113	1.4	102	14.4
Vinclozolin	103	13.0	125	1.3	114	9.7
Average Recovery (%)	108		111		106	
Std. Dev.	7.1		8.0		6.9	
RSD (%)	6.6		7.2		6.5	

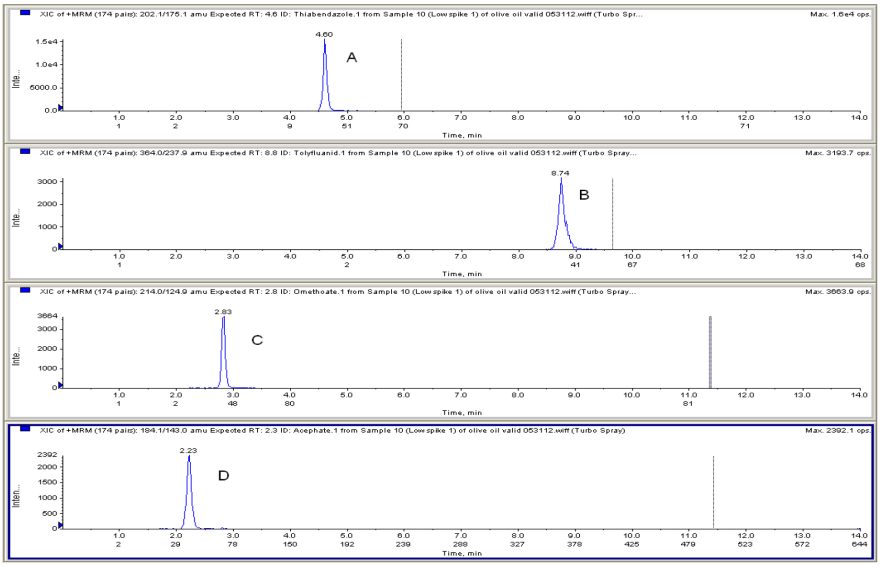

Figure 2. LC-MS/MS Chromatogram (MRM) of thiabendazole (A), tolyfluanid (B), omethoate (C), and acephate (D) spiked in blank olive oil at 200 ng/g. The sample concentration is 0.0167 g sample/mL solvent with 1 μL injection volume.

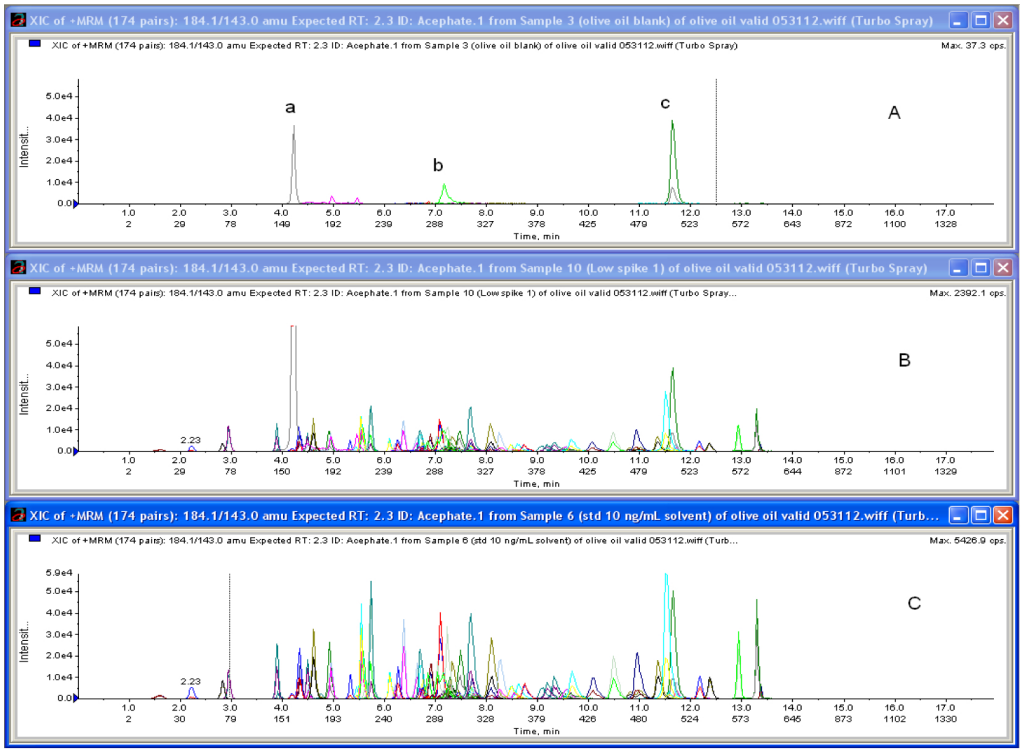

Figure 3. Reconstructed LC-MS/MS chromatogram of olive oil blank (A), olive oil blank fortified with standard mix at 200 ng/g (B), and 10 ng/mL standard mix in acetonitrile (C).The sample concentration is 0.0167 g sample/mL solvent with 1 μL injection volume. Peak a and b are compounds found in olive oil. Peak c is d10-chlorpyrifos (internal standard).

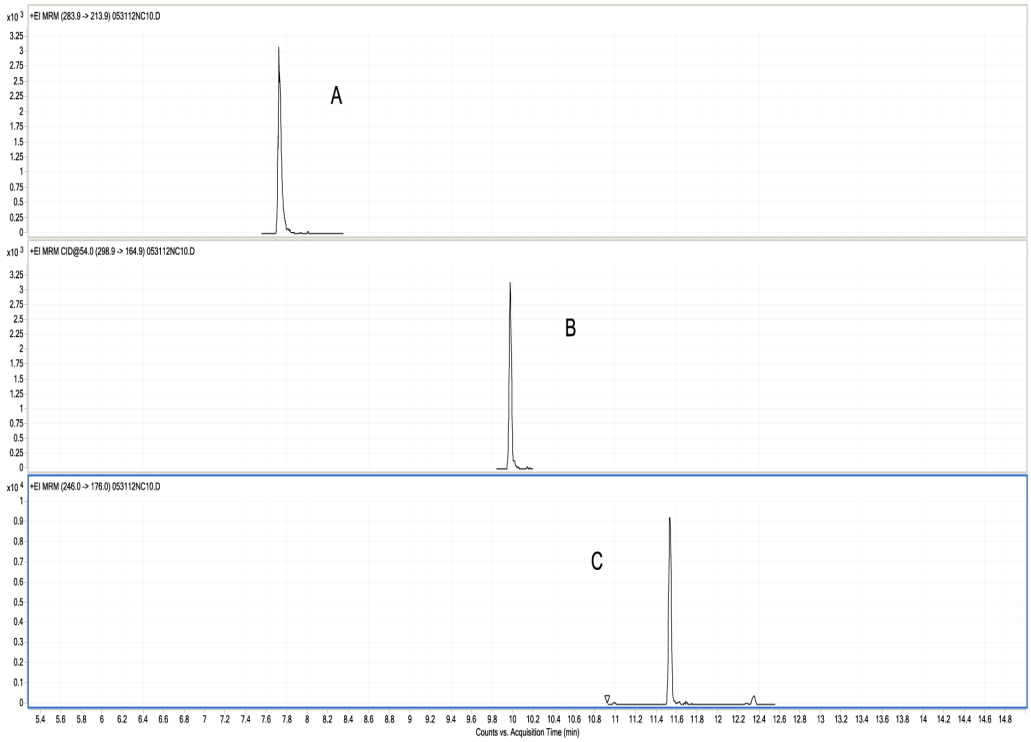

Figure 4. GC-MS/MS chromatogram (MRM) of hexachlorobenzene (A), dacthal (B), and o,p DDE (C) spiked in olive oil blank at 200 ng/g. The extract concentration is 0.0167 g/mL with 1 μL injection volume.

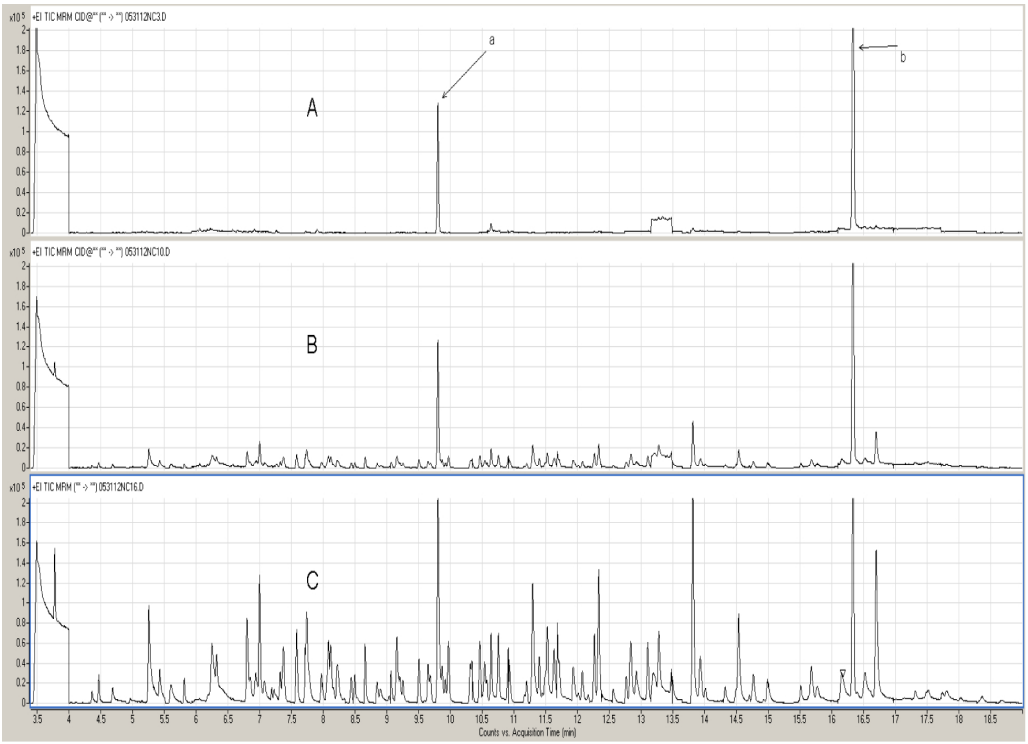

Figure 5. Reconstructed GC-MS/MS chromatograrns of olive oil blank (A), olive oil blank fortified with standard mix at 200 ng/g (B), and at 1000 ng/g (C). The sample concentration is 0.0167 g/mL with 1 μL injection volume. Peak "a" is d10-chlorpyrifos (internal standard). Peak "b" is an unknown compound found in olive oil.

umn inlet performance. The sample extract in acetonitrile is directly injected into GC- MS/MS after the dispersive cleanup with column back flush after each run. This 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 the multiple reaction monitoring mode) of hexachlorobenzene, dacthal, and o,p-DDE spiked in olive oil blank at 200 ng/g fortifying 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 200 ng/g fortifying level using 0.0167 g sample/mL solvent for extraction with minimum interference. Figure 5 shows the comparison of total ion chromatograms between olive oil blank and those from the blank fortified at 200 and 1000 ng/g. GC-MS/MS has a few draw backs 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 samples. In order to solve this problem, we used standards in matrix that is similar to the sample to screen the type of pesticide found and estimate the concentration from the calibration curve. The standard addition method of the particular sample should be used in order to accurately determine the concentration for regulatory purposes. This will correct for the matrix effect without the need to obtain pesticide-free matrix of the same kind.

3.4. Method validation

The proposed modified QuEChERS procedure was used to evaluate 138 pesticides listed in Tables 5 and 6 (chlorpyrifos was in both Tables). A wide range of polarity from very polar pesticides such as methamidophos 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, e.g. poor extractability, poor LC/MS and/or GC/MS responses, selectivity, and instability in extraction and/or cleanup procedure. The proposed method has major advantages such as the following: a) utilizes the simplicity of acetonitrile 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) injects minimum amount of sample extract to LC-MS/MS which minimizes matrix effect, d) no need for matrix matched standard for LC/MS analysis, e) uses quick dispersive SPE to remove lipid residue from sample extract prior to GC analysis, and f) uses GC column back flush program to maintain system integrity and reduces instrument downtime. LC-MS/MS was used not only for LC amenable pesticides, but also for some of the GC amenable compounds that exhibited acceptable responses to LC-MS/MS (about 60% of the entire list). The LC-MS/MS procedure is selective/sensitive, quick (shake-and-shoot), does not need matrix

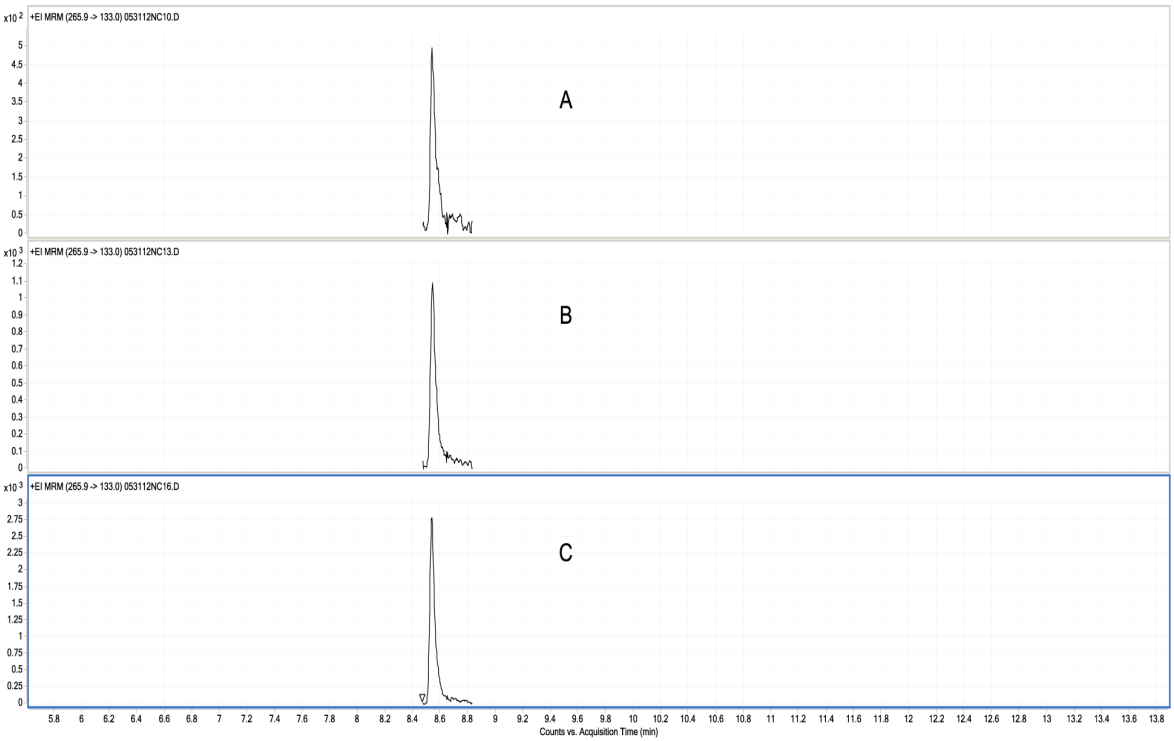

Figure 6. GC-MS/MS chromatogram (MRM) of chlorothalonil fortified in olive oil blank at 200 ng/g (A), at 500 ng/g (B), and at 1000 ng/g (C). The sample concentration is 0.0167 g/mL with 1 μL injection volume.

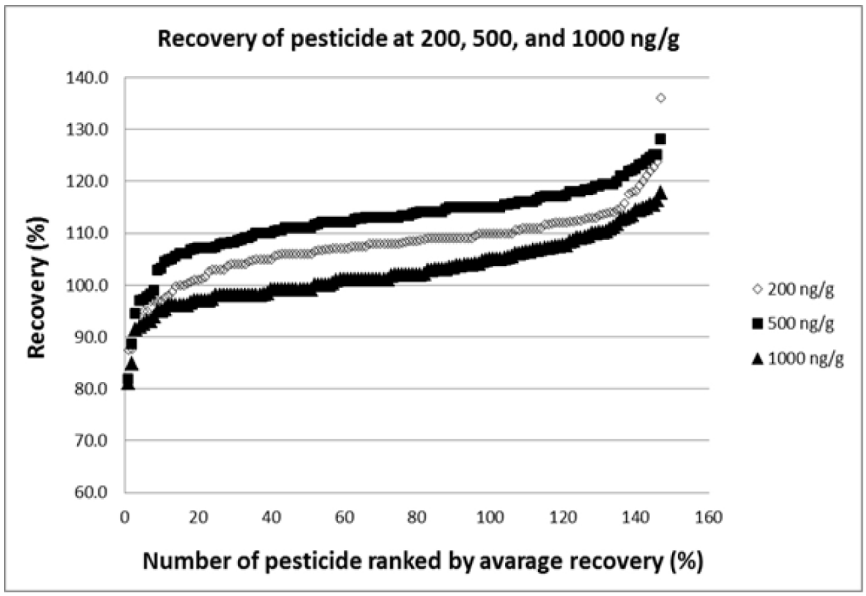

Figure 7. Average recovery of all pesticides spiked in blank olive oil at 200, 500, and 1000 ng/g.

matched standard, and requires minimal sample cleanup (improved recovery). 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 [8].

By using the shake-and-shoot method with LC-MS/MS without using PSA, the recovery of dichlorfluanid and tolyfluanid are at least 98% at all levels, a significant improvement.

The recoveries and RSDs for all analytes quantified by LC-MS/MS method at 200, 500, and 1000 ng/g (three replicates per each level) are excellent at 108 ± 5.5, 114 ± 3.6, and 100 ± 3.8, respectively (Table 5). It has been demonstrated that more than 200 LC amenable pesticides in high fat samples including olive oil, olive oil, fish, milk, and almond nuts can be determined by using LC-MS/MS with acceptable results [16]. The lowest fortification level in this method was 200 ng/g. The signal/noise data obtained from Figure 2 suggest that lowest fortification level of close to one third of 200 ng/g can be achieved. No significant interference from sample matrix that may cause peak identification or quantification problem was observed. The majority of pesticides were determined by LC-MS/MS, while GC-MS/MS was used to cover the rest of pesticides that give poor response and retention by LC-MS/MS. For GC-MS/MS method, acetonitrile extraction with salting-out procedure alone is not sufficient to eliminate lipid interference that may be harmful to the GC injector port and analytical column. Dispersive SPE cleanup technique with $MgSO_4$-PSA-C18 is a suitable mean to trap fatty acids, water and lipid residue remaining in acetonitrile without the loss of planar structure pesticides [19]. The final concentration of matrix in sample extract at 0.0167 g sample/mL solvent is relative lower than the conventional QuEChERS method with GC-MS/SIM (about 2-4 g sample/mL solvent). This method relies on the more sensitive instrument of GC-MS/MS to detect low level pesticide residue 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 50 injections of olive oil extract were analyzed on the GC-MS/MS with no significant peak deterioration or sensitivity. The recoveries and RSDs for 59 analytes quantified by GC-MS/MS method at 200, 500, and 1000 ng/g (n = 3) are 108 ± 6.6, 111 ± 7.2, and 106 ± 6.5%, respectively (Table 6). The accuracy and precision of GC-MS/MS is not as good as the LC-MS/MS method for a few reasons. The Table 6 has included some difficult compounds including amitraz [20] and L- cyhalothrin that are well known for stability issue in solvent [21] and matrix effect in the GC injector port [22]. A few compounds such as iprodione, fenvalerate, endosulfan, and chlorothalonil have poor sensitivity at 200 ng/g fortifying level, which resulted in unreliable data at this level. Figure 6 shows the chromatograms of chlorothalonil in olive oil fortified at 200, 500, and 1000 ng/g. The signal/noise ratio at 200 ng/g fortifying level is approximately 10:1 representing the limit of quantification level for chlorothalonil. In order to improve the LOQ of some these compounds detected by GC-MS/MS, one may choose to increase sample size from 0.5 g to 2 g and take a risk of contaminating injector insert or analytical column.

Increasing solvent/sample ratio has improved the recovery of very lipophilic over the previous QuEChERS method for high fat samples [10, 15]. These troublesome pesticides include 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 acetonitrile extraction and salting-out step, for high fat sample, fat layer is formed between the bottom aqueous layer and top acetonitrile layer. Hexachlorobenzene is very lipophilic and tends to partition between the fat layer and the acetonitrile layer. By increasing the solvent/sample ratio, the phase ratio of fat layer/acetonitrile is increased, hence partitioning of hexachlorobenzene to acetonitrile layer in increased. In this method, average recoveries of hexachlorobenzene at 200, 500, and 1000 ng/g (n=3) are 88, 89, and 85% with RSDs of 11.7, 2.0 and 12.7%, respectively. Recovery of dieldrin, endrin, o,p′DDT, and BHC are consistently higher than 97% across the board. These compounds demonstrate poor responses by LC-MS/MS due to their poor ionization under the positive ESI. The average recoveries for all pesticides analyzed by both LC-MS/MS and GC-MS/MS at 200, 500, and 1000 ng/g are plotted against the number of pesticides ranked by average recovery (%) (Figure 7). It shows excellent recovery at the level of 1000 ng/g fortification, when a compound out of 138 has its recovery outside of 70-120% range. The recoveries of all 138 compounds are within 70-120% range. At 500 ng/g fortifying level, 12 out of 138 compounds have recovery that are outside 70-120% range with a much tighter standard deviation than the 200 ng/g fortifying levels which has 5 out of 138 compounds have recovery falls outside 70-120% range. GC-MS/MS should only be used to determine pesticides that cannot be analyzed by LC-MS/MS such as hexachlorobenzene and other OC compounds. LC- MS/MS should be used to analyze the rest of the pesticides for its simplicity and reliability. Ultimately, the method was designed as a screening tool to cover a wide range of pesticides in fatty matrix with reasonable limit of quantification in a very short time. It requires minimal sample preparation as compared with other previous methods such as PAM [23] and yields improved recovery of very lipophilic pesticides which were problematic with regular or buffered QuEChERS methods [15]. The current method will be further evaluated to cover different fatty matrices samples such as egg, salmon, and milk.

4. Declaration of conflicting interest

The authors declare that there is no conflict of interest. Research was funded by U.S. Food and Drug Administration.

5. Disclaimer

The views expressed are those of the authors and should not be construed to represent the views or policies of the U.S. Food and Drug Administration. Any reference to a specific commercial product, manufacturer, or otherwise, is for the information

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6. Acknowledgments

The authors gratefully acknowledge Dr. Greg Mercer for his technical assistance and suggestions. 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.

7. Article information

The article was received on July the 2nd, 2015 and available on-line August the 12th, 2015.

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