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ARTICLE

Method for the Determination of Organophosphorus and Pyrethroid Pesticides in Food via Gas Chromatography with Electron-Capture Detection

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We have developed a rapid, high-throughput, accurate, multiresidue method for the analysis of selected organophosphorus and pyrethroid pesticides in a variety of food samples suitable for use in public health and epidemiologic investigations of high-use pesticides using modifications of existing methods. The procedure involves a pesticide extraction from the food sample with acetonitrile followed by a salting-out with NaCl and cleanup of the extract with a multilayer solid-phase extraction cartridge composed of a Supelclean ENVI-CARB-II top layer and a primary−secondary amine bottom layer separated by a polyethylene frit. To evaluate the method, we performed fortification studies at 50, 100, and 200 ng/g for 3 organophosphorus and 4 pyrethroid pesticides in 16 different foods. Instrumental analysis was carried out by capillary gas chromatography with electron-capture detection (GC-ECD). Confirmatory analysis was performed by GC coupled with mass spectrometry (MS) in the selected-ion monitoring (SIM) mode. Average recoveries for each fortification level ranged from 49 to 146% with 80% of recoveries between 80 and 120%.

KEYWORDS: Organophosphorus pesticides; pyrethroid pesticides; multiresidue; food samples; electron-capture detection; QuEChERS; GC-ECD; GC-MS

INTRODUCTION

Regulatory agencies and contract, industrial, and academic laboratories often conduct global surveillance of pesticides in food. Utilizing a variety of methods, researchers analyze thousands of samples annually for a variety of purposes including regulatory enforcement and surveillance monitoring (*1*). Many researchers are focused on investigating and developing multiresidue methods (MRMs) with optimal recovery for tens or hundreds of pesticides for only one food (*2*−*5*). In addition, many MRMs are currently focused on fruits and vegetables (*6*−*9*). Pesticides are not only found in fruits and vegetables but also in grain products, dairy, some meats, and beans/legumes (*10*, *11*). In the United States, pesticides are regularly monitored in domestically grown foods to ensure compliance with residue limits or tolerances set by the U.S. Environmental Protection Agency. The U.S. Department of Agriculture (USDA)'s International Maximum Residue Limit Database includes U.S. tolerance limits for various foods as well as maximum acceptable levels in 70 other countries for a variety of pesticides (*12*). There are still countries with limited or no control over pesticide residues in food, and the U.S. increasingly imports food from these countries (*13*). Pesticides in food are potentially harmful to the developing fetus (*14*−*17*) and to children (*18*). These factors warrant further development of methods to assess dietary exposure (i.e., food as actually eaten by individuals) and the need for a quick, high-throughput, low-cost MRM able to quantify pesticide residues in various types of food products at low ng/g levels.

Historically, two extraction methods have been used for pesticide residue analyses in fruits and vegetables (*19*): the Luke method, involving acetone extraction followed by partitioning with a mixture of dichloromethane and light petroleum (*20*), and a method involving ethyl acetate extraction in the presence of sodium sulfate (Na_2SO_4) as a drying agent (*21*). Both methods have been modified in recent years to be less labor- and time-intensive and less environmentally hazardous (*19*). For example, Anastassiades et al. (*22*) developed QuEChERS (quick, easy, cheap, effective, rugged, and safe), a method using acetonitrile extraction with sodium chloride (NaCl) as a salting-out agent and magnesium sulfate ($MgSO_4$) as a drying agent followed by dispersive primary−secondary amine (PSA) sorbent solid-phase extraction (SPE) cleanup instead of a SPE column elution of the extract. In this case and many others, instrumental analysis was carried out via GC (*4*, *23*). A number of published methods utilize GC-ECD to investigate pesticide residues (*23*−*26*) and GC-MS to confirm pesticide identity (*23*, *25*, *26*).

Acetonitrile has become a favored extraction solvent because it (a) is easily separated from water upon salt addition; (b) leads to increased recovery of polar compounds such as organophosphorus (OP) pesticides; and (c) minimizes the number of coextractives, such as lipids and wax materials (*19*). The Dutch Inspectorate for Health Protection validated the QuEChERS method for 400 pesticides in produce (*19*), and Lehotay et al. validated the QuEChERS method for the determination of more

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Table 1. GC-ECD and GC-MS Recovery Ranges, Molecular Masses, and Selected Ions of All Extracted Baby Food Samples Fortified at 50 ng/g

pesticide	GC-ECD % recovery range (n = 24)	molecular mass	selected ion (m/z) range	GC-MS % recovery range (n = 7)
diazinon	83.0−98.4	304	303.6005−304.6005	18.0−71.4
malathion	62.8−135.5	330	172.5808−173.5808	47.0−103.5
chlorpyrifos	82.0−122.6	351	313.4569−314.4569	32.8−74.4
cis/*trans*-permethrin	86.1−107.7	391	182.5804−183.5804	48.3−100.1
cyfluthrin	78.8−104.1	434	205.5600−206.5600	50.5−100.0
cypermethrin	83.4−122.0	416	180.5648−181.5648	54.3−135.9
deltamethrin	81.1−113.8	505	252.4045−253.4045	44.5−110.7

than 200 pesticides in produce (*27*). Lightfield et al. modified it to improve extraction and the stability of fungicides via using 1% acetic acid to protonate any deprotonated compounds in the acetonitrile extraction (*28*). Moreover, the QuEChERS method has been used successfully with a combination C_{18} and PSA sorbent in a variety of food matrixes (*29*). Investigators at Agriculture and Agri-Food Canada used the QuEChERS method coupled with Supelclean ENVI-CARB-II SPE (Sigma-Aldrich, Inc., Bellefonte, PA) cartridges to reduce background interference (*30*). Recent methods for determining pesticide residues in produce use a tandem configuration of two or three SPE columns for the cleanup of raw extract (graphitized carbon black, C_{18}, aminopropyl bonded silica, PSA, and strong anion exchange (*19*)).

In spite of these developments, there is a need for improvement of MRMs for an assortment of foods. QuEChERS has primarily been used and validated only in the analysis of fruits and vegetables (*22*−*24*, *26*). Moreover, Anastassiades et al. assert that the QuEChERS method preferentially removes many polar matrix components such as organic acids, certain pigments, and sugars, to some extent (*22*). This may lead to the accumulation of deposit in the instrumentation used, possibly resulting in a decrease in analytical sensitivity with increasing sample size and an increase in time needed for instrument maintenance. Lightfield et al. altered the QuEChERS methods to include a buffering step to obtain specific pesticides (*24*). Moreover, Anastassiades et al., Lehotay et al., and Fillion et al. used large sample sizes (10−50 g), and such a sample size may not be readily available (*22*, *23*, *26*). In this work, we present a customized procedure based upon QuEChERS-type methods developed by Anastassiades et al. (*22*) and Fillion et al. (*30*) for the rapid, high-throughput, inexpensive multiresidue determination of OP and pyrethroid pesticides in 16 different types of foods requiring only a 1 g sample. Quantitation was carried out by GC-ECD, and confirmatory analysis was carried out by GC-MS in selected-ion monitoring (SIM) mode.

MATERIALS AND METHODS

Reagents and Materials. Acetonitrile (HPLC grade), toluene (HPLC grade), and Na_2SO_4 (ACS grade) were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). NaCl (ACS grade) was purchased from J. T. Baker (Phillipsburg, NJ). The Supelclean ENVI-CARB-II/PSA SPE cartridges (bed A, 500 mg of ENVI-CARB-II; bed B, 300 mg of PSA) were purchased from Sigma-Aldrich, Inc. (Bellefonte, PA). Helium (zero grade) and nitrogen (zero grade) gas were of 99.999% ultra high purity obtained from Specialty Gases Southeast, Inc. (Suwanee, GA). The water used was obtained from an ultrapure 18.2 MΩ·cm Milli-Q water (Millipore, Billerica, MA) system.

The TurboVap LV, an evaporative concentrator, was obtained from Zymark (Hopkinton, MA). The 15-mL glass centrifuge tubes and snap caps were purchased from VWR (Suwanee, GA). Adjustable single-channel pipetters were obtained from Eppendorf North America (Westbury, NY; Calibrated Nov 2007). The Vortex-Genie 2 was purchased from Scientific Industries, Inc. (Bohemia, NY). The centrifuge used was obtained from International Equipment Co. (Needham Heights, MA).

Standards. Pesticide reference standards were obtained from the National Center for Environmental Health, Center for Disease Control and Prevention (CDC; Atlanta, GA), or Chem Service, Inc. (West Chester, PA). Stock solutions and working standard solutions were prepared in acetonitrile. Mixed fortification standards, each containing 3 OP (diazinon, malathion, and chlorpyrifos) and 4 pyrethroid (permethrin, cyfluthrin, cypermethrin, and deltamethrin) pesticides at 5.0 μg/mL, were prepared in acetonitrile from stock standard solutions.

Food Samples. For fortification recovery studies, foods were obtained from the local grocery store. We purchased the baby food forms of green beans, butternut squash, carrots, sweet potatoes, apple sauce, bananas, beef, and chicken. Baby food was selected as it is prehomogenized, minimizing the variability associated with the heterogeneity of these foods. We also bought apple juice, beer, bread crumbs, oats, skim milk, plain yogurt, black beans, and soy milk. All foods were used as purchased, and none of the foods was labeled organic. We did not conduct any type of homogenization on any of the samples. Some of the foods, such as apple juice, bananas, carrots, apple sauce, and green beans, were chosen upon the basis of the fact that they are consumed in large amounts by children and/or are important parts of their diets (*31*). All fortified samples were analyzed for background pesticide concentrations as well as in fortified form. With the exception of baby food carrots, no detectable background levels were noted. For baby food carrots, a background malathion concentration of 36.0 ± 6.6 ng/g (n = 3) was measured.

Instrumental Analysis. A Hewlett-Packard Model 5890A Series II GC equipped with an Agilent Technologies (Santa Clara, CA) model electron-capture detector (ECD) and a 7683B Series Injector autosampler (Agilent Technologies, Inc., Santa Clara, CA) was used. The DB-5 (Agilent Technologies, Inc., Santa Clara, CA) GC column used was 30 m, 0.25 mm i.d., and 0.25 μm film thickness [5% phenyl and 95% dimethylpolysiloxane]. The temperature programming began at 80 °C, held for 2 min, 80−280 at 10 °C/min to 280 °C, then held for 13 min. The helium carrier gas was at a constant flow of 2 mL/min, and nitrogen makeup gas flow was 60 mL/min. The injection was 1.0 μL (splitless). Other relevant analytical parameters included 2 mm i.d. single taper injection liner, injection port temperature of 240 °C, and detector temperature of 280 °C.

To confirm the identities of the pesticide residues in all matrixes, a Model 6890 GC (Agilent Technologies, Inc., Santa Clara, CA) equipped with a MAT 95XL (ThermoFinnigan, Bremen, Germany; 5 kV) magnetic sector mass spectrometer was used. The GC column was a 30 m (0.25 mm i.d. by 0.25 μm film thickness) DB-5MS column (J&W Scientific, Folsom, CA). The initial column temperature was 100 °C and was held for 1 min. Then the oven was heated to 320 at 10 °C/min. The temperature was then held at 320 °C for 5 min. The GC system was operated in splitless injection mode with a 1.0 μL injection and a constant flow of 1 mL/min of helium. The screening analysis was performed in the SIM mode, monitoring at least two characteristic ions for each pesticide compound (**Table 1**). For diazinon, malathion, and chlorpyrifos, we observed only one peak, while multiple peaks were observed for permethrin, cyfluthrin, cypermethrin, and deltamethrin.

Procedure. Samples were handled with trace-cleaned glass or metal equipment. Trace-cleaning consisted of washing with warm tap water and a 1% Alconox solution (Alconox, Inc., White Plains, NY), followed by thorough rinsing with tap water, then three times with deionized water, and a final time with ultrapure Milli-Q water. Equipment was left to dry in an oven at 150 °C, then rinsed once with HPLC-grade acetonitrile (Sigma Aldrich, St. Louis, MO).

Briefly, the food matrix (1 g for solid food or 1 mL for liquid) was placed in a 15-mL disposable glass centrifuge tube to which 5 mL of acetonitrile and 1 g of NaCl were added. The mixture was vortexed for 3 min, then centrifuged for 5 min. The ENVI-CARB-II/PSA cartridges were conditioned with 5 mL of 25% v/v toluene in acetonitrile. Na_2SO_4 was added on top of each SPE cartridge to a depth of ∼2 mm.

Table 2. Percent Recoveries of OP and Pyrethroid Pesticides Extracted from Baby Foods Fortified at 50, 100, and 200 ng/g[a]

	apple sauce						bananas						butternut squash					
	50 ng/g		100 ng/g		200 ng/g		50 ng/g		100 ng/g		200 ng/g		50 ng/g		100 ng/g		200 ng/g	
diazinon	92.3	(2)	97.1	(8)	97.2	(1)	89.8	(5)	100.5	(1)	98.4	(1)	91.5	(2)	95.0	(8)	94.2	(6)
malathion	125.5	(8)	131.7	(6)	128.9	(4)	131.9	(7)	138.3	(3)	130.7	(6)	126.8	(2)	130.7	(5)	126.1	(7)
chlorpyrifos	112.5	(4)	108.0	(6)	107.4	(5)	121.1	(11)	116.0	(7)	109.1	(6)	122.6	(1)	116.9	(7)	110.9	(6)
permethrin	101.4	(5)	102.4	(4)	99.1	(1)	107.4	(9)	106.7	(1)	103.5	(1)	107.3	(6)	105.1	(6)	94.0	(3)
cyfluthrin	102.4	(8)	104.6	(4)	96.6	(4)	104.1	(5)	109.2	(1)	102.0	(9)	104.0	(4)	98.0	(2)	102.8	(5)
cypermethrin	102.8	(3)	96.8	(3)	97.5	(0)	112.9	(22)	100.3	(1)	98.0	(7)	119.9	(7)	101.2	(3)	100.3	(5)
deltamethrin	91.0	(18)	107.6	(7)	82.5	(11)	108.5	(5)	132.9	(2)	122.6	(8)	113.8	(2)	103.1	(4)	104.6	(10)

	carrot						sweet potatoes						beef					
	50 ng/g		100 ng/g		200 ng/g		50 ng/g		100 ng/g		200 ng/g		50 ng/g		100 ng/g		200 ng/g	
diazinon	89.6	(7)	98.2	(2)	93.9	(3)	87.2	(4)	93.3	(9)	77.4	(7)	83.0	(5)	91.2	(12)	91.3	(17)
malathion	62.8	(24)	107.6	(3)	125.1	(4)	85.4	(8)	93.9	(1)	92.1	(9)	108.4	(17)	134.7	(11)	129.9	(14)
chlorpyrifos	97.1	(6)	104.0	(4)	104.9	(3)	82.0	(15)	92.2	(13)	90.0	(3)	93.1	(11)	104.4	(12)	106.2	(10)
permethrin	107.7	(15)	109.3	(2)	100.4	(2)	86.1	(10)	100.6	(8)	88.9	(6)	94.0	(7)	101.7	(8)	98.5	(5)
cyfluthrin	85.9	(17)	90.4	(5)	93.1	(4)	78.8	(13)	91.5	(7)	93.3	(2)	78.9	(18)	98.8	(7)	99.9	(8)
cypermethrin	87.3	(15)	102.4	(3)	98.9	(8)	83.4	(8)	92.5	(3)	94.4	(4)	85.6	(14)	97.3	(3)	101.6	(8)
deltamethrin	98.8	(8)	100.7	(15)	99.1	(2)	81.1	(14)	94.0	(7)	94.5	(7)	95.3	(17)	113.5	(3)	108.9	(13)

	green beans						chicken					
	50 ng/g		100 ng/g		200 ng/g		50 ng/g		100 ng/g		200 ng/g	
diazinon	93.7	(1)	97.4	(6)	80.6	(9)	98.4	(4)	103.6	(5)	99.3	(4)
malathion	97.6	(6)	124.3	(6)	118.6	(9)	135.5	(0)	146.0	(6)	138.6	(4)
chlorpyrifos	91.6	(4)	103.3	(9)	95.0	(10)	107.6	(3)	110.8	(3)	108.2	(5)
permethrin	93.8	(5)	103.3	(4)	99.9	(1)	95.3	(5)	91.9	(7)	98.3	(12)
cyfluthrin	81.1	(13)	96.7	(3)	93.0	(6)	91.3	(8)	93.5	(15)	99.9	(10)
cypermethrin	122.0	(16)	120.6	(5)	101.7	(6)	95.6	(7)	97.2	(14)	104.8	(9)
deltamethrin	83.6	(13)	115.4	(11)	97.2	(7)	89.7	(3)	110.2	(18)	110.8	(9)

[a] $n = 3$; three samples were fortified at each of the fortification levels. Values in parentheses are coefficients of variation.

A 2 mL aliquot of the organic extract was loaded onto the cartridge, which was then eluted with 10 mL of 25% v/v toluene in acetonitrile. The eluant was collected in a 15-mL disposable glass centrifuge tube and placed in a TurboVap LV and evaporated under a stream of air at 10 psi and 35 °C for 15 min and again at 25 psi and 35 °C for 30 min to an approximate volume of 800 μL. We eluted the cartridge once more with 10 mL of 25% v/v toluene in acetonitrile, adding it to the reduced volume of the first eluant. The combined eluants were then evaporated to dryness using the TurboVap LV first at 10 psi and 35 °C for 15 min and then at 25 psi and 35 °C. Samples were reconstituted in 1 mL of acetonitrile and stored at −20 °C.

Fortification. Twelve 1 g samples of each food type were weighed into 15-mL disposable glass centrifuge tubes. Nine of theses samples ($n = 3$ for each fortification level) were fortified with fortification standard solutions and vortexed for 3 min to achieve final concentrations of 50, 100, and 200 ng/g, respectively. The nine samples were extracted and the extracts transferred to GC sample vials. Three blank (unfortified) samples for each food type were prepared by adding 1 g samples of each food type to 15-mL disposable glass centrifuge tubes.

Identification, Quantification, and Confirmation of Pesticides in Food Samples. Solvent standards were prepared from more concentrated standards at various concentrations (1, 5, 10, 25, 50, 100, 150, 200, 250, 500, and 1000 ng/g) and used to create an 11-point calibration curve for quantification. Method detection limits (MDLs) were calculated for each analyte using a power regression model. We defined the lowest concentration used in the calibration curve, 1 ng/g, to be the limit of detection (LOD) for each pesticide. Detection limits were verified by injection of the samples prepared at 1 ng/g to ensure that discernible peaks had a signal-to-noise ratio >3.

Sample extracts and standards were injected on the GC-ECD. Peaks were identified by comparing their retention times to the retention times of the standards. All peaks were integrated manually. In the case that an analyte was composed of multiple isomers presenting multiple chromatographic peaks, we integrated the entire peak complex as a whole peak area instead of each individual peak or isomer. The method of standard addition was employed to account for any matrix effects. We also used the method of standard addition to determine the concentration of unfortified and fortified samples on the basis of the standard calibration curve (*32*). We conducted a qualitative confirmation analysis of the baby food samples fortified at 50 ng/g using GC-MS. Qualitative confirmation analysis was carried out using an internal standard, $^{13}C_6$ PCB-156, at a single concentration. The concentration of the $^{13}C_6$ PCB-156 we added to the samples was 200 pg/μL. We added 10 μL to each sample and diluted with 100 μL of sample to give a concentration of 1.82 pg/μL in the diluted extract analyzed.

RESULTS

Recoveries. Tables 2 and **3** summarize recoveries by food types and fortification levels. Recoveries ranged from 49−146% across all foods and replicates, with 80% of recoveries between 80 and 120%. The values for the coefficients of variance ranged from 0 to 37% across all foods, pesticides, and replicates with the majority of coefficients of variance below 10%. All recoveries were $<80\%$ for the more polar OP pesticide, diazinon, in apple juice samples. Percent recoveries in black beans were also $<80\%$ for chlorpyrifos, permethrin, and cyfluthrin, and were generally lower for all fortified pesticides in comparison to those of the other food samples. Also, the widest range of percent recovery, 49.1−84.9%, occurred with black beans. In general, malathion recoveries were higher than 120% across most foods and most replicate samples.

Chromatography. Most of the GC chromatograms showed little interference from the sample matrix. **Figures 1** and **2** show the chromatograms of the 7 pesticides in black beans and baby food beef, respectively. These two chromatograms are indicative of the extremes in interference observed during study in which both show multiple peaks of interference. None of the multiple peaks coeluted or interfered with peaks of target analytes. Stable chromatographic retention times allowed for reliable identification of unknown peaks. For example, the retention time of diazinon (~16.544 min) did not vary by more than ±0.004 min during the

Table 3. Percent Recoveries of OP and Pyrethroid Pesticides Extracted from Foods Fortified at 50, 100, and 200 ng/g[a]

	apple juice						beer						black beans					
	50 ng/g		100 ng/g		200 ng/g		50 ng/g		100 ng/g		200 ng/g		50 ng/g		100 ng/g		200 ng/g	
diazinon	70.1	(17)	75.5	(2)	71.3	(4)	91.5	(3)	89.2	(5)	89.9	(2)	49.1	(24)	84.9	(22)	64.0	(18)
malathion	87.6	(12)	92.1	(3)	88.9	(7)	90.2	(7)	107.1	(4)	111.3	(5)	77.5	(8)	85.4	(2)	89.1	(4)
chlorpyrifos	86.5	(7)	78.5	(1)	73.7	(7)	77.0	(19)	84.3	(3)	86.3	(4)	54.7	(4)	52.9	(5)	55.6	(6)
permethrin	105.5	(8)	115.6	(7)	102.3	(2)	106.6	(9)	102.9	(5)	101.6	(7)	77.0	(6)	75.5	(6)	75.3	(7)
cyfluthrin	89.5	(10)	98.4	(4)	95.1	(4)	92.6	(8)	94.0	(5)	95.7	(3)	74.8	(9)	79.8	(4)	75.9	(9)
cypermethrin	91.1	(7)	103.7	(1)	95.8	(3)	99.8	(12)	98.4	(5)	97.4	(1)	80.6	(6)	79.8	(1)	78.1	(10)
deltamethrin	107.2	(8)	99.7	(5)	101.8	(7)	108.7	(21)	107.6	(3)	98.4	(8)	80.4	(8)	82.3	(5)	84.7	(10)

	bread crumbs						oats						milk					
	50 ng/g		100 ng/g		200 ng/g		50 ng/g		100 ng/g		200 ng/g		50 ng/g		100 ng/g		200 ng/g	
diazinon	80.2	(10)	111.4	(7)	99.6	(10)	94.5	(10)	103.9	(6)	102.0	(2)	77.1	(3)	76.6	(3)	81.3	(4)
malathion	135.2	(18)	96.6	(13)	101.3	(5)	116.4	(13)	123.0	(2)	115.8	(3)	101.3	(8)	112.0	(5)	115.1	(7)
chlorpyrifos	82.3	(2)	101.3	(3)	103.8	(5)	116.0	(8)	117.2	(2)	111.6	(3)	94.2	(4)	95.2	(5)	95.6	(0)
permethrin	112.4	(25)	109.1	(14)	101.0	(4)	97.2	(4)	100.8	(5)	96.2	(3)	91.4	(4)	97.2	(7)	97.9	(5)
cyfluthrin	100.3	(8)	93.9	(10)	85.3	(8)	75.5	(37)	94.8	(4)	101.1	(1)	81.2	(6)	90.4	(8)	92.4	(2)
cypermethrin	135.5	(7)	102.2	(6)	78.6	(3)	92.9	(1)	99.5	(3)	101.4	(1)	93.2	(12)	96.9	(3)	97.4	(4)
deltamethrin	110.5	(12)	111.4	(15)	101.2	(7)	83.6	(5)	94.7	(4)	96.1	(10)	79.8	(3)	97.2	(6)	96.1	(3)

	soy milk						yogurt					
	50 ng/g		100 ng/g		200 ng/g		50 ng/g		100 ng/g		200 ng/g	
diazinon	88.6	(7)	89.9	(6)	83.1	(4)	106.4	(10)	95.4	(4)	97.2	(7)
malathion	114.6	(11)	129.0	(4)	121.0	(0)	112.3	(6)	119.4	(3)	129.1	(8)
chlorpyrifos	105.3	(9)	117.3	(7)	108.9	(4)	112.4	(6)	104.3	(2)	106.7	(4)
permethrin	117.7	(9)	106.0	(9)	100.6	(6)	94.5	(1)	102.6	(3)	97.8	(2)
cyfluthrin	97.8	(11)	95.6	(9)	97.3	(2)	91.7	(6)	92.8	(5)	96.5	(5)
cypermethrin	119.0	(12)	106.6	(7)	105.8	(1)	93.7	(5)	95.9	(5)	99.1	(3)
deltamethrin	98.0	(19)	97.7	(12)	98.6	(4)	100.2	(16)	100.0	(1)	96.7	(9)

[a] $n = 3$; three samples were fortified at each of the fortification levels. Values in parentheses are coefficients of variation.

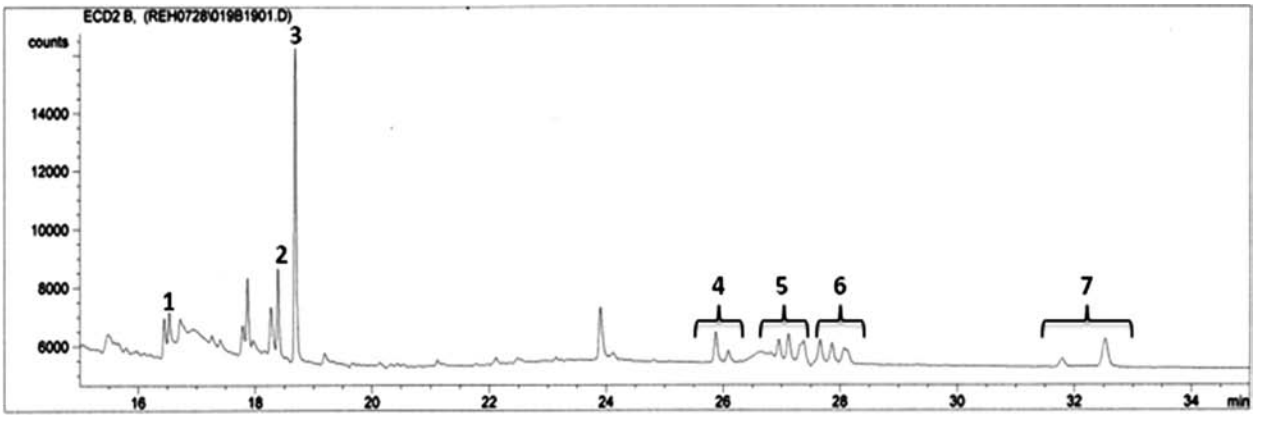

Figure 1. GC-ECD chromatogram of a black bean extract fortified with 50 ng/g OP and pyrethroid pesticides. *X*-axis = time in min. *Y*-axis = area counts. **1**, diazinon; **2**, malathion; **3**, chlorpyrifos; **4**, permethrin; **5**, cyfluthrin; **6**, cypermethrin; **7**, deltamethrin.

course of a 28-h analytical run. Minimal peak broadening, tailing, and peak matrix interference were observed. Baseline resolution was achieved the majority of the time affording separation of peaks differing in retention times by <0.3 min. Consequently, retention times were used to accurately estimate the identity of unknown peaks.

Confirmatory Analysis. For qualitative confirmation analysis of interested analytes, we present selected ions, molecular weights, and recovery ranges for GC-ECD and GC-MS in **Table 1**. Recoveries were averaged for each pesticide and the relative standard deviations calculated. Across all baby food matrixes and pesticides fortified at 50 ng/g, overall GC-ECD percent recovery ranged from 62.8 to 135.5%, while the same range obtained during confirmatory analysis was 18.0−135.9%.

DISCUSSION

Extraction Procedure. Fillion et al. used an acetonitrile extraction with a first cleanup with a C_{18} cartridge followed by an additional cleanup with a carbon SPE cartridge coupled to an aminopropyl cartridge cleanup to remove coextractives. Determination of pesticides was by GC with mass-selective detection in the selected-ion monitoring mode and liquid chromatography with postcolumn reaction and fluorescence detection for *N*-methyl carbamates (*30*). We used a 1 g sample versus the 50 g sample used by Fillion and co-workers; thus, our method required less solvent (30 mL vs 105 mL) and a smaller sample size. This small sample size and solvent volume requirements were effective with homogeneous samples, such as baby food, but was not as effective with more heterogeneous samples, such as black beans, where homogenization is more critical.

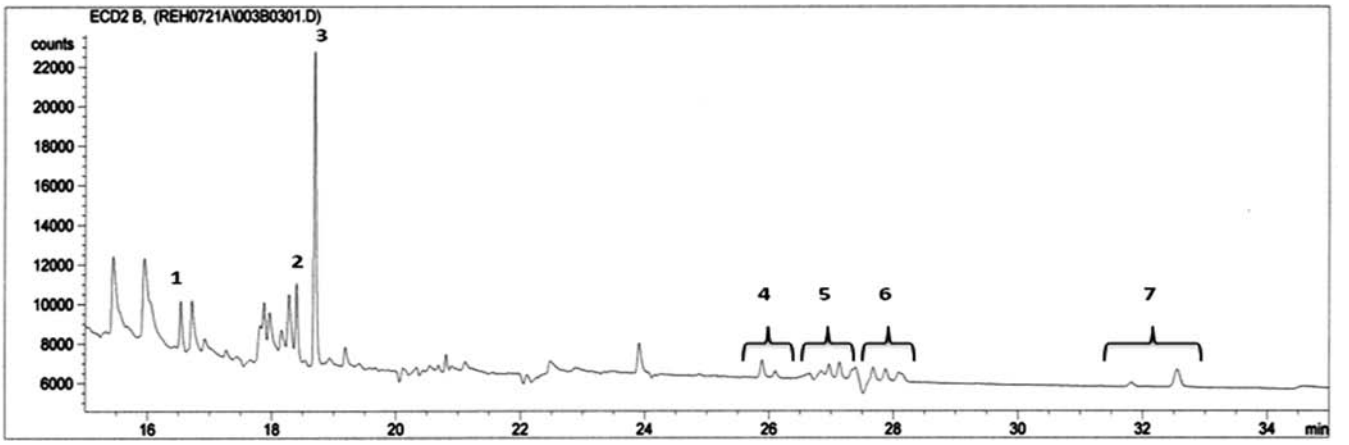

Figure 2. GC-ECD chromatogram of a baby food beef extract fortified with 50 ng/g OP and pyrethroid pesticides. *X*-axis = time in min. *Y*-axis = area counts. **1**, diazinon; **2**, malathion; **3**, chlorpyrifos; **4**, permethrin; **5**, cyfluthrin; **6**, cypermethrin; **7**, deltamethrin.

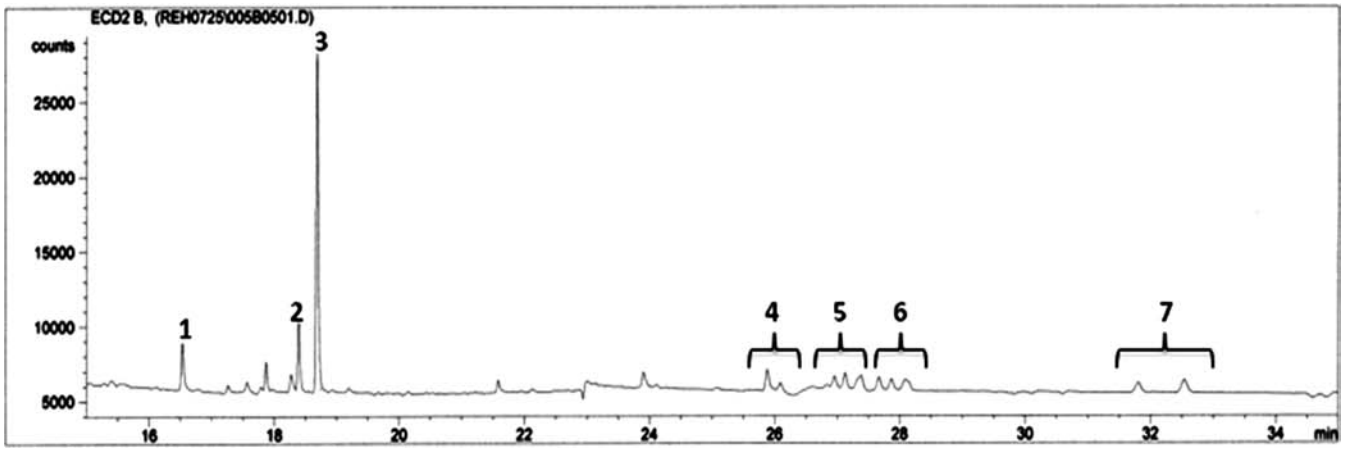

Figure 3. GC-ECD chromatogram of a plain yogurt extract fortified with 50 ng/g OP and pyrethroid pesticides. *X*-axis = time in min. *Y*-axis = area counts. **1**, diazinon; **2**, malathion; **3**, chlorpyrifos; **4**, permethrin; **5**, cyfluthrin; **6**, cypermethrin; **7**, deltamethrin.

We also modified the QuEChERS and Fillion methods (*22*, *30*) to include a NaCl partitioning step during the acetonitrile extraction. We found the salting-out of the aqueous phase in the sample and acetonitrile to be efficient, eliminating the need for a drying agent such as $MgSO_4$. Moreover, we were able to analyze OP and pyrethroid pesticides via GC-ECD without further workup (i.e., solvent exchange or internal standard addition) after reconstitution.

Co-extracted sample matrix components frequently produce coeluting chromatographic peaks that preclude the accurate detection of low (< 20 ng/g) pesticide residue levels in the sample extract (*33*). The removal of interferences, such as pigments and fats, with the ENVI-CARB-II/PSA cartridge reduced the occurrence of coextractives and matrix enhancement effects in our method (*34*). Coupling a rapid, economical, high-throughput sample cleanup with a selective and sensitive detector aids in trace-level analysis (*33*). The instrumentation run becomes more reliable since multiple samples can be analyzed without constant instrument maintenance. We also reduced our waste by streamlining the Fillion et al. method to use only one SPE cartridge instead of three. Finally, Fillion et al. stated that generally they can prepare 42 samples for analysis each week (*30*). Using the method we present allows for a comparable throughput in excess of 40 samples per week. Moreover, the typical material costs for this multiresidue method was approximately $6 per sample, while other MRMs can cost up to twice that amount per sample (*35*).

Recoveries. Recoveries of fortified levels were generally accurate for the majority of samples analyzed. Apple juice, however, gave lower overall recoveries for OPs in comparison to those of the other foods. For example, whereas the peak height was between 22000 and 27000 units for yogurt and beef samples, the peak height for the apple juice sample was a little over 19000 units for an identical spiking concentration (**Figure 4**). Recoveries were lower for the more polar OP pesticides in apple juice samples likely due to the propensity of these molecules to undergo acid hydrolysis causing the formation of OP degradation products of the parent compound under acidic conditions. As we were evaluating only the parent compound, we can only speculate that degradation may account for the lower percent recovery.

The black bean chromatogram (**Figure 1**) displayed peak heights < 16000 units. Standard addition analysis of the fortified samples suggests residual matrix effects for this food. The percent recovery was $< 80\%$ for most of the OP and pyrethroid pesticides in black beans. We speculate that this is due to the heterogeneity of the sample because heterogeneity may result in variable recovery due to the occurrence of preferential binding of pesticide to varied parts of the heterogeneous sample. We also observed lower recovery of the OP versus the pyrethroid pesticides in black beans. The mean recovery of malathion from black beans was $84.0 \pm 5.9\%$. In the homogeneous foods, the recovery was generally higher, sometimes exceeding 100%. Also, black beans gave the widest range of recovery for the target pesticides.

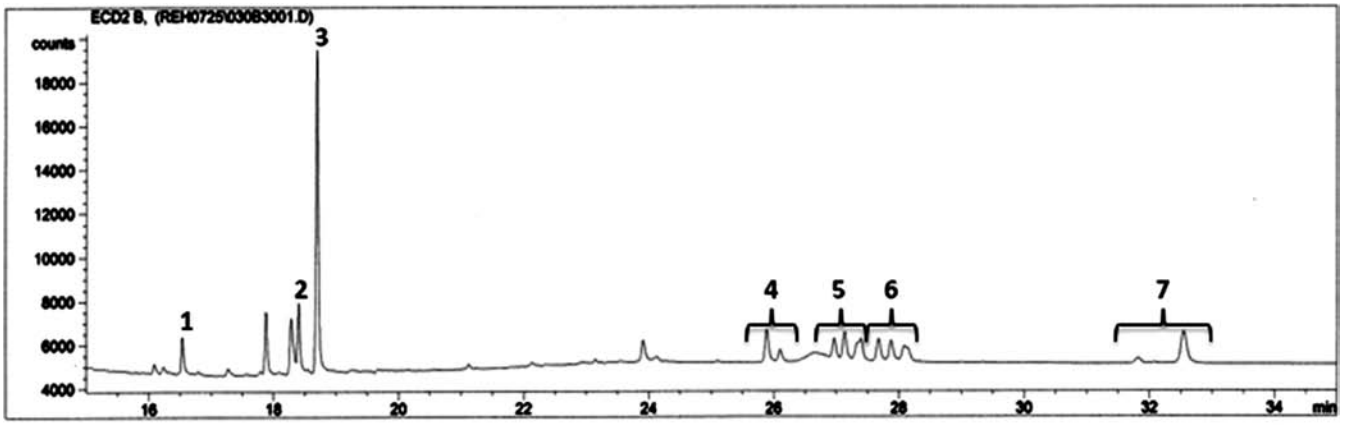

Figure 4. GC-ECD chromatogram of an apple juice extract fortified with 50 ng/g OP and pyrethroid pesticides. *X*-axis = time in min. *Y*-axis = area counts. **1**, diazinon; **2**, malathion; **3**, chlorpyrifos; **4**, permethrin; **5**, cyfluthrin; **6**, cypermethrin; **7**, deltamethrin.

Heterogeneous distribution of pesticide residues within a particular food sample may be due to uneven application of pesticides on the original crop, uneven uptake into the plant matrix, or other factors. We purchased black beans as canned, whole beans. In this case, heterogeneity in the sample may have resulted from application/uptake heterogeneities and/or to our fortification method. We fortified samples by adding pesticide and vortexing for three minutes. The fortified pesticide may have bound heterogeneously to the different components of the bean matrix, e.g., to pieces of waxy skin instead of starchy flesh. This in turn may have precluded the extraction from occurring uniformly, resulting in varied recoveries among the fortified black beans samples. When analyzing black beans and similar samples in the future, we recommend they be homogenized prior to fortification.

Method Advantages and Limitations. This procedure was applied to 16 foods collected from local grocery stores. Unfortified samples were analyzed via injection on the GC-ECD for pesticides. Unfortified-sample chromatograms had few matrix interference peaks, and we detected no residues in unfortified samples with the exception of malathion in baby-food carrots. Consequently, we could determine if the pesticides used to fortify samples were present in unfortified-sample chromatograms via visual inspection; qualitatively, the presence of a peak at the appropriate retention time indicates the presence of the spiked pesticide.

One potential shortcoming of the standard QuEChERS method is its lack of utility in analyzing matrixes with moderate fat content such as some dairy products or meats (*22*). Some dairy products, including milk, exist as an emulsion and have often proven to be difficult to extract because of the fact that organic extraction breaks down the emulsion resulting in a heterogeneous sample. Using our method on fortified yogurt samples resulted in a chromatogram with easily discernible peaks and few matrix interferences, confirming the utility of our cleanup procedure. Similarly, many researchers are reluctant to test red meat because of its high fat content. Unlike the yogurt chromatogram (**Figure 3**), the baby food beef chromatogram (**Figure 2**) showed more matrix interference in the early retention time region. Nonetheless, the chromatogram is relatively clean, and the peaks of interest are clearly discernible. During our experimentation, we also tried to apply this method to 100% fat and oil matrixes (e.g., canola oil) without success.

In general, our cleanup method was effective since the chromatograms displayed little matrix effect in the region of interest. An alternative strategy would involve the use of an internal standard, preferably an isotopically labeled version of one or more of the measured pesticides. This would be considerably more expensive, however. Given our goals of low-cost and rapid-throughput, we opted not to use labeled standards.

Malathion generally showed an augmented percent recovery (i.e., >120%) in many of the samples. This did not diminish with concentration, and the coeluting peaks did not change in size across the fortification levels. In confirmatory analysis, we did not observe a similar enhancement. Thus, we attribute the increased recovery to an interference caused by an enhancement of electron-capture-detector response and/or coelution of another compound with a retention time similar to that of malathion during GC-ECD analysis.

We chose a routine confirmatory technique that used a different detection system (*36*). Since GC-MS is regarded as the recommended reference method because of its accurate sensitivity and specificity (*36*), we used it to qualitatively confirm peak identities in baby food samples fortified at 50 ng/g (*23*). Although we achieved adequate separation and detection in minimal time using a capillary column and an ECD, we wanted to preclude any significant probability of false positive results from potential interference. The qualitative confirmation analysis ruled out this probability since peaks apparent in the ECD chromatograms also appeared in the MS chromatograms.

We demonstrated successful application of our method to a variety of food matrixes. Future studies may extend the method to increase the number of pesticides analyzed, evaluate additional classes of pesticides, or investigate additional food matrixes. Continued work focusing on high-throughput, low-cost methods will be of importance in assessing the public health impact of pesticides.

ABBREVIATIONS USED

ECD, electron-capture detector; GC, gas chromatography; LOD, limit of detection; MDL, method detection limits; $MgSO_4$, magnesium sulfate; MRMs, multiresidue methods; MS, mass spectrometry; Na_2SO_4, sodium sulfate; NaCl, sodium chloride; OP, organophosphorus; PSA, primary-secondary amine; PSI, pounds per square inch; QuEChERS, quick, easy, cheap, effective, rugged, safe; SIM, selected-ion monitoring; SPE, solid-phase extraction.

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