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ARTICLES

Development of an Analytical Scheme for the Determination of Pyrethroid Pesticides in Composite Diet Samples

ANNE P. VONDERHEIDE,[†] PETER E. KAUFFMAN,[§] THOMAS E. HIEBER,[§] JUDITH A. BRISBIN,[#] LISA JO MELNYK,[†] AND JEFFREY N. MORGAN*,[†]

National Exposure Research Laboratory, Microbiological and Chemical Exposure Assessment Research Division, Chemical Exposure Research Branch, and National Council on the Aging, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268; and Office of Ground Water and Drinking Water, Technical Support Center, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268

Analysis of an individual's total daily food intake may be used to determine aggregate dietary ingestion of given compounds. However, the resulting composite sample represents a complex mixture, and measurement of such can often prove to be difficult. In this work, an analytical scheme was developed for the determination of 12 select pyrethroid pesticides in dietary samples. In the first phase of the study, several cleanup steps were investigated for their effectiveness in removing interferences in samples with a range of fat content (1−10%). Food samples were homogenized in the laboratory, and preparatory techniques were evaluated through recoveries from fortified samples. The selected final procedure consisted of a lyophilization step prior to sample extraction. A sequential 2-fold cleanup procedure of the extract included diatomaceous earth for removal of lipid components followed with a combination of deactivated alumina and C_{18} for the simultaneous removal of polar and nonpolar interferences. Recoveries from fortified composite diet samples (10 μg kg^{-1}) ranged from 50.2 to 147%. In the second phase of this work, three instrumental techniques [gas chromatography-microelectron capture detection (GC-μECD), GC-quadrupole mass spectrometry (GC-quadrupole−MS), and GC-ion trap-MS/MS] were compared for greatest sensitivity. GC-quadrupole-MS operated in selective ion monitoring (SIM) mode proved to be most sensitive, yielding method detection limits of approximately 1 μg kg^{-1}. The developed extraction/instrumental scheme was applied to samples collected in an exposure measurement field study. The samples were fortified and analyte recoveries were acceptable (75.9−125%); however, compounds coextracted from the food matrix prevented quantitation of four of the pyrethroid analytes in two of the samples considered.

KEYWORDS: Pesticides; composite diet; GC-MS; pyrethroids; cleanup

INTRODUCTION

The U.S. Environmental Protection Agency's (USEPA) National Exposure Research Laboratory (NERL) conducts aggregate exposure studies to determine an individual's exposure to various environmental contaminants (*1*). Thus, NERL exposure-monitoring programs require the ability to determine a broad range of target analytes in dietary samples. For economic reasons, dietary sampling is usually composed of a food composite, and the number of analytes determined in a single analysis is maximized. However, analytical methods for the determination of contaminants in foods have historically been developed in support of regulatory programs and are specific to food items or food groups (*2*). Most of the available methods have been developed, tested, and validated for relatively few analytes and food items (*2*). Method performance for composite duplicate diet samples, as collected in NERL's residential-based exposure-monitoring programs, is largely unknown. Due to the lack of validated methods for a wide range of pesticides and other contaminants in composite food samples, NERL is

* Address correspondence to this author at the U.S. Environmental Protection Agency, MS-564, 26 W. Martin Luther King Dr., Cincinnati, OH 45268 [e-mail Morgan.jeffrey@epa.gov; telephone (513) 569-7738; fax (513) 569-7757].

[†] National Exposure Research Laboratory, U.S. Environmental Protection Agency.

[§] National Council on the Aging.

[#] Office of Ground Water and Drinking Water, U.S. Environmental Protection Agency.

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developing methods for a variety of contaminants in composite foods to effectively support exposure-monitoring programs.

Synthetic pyrethroid pesticides are a class of analytes of current interest to NERL exposure-monitoring programs. These insecticides are used extensively worldwide in agriculture and as pest-control agents in household applications (*3*). This widespread use is in part due to the USEPA's restrictions on their predecessors, the organophosphate pesticides, as well as their low mammalian toxicity as compared to previously used insecticides. Additionally, they are effective (at low dosages) against a broad range of pests and are stable under field conditions. Such characteristics have popularized their use and, as a consequence, residues have been found in a variety of food items (*4*). Pyrethroid compounds present in the diet may result from crops treated with insecticides during the growing season, postharvest treatment, and/or animals treated against disease vectors, as well as by contamination during preparation and handling of foods in residential environments where pyrethroid pesticide have been applied (*5*). Although they exhibit relatively low toxicity, studies have identified pyrethroid pesticides as potential neurotoxicants (*6*), and one popular member of this class (permethrin) is also suspected to possess endocrine-disrupting properties (*7*). Consequently, there is interest in determining human exposure to these compounds.

Dietary sampling, as practiced by NERL, requires the analysis of a food dietary composite sample containing a representation of all food items consumed during a monitoring period (typically 24 h). In this type of sampling, study participants collect a duplicate (same food items in same amounts) of everything they consume in a 24 h period. The entire 24 h collection is combined into one sample used for analysis. Such a sample may consist of a combination of grains, fruits, vegetables, meats, and/or dairy products. Extraction of pesticides from vegetable samples is fairly straightforward (*8*−*14*). However, extraction of pesticides from fatty foods typically requires several cleanup steps to optimize chromatographic results while preserving instrument integrity (*15*−*18*). Removal of the matrix from sample extracts also enables more reproducible results and minimizes any effects on detector response (*19*). Additionally, minimizing the amount of coextractives lessens the chance for pyrethroid isomerization (*20*).

Several multiresidue methods that measure pyrethroid pesticides in foods were reviewed for their applicability to the analysis of composite diet samples. Pyrethroid pesticides are lipophilic compounds, so complete extraction from fatty matrices must be accompanied by the simultaneous extraction of considerable amounts of fatty material. Generally, additional cleanup steps are necessary to remove coextractives that might otherwise interfere with analyte separation and detection. Published methods for the determination of pyrethroid pesticides have employed such polar adsorbents as silica gel (*11*, *12*), florisil (*9*), and alumina (*18*, *21*), alone, or in combination (*14*, *16*, *17*). A novel combination of alumina and C_{18} solid phase extraction (SPE) was used by Esteve-Turrillas et al. (*15*) in the analysis of pyrethroid residues in vegetable oils. Acetonitrile was used as the elution solvent to minimize the amount of coextracted material in the final sample. In an alternate approach, Columé et al. (*11*, *12*) used lyophilization as a sample pretreatment technique in the analysis of agricultural samples. They reported that the lyophilization process minimized the presence of natural pigments and other coextracted materials.

Instrumental analysis of these analytes can also prove to be complicated. In using gas chromatographic separation, very late elution of some analytes is observed due to their high boiling points. In the realm of detection, electron capture has proven to be very sensitive in the analysis of these compounds (*8*, *11*, *12*, *17*, *18*), although complicated matrices may yield interferences visible to this detector, thus enhancing the need for preparatory extract cleanup. Mass spectrometry (MS) is a popular choice for pyrethroid analysis (*16*) as it yields a high level of confidence in identification; however, extensive fragmentation is encountered when using electron impact ionization. This fragmentation has been attributed to the labile ester linkage common to the entire class (*22*) that results in fragments of low molecular weight, which can ultimately complicate the choice of primary and qualifier ions. Selected ion monitoring is often performed with quadrupole MS to increase sensitivity (*9*, *14*, *21*). Ion trap mass spectrometry is another instrumental choice that is gaining in popularity due to its ability to perform MS^n analysis (*10*, *13*, *15*). Finally, detection is complicated by the numerous stereoisomers (pyrethroids may contain two to three asymmetric carbons atoms or chiral centers, and therefore have two to four diastereoisomeric pairs of enantiomers) that generally separate chromatographically, dividing the signal and subsequently lowering the signal-to-noise ratio.

The objective of this work was to develop an extraction technique coupled with an instrumental method that would yield detection limits in the low micrograms per kilogram range for the pyrethroid pesticides of interest in composite dietary samples collected during exposure-monitoring field studies (i.e., samples collected from random or targeted subsets of the U.S. population for the purpose of measuring an individual's exposure to environmental contaminants). Such low detection limits would allow food concentrations to be more comparable with other environmental samples collected within the same monitoring period, thereby allowing dietary exposures to be compared with dermal and inhalation exposures. An existing pressurized liquid extraction (*23*) method for the analysis of a broad range of pesticides in composite diet samples was used as the starting point (*24*). The applicability of various cleanup procedures in the analysis of the pyrethroid pesticides was explored. Compound separation was accomplished by gas chromatography. Detection by three different means was investigated for highest sensitivity: electron capture (ECD), quadrupole mass spectrometry (MS), and ion trap MS-MS. The quadrupole instrument was operated in the selected ion monitoring (SIM) mode; additionally, the gas chromatograph inlet for this instrument was equipped with a temperature-programmable large volume injector (*25*) with a preseparation column (*21*). The performance of the final method was tested by the analysis of several composite diet samples, both prepared in the laboratory and collected in a field study.

MATERIALS AND METHODS

Reagents and Standards. All solvents (Tedia, Fairfield, OH) were of Absolv grade. All analytical standards were purchased from Absolute Standards, Inc. (Hamden, CT). The internal standard 9,10-dichloroanthracene was purchased from Aldrich Chemical Co. (Milwaukee, WI). A second internal standard, PCB-195, was obtained from Chem Service (West Chester, PA). Acetonitrile was used for standard dilutions performed in preparation of the calibration curve.

Both tralomethrin and deltamethrin were included in the standard solutions, although it is widely known that tralomethrin is partially transformed into deltamethrin during gas chromatographic analytical schemes (*26*). The phenomenon is also apparent from the much higher limits of detection of tralomethrin as demonstrated under Results and Discussion.

Composite Diet Samples. Commercially available frozen, refrigerated, and packaged solid foods were combined to form low-, medium-, and high-fat composite diets containing approximately 1, 5, and 10% fat, respectively (*21*). The composite diets were intended to be representative of 24 h duplicate diets collected in exposure studies and included ready-to-eat meats, cheeses, fruits, vegetables, and starches, all purchased at local markets. The composite diets did not include beverages. Each composite was homogenized in a Robot Coupe, model R2 food processor (Robot Coupe USA, Ridgeland, MS) and stored in screw-capped jars with Teflon-lined lids at −70 °C until used. Solid food composite diet samples, previously collected in a USEPA-sponsored exposure-monitoring field study and held at −70 °C, were also analyzed to assess performance of the method with typical dietary samples.

All samples were lyophilized prior to extraction. First, samples were weighed and frozen at −70 °C for 2 h. They were then freeze-dried overnight or until a constant weight, of <1% moisture, was obtained. Once this point was reached, samples were ground to a fine powder using a glass mortar and pestle. Ground samples were transferred to glass jars, sealed with Parafilm M, and stored in a desiccator.

Extraction Procedure. An amount of 3.2 g of lyophilized composite diet sample (approximately 10.0 g wet weight equivalent) was mixed with 8.0 g of diatomaceous earth [Hydromatrix (HMX), Varian, Harbor City, CA] by mortar and pestle (*27*). The mixture was transferred to a 33 mL Dionex 200 accelerated solvent extractor (ASE) (Sunnyvale, CA) extraction cell that contained a filter pad on the bottom. The surrogate standard (mirex) was added (100 ng mL^{-1}), and a filter pad was placed over the top of the sample and lightly tamped down. The remaining space in the cell was filled with clean solid glass beads. The extraction solvent was hexane, and a 100% flush volume was employed. Nitrogen was used at a pressure of 1500 psi. Specific cycle times were 5 min, 10 min, and 60 s for heat, static, and purge times, respectively. Additionally, a preheat time of 1 min was employed. The extraction temperature was 100 °C, and the samples were subjected to two static extraction cycles. After extraction, the extracts were transferred to Zymark TurboVap II (Zymark Corp., Hopkinton, MA) concentrator tubes. The 60 mL collection tubes were rinsed with 25 mL of hexane; this was added to the concentrator tubes, and the entire volume was reduced to 0.5 mL under nitrogen.

Cleanup Procedure—Partitioning on HMX To Remove Nonpolar Residue. Glass chromatography columns were filled with 10 g of HMX. The concentrated extracts were transferred to the glass chromatography columns using 5−10 mL of hexane and were allowed to stand for 30 min. The columns were eluted with an 80 mL volume of acetonitrile saturated with hexane, and the eluent was collected in a TurboVap II concentrator tube. The extracts were evaporated to dryness under nitrogen.

Cleanup Procedure—Solid Phase Extraction (SPE) on Alumina/C_{18} To Remove Polar Materials. A 20 mL polypropylene SPE tube prepacked with 5 g of C_{18} Mega Bond Elut (Varian) was washed with acetonitrile. An amount of 10 g of 10% deactivated neutral alumina (Neutral Brockman Activity I, Certified 60-325 mesh) (Fisher Scientific, Pittsburgh, PA), slurried in acetonitrile, was added to the prepacked C_{18} SPE tube. The HMX concentrate was dissolved in 20 mL of acetonitrile and transferred to the alumina/C_{18} column. It was allowed to flow through the column, and all of the effluent was collected in a fresh concentrator tube. A volume of 20 mL of acetonitrile was added to the original concentrator tube as a wash and transferred to the cleanup column. All of the column effluent was collected, concentrated, and exchanged to ethyl acetate. Internal standards (50 ng mL^{-1}) were added, and the sample was diluted to 5.0 mL with ethyl acetate. Internal standards used included ronnel, 9,10-dichloroanthracene, perylene-d12, and PCB-195. Note: Perylene-d12 was not employed in the μECD analysis.

Instrumentation. *GC-μECD.* One microliter of sample extract was analyzed by an HP 6890 GC-μECD (Agilent Technologies, Palo Alto, CA) equipped with a 30 m × 0.25 mm RTX-5 cross-linked 95% dimethyl and 5% diphenylpolysiloxane chromatography column with a film thickness of 0.25 μm (Restek Corp., Bellefonte, PA). The oven was held at an initial temperature of 100 °C for 2 min and then increased at a rate of 25 °C min^{-1} to 215 °C and held for 1 min. The oven temperature was then increased at a rate of 15 °C min^{-1} to 290 °C and held for 1 min followed by an increase at a rate of 5 °C min^{-1} to 315 °C and held for 3 min. The flow rate for the helium carrier gas (99.999% purity) was maintained at 1.4 mL min^{-1} throughout the entire run (21.6 min). The makeup gas was 5% methane/95% argon, 99.999% purity. The injection was split 5:1. The injection port temperature was maintained at 260 °C, and the detector temperature was set to 340 °C.

GC-Quadrupole-MS. The instrument was an HP 6890 GC coupled to an HP 5973 mass spectrometer (Agilent Technologies). Instrument operating conditions are detailed in **Table 1**. The mass spectrometer was operated in the electron ionization (EI) mode. The separation column was purchased from Restek and allowed the further separation of the allethrin isomers (isomers were separated into four peaks rather than two as on the GC-ion trap and GC-μECD instruments). The injector was an Apex ProSep 800 PreColumn Separation Inlet (Apex Technologies, Inc., Independence, KY), and specific conditions are given in **Table 1**. Target and qualifier ions for each of the pyrethroid pesticides

Table 1. Apex Injector and GC-Quadrupole-MS Operating Conditions

parameter	setup
instrument	Agilent 6890 GC and 5973 MS
column	RTX-5 SIL MS, 30 m × 0.25 mm i.d. × 0.25 μm
carrier gas	helium (99.999% purity)
column head pressure	initial 8.2 psi, 1.0 mL min^{-1}, constant flow
injector type	Apex ProSep 800, split/splitless
injection liner	Apex ProSep glass liner 4.0 mm id × 24.0 cm, HT-5 wall coated or BPX5 fiber coated
injection volume/ solvent	20 μL using ethyl acetate, 50 μL syringe
GC split ratio	30:1
MS detector temperatures	MS quad = 150 °C, MS source = 230 °C
transfer line temperature	290 °C
MS setup	SIM (see **Table 2**)
oven temperature program	initial temperature = 60 °C, hold time = 3.0 min ramp: 40 °C min^{-1} second temperature = 215 °C, hold time = 1.0 min ramp = 2.5 °C min^{-1} third temperature = 280 °C ramp = 30 °C min^{-1} fourth temperature = 300 °C, hold time = 10.45 min
total run time	45 min

Apex Precolumn Mode Program

stage	inlet mode	mode time (min)
initial	GC split	0.00
1	splitless	0.30
2	GC split	3.29
3	ProSep split (100 mL min^{-1})	3.40
4	GC split	28.0

Apex Precolumn Temperature Program

stage	rate	target temperature (C°)	duration (min)
initial		90	0.30
1	150	270	3.29
2	100	310	31.00
3	100	250	7.00
4	100	90	0.00

Table 2. SIM Target and Qualifier Ions for GC-Quadrupole-MS

analyte	target ion (*m*/*z*)	retention time (min)	qualifier ion 1 (*m*/*z*)	% relative abundance	qualifier ion 2 (*m*/*z*)	% relative abundance
ronnel (IS[a])	285	9.67	287	69.9	289	14.9
allethrin 1a	123	10.84	107	27.0	136	23.4
allethrin 2a	123	10.87	107	26.6	136	21.9
allethrin 1b	123	10.95	107	23.3	136	19.4
allethrin 2b	123	10.98	107	22.7	136	20.4
9,10-DCA (IS)	246	12.97	248	66.1	176	40.1
resmethrin	123	15.88	171	54.7	143	36.4
bifenthrin	181	16.89	166	28.2	165	26.9
tetramethrin	164	17.04	123	33.2	165	11.2
sumithrin	123	18.28	183	61.9	184	12.7
L-cyhalothrin 1	181	18.98	197	69.9	208	53.2
mirex (surrogate)	272	19.34	274	81.2	237	49.7
L-cyhalothrin 2	181	19.48	197	72.8	208	54.8
PCB-195 (IS)	430	20.90	428	89.5	358	71.9
cis-permethrin	183	21.62	163	16.7	165	15.4
trans-permethrin	183	22.03	163	22.2	165	19.1
cyfluthrin 1	163	23.38	206	88.4	226	66.3
cyfluthrin 2	163	23.70	206	70.5	226	44.4
cyfluthrin 3	163	23.90	206	86.1	226	63.7
cyfluthrin 4	163	24.05	206	73.2	226	47.4
cypermethrin 1	181	24.38	163	99.3	209	29.6
cypermethrin 2	181	24.75	163	121.6	209	28.1
cypermethrin 3	181	24.87	163	101.5	209	29.7
cypermethrin 4	181	25.03	163	121.0	209	28.8
perylene-d12 (IS)	264	25.66	260	25.4	265	22.1
fenvalerate	167	27.16	125	96.4	181	59.2
esfenvalerate	167	27.89	125	95.4	181	59.8
tralomethrin	181	29.08	253	71.6	251	38.1
deltamethrin	181	29.72	253	78.0	251	40.4

[a] IS = internal standard.

are given in **Table 2**. Fragment ions chosen as qualifiers for some analytes (i.e., permethrins) represent the monitoring of two halogenated isotopes. For these analytes, isotope ratios were calculated to ensure proper identification (*14*, *28*).

GC-Ion Trap-MS-MS. The GC-ion trap-MS-MS system consisted of a Varian 3800 gas chromatograph coupled to a Saturn 2200 mass spectrometer (Varian, Inc., Walnut Creek, CA). Five microliters of sample extract was injected using a Varian CP-8400 autosampler and a Varian 1079 injector operated in the standard split/splitless mode. A Focus Liner (SGE, Ringwood, Australia) and a Varian Factor Four capillary column (VF-5 ms, 30 m × 0.25 mm id, 0.25 μm film thickness) were used. The carrier gas was helium (99.999% purity) at a constant flow of 1 mL min^{-1}. The injector was held at an initial temperature of 75 °C for 0.5 min, then ramped at 200 °C min^{-1} to 270 °C, and held for the duration of the run. Initially, the split state was turned on at a ratio of 20:1. At 0.5 min, the split state was turned off; at 4.0 min, the split state was returned to on at a ratio of 20:1 for the duration of the program. The column oven was initially held at 60 °C for 3.0 min, then ramped at 40 °C min^{-1} to 215 °C, held for 1.00 min, then ramped at a rate of 2.5 °C min^{-1} to 280 °C with no hold time, and finally ramped at 30 °C min^{-1} to 300 °C and held for 10.46 min (45.00 min total run time). The transfer line was held at 280 °C. The mass spectrometer was operated in the EI mode. The GC-ion trap-MS-MS parameters are listed in **Table 3**.

RESULTS AND DISCUSSION

Phase 1. A method developed previously in our laboratory for the extraction of the composite diet matrix included only one of the pyrethroids, the *cis*/*trans* isomers of permethrin (*24*). This method employed ASE utilizing a hexane and acetone solvent combination. The first cleanup step utilized diatomaceous earth to eliminate coextracted lipid materials. Hexane extracts were placed on this cleanup column; analytes were then eluted with acetonitrile, leaving the fatty compounds on the column. The second cleanup used alumina to remove the unwanted polar compounds, and analytes were collected with a mixture of methylene chloride/hexane (70:30). Analysis was performed with GC-quadrupole-MS operating in SIM mode.

Increasing the analyte list to include additional pyrethroids resulted in chromatograms with a significant number of interfering peaks by GC-quadrupole-MS, even when analysis was performed in SIM mode, implying that interferent concentrations may be quite large. Full-scan analysis was performed with inconclusive identification of the interferents. Experimental changes/additions to the preparatory procedure were investigated as a means to eliminate interferents. For all modifications, the presence of coextractives and pyrethroid pesticides in extracts was monitored by weighing the residues after evaporation and by GC-quadrupole-MS, respectively. The residue weight (the total material extracted) was used as a measure of the thoroughness of a given method in separating organic-soluble material from a matrix (see **Table 4**). As such, reduction with no accompanying loss in analyte would signify removal of matrix constituents or potential interferents. Additionally, smaller residues would aid in minimizing the need for long-term instrument maintenance.

The extraction solvents of the existing method included a hydrophobic solvent (hexane) and a water-miscible solvent (acetone). This combination permitted matrix penetration; however, it also allowed the simultaneous extraction of water and water-soluble coextractives with the pyrethroid analytes. Others have noted that the inclusion of lyophilization prior to the extraction procedure reduced interfering peaks in subsequent chromatography while retaining high recoveries for the pyrethroids (*11*, *12*). In the analysis of ground meat (20% fat) fortified with 10 pyrethroids, Argauer and co-workers (*29*) demonstrated higher analyte recoveries when water was removed by freezing. Therefore, both wet and lyophilized samples (equivalent initial sample amounts) were extracted with the existing protocol, and **Figure 1** compares the chromatograms obtained from each. As is shown, the number of extraneous peaks between 12 and 20 min (the elution period of allethrin, resmethrin, tetramethrin, bifenthrin, phenothrin, and cyhalothrin) was considerably reduced. Furthermore, no accompanying loss in analyte recovery was observed, and this step was included in all subsequent experiments.

The use of diatomaceous earth in conjunction with acetonitrile elution is a well-known technique for separating pesticide compounds from fat. Therefore, attention was primarily directed to the second cleanup step of the existing method, specifically toward modification of the adsorbent. Because the polarity of the interferents was not known, four alternative adsorbents of various polarities were evaluated. In the first modification, silica gel (for the removal of additional polar interferents) was investigated to replace or be used in conjunction with the existing alumina cleanup. Because the composition of the elution solvent is a critical factor in analyte recovery, various ratios of methylene chloride/hexane were examined for optimal recovery of the pyrethroid analytes. These included 100:0, 90:10, 70:30, 50:50, 30:70, 10:90, and 0:100 methylene chloride/hexane. A ratio of 70:30 showed the highest recovery of the pyrethroids (data not shown) from the silica gel column, as it did with the alumina column; however, analyte recoveries were poorer than those obtained with only the alumina cleanup.

In the second modification, analyte behavior was examined with neutral alumina that had not been deactivated (alumina used in the existing method was deactivated with 10% water prior to use). Recoveries for the pyrethroid analytes from a standard ranged from 69.2 to 112% (data not shown). Fortified

Table 3. GC−Ion Trap−MS-MS Parameters

pesticide	retention time (min)	parent mass (*m*/*z*)	segment technique range (*m*/*z*)	ion preparation[a]	waveform type	excitation level (*m*/*z*)	excitation amplitude (V)	quantitation (*m*/*z*)
ronnel (IS)	9.17	285	230–290	MS/MS	nonresonant	100.6[b]	92.50	239.9 + 241.9
allethrin	10.30 + 10.41	123	70–135	MS/MS	nonresonant	54.0	46.00	80.9 + 95.0
resmethrin	15.08	123	70–135	MS/MS	nonresonant	54.0	46.00	80.9 + 94.9
tetramethrin	15.79 + 16.17	164	95–195	MRM	nonresonant	72.1	65.00	106.8 + 120.0
bifenthrin	16.05	181	95–195	MRM	nonresonant	79.7	72.50	164.3 + 165.0
phenothrin	17.44	123	70–135	MS/MS	nonresonant	54.0	46.00	80.9 + 94.9
L-cyhalothrin	18.06 + 18.55	197	130–285	MRM	nonresonant	86.7	75.00	141.1 + 159.0
mirex (surrogate)	18.32	272	130–285	MRM	nonresonant	98.9[b]	83.50	235.0 + 236.9
PCB-195 (IS)	19.86	430	70–440	MS/MS	resonant	189.8	1.45	393.0 + 394.8
cis-permethrin	20.64	183	155–195	MS/MS	nonresonant	80.5	79.25	165.1 + 168.0
trans-permethrin	21.03	183	155–195	MS/MS	nonresonant	80.5	79.25	165.0 + 168.0
cyfluthrin	22.33 + 22.70 + 22.84 + 23.02	163	80–175	MS/MS	nonresonant	71.7	62.00	91.0 + 126.8
cypermethrin	23.31 + 23.67 + 23.81 + 23.96	163	80–175	MS/MS	nonresonant	71.7	62.00	91.0 + 126.8
fenvalerate	26.04	225	100–235	MS/MS	nonresonant	99.1	90.00	118.9 + 141.0
esfenvalerate	26.76	225	100–235	MS/MS	nonresonant	99.1	90.00	118.9 + 141.0
tralomethrin	27.93	253	40–265	MS/MS	nonresonant	111.5	89.00	171.8 + 173.9
deltamethrin	28.59	253	40–265	MS/MS	nonresonant	111.5	89.00	171.8 + 173.9

[a] MS/MS = tandem mass spectrometry; MRM = multiple reaction monitoring. [b] Used an excitation storage level that was 80% of the calculated value based on the parent ion and a desired *q* value of 0.4.

Table 4. Coextractive Weights after Various Cleanup Processes[a]

cleanup	coextractive weight (mg)
none	278 ± 4 (n = 10)
1. HMX 2. alumina; elution with 70:30 hexane/methylene chloride	23 ± 5 (n = 3)
1. HMX 2. alumina; elution with 70:30 hexane/methylene chloride; GCB/PSA	26 ± 2 (n = 6)
1. HMX 2. alumina; elution with acetonitrile	25 ± 4 (n = 3)
1. HMX 2. alumina/C_{18}; elution with acetonitrile	0.7 ± 0.2 (n = 10)

[a] All samples were medium fat and underwent lyophilization pretreatment. Hexane was used as the extraction solvent in all experiments.

medium-fat composite diet samples were then processed with neutral alumina. However, recoveries from this matrix varied unacceptably between 13.3 and 141% (data not shown). Furthermore, the third and fourth isomers of cyfluthrin could not be quantitated due to matrix interference.

In the third experiment, a graphitized carbon black/primary secondary amine-bonded silica adsorbent (GCB/PSA) (for the removal of additional fatty acids) was investigated for use after the two existing cleanup steps (HMX and alumina). However, recoveries were significantly diminished (by approximately 50%), and the residue weights were not significantly improved (see **Table 4**). This was attributed to pyrethroid adsorption; however, variation of elution solvent was not explored. The graphitized carbon black did remove colored interferents as the eluted extract was clear and colorless; others have noted the success of this cleanup scheme in the elimination of pigments (*16*).

Prior to investigation of the last solid phase, a change of solvent was explored. The existing method utilized a 70:30 methylene chloride/hexane mixture for the elution of the analytes from the alumina column. Others have noted that the use of acetonitrile as the elution solvent reduced the coextracted material in the final extract (*15*). Additionally, change of solvent from the methylene chloride mixture to acetonitrile decreased the safety hazards of the extraction protocol. Pyrethroid recovery was monitored to ensure that no analyte loss was encountered. Aliquots of calibration standards were placed on alumina columns and eluted with acetonitrile. The percent recoveries for the pyrethroids of interest ranged from 80 to 150% (data not shown). No significant reduction in residue weight was observed (see **Table 4**).

Finally, a nonpolar solid phase was examined for inclusion in the cleanup step. The alumina stationary phase was placed on top of the C_{18} stationary phase in the prepacked Mega Bond Elut columns. The volume of acetonitrile was increased from the 30 mL utilized in previous experiments to 40 mL to compensate for the larger column volume. Standard recoveries were acceptable, ranging from 60 to 140%, and residue weights were decreased by approximately a factor of 40 (see **Table 4**). Pyrethroid recovery was then monitored in actual matrix samples. Samples of a low-fat, a medium-fat, and a high-fat composite diet were fortified with 10 and 50 μg kg^{-1} of each of the pyrethroid compounds, and recoveries ranged from 50.2 to 147% and from 26.2 to 124%, respectively. Recovery data are given in **Table 5**. Values for all pyrethroids investigated ranged from 1.1 to 29% relative standard deviation (RSD). Three of cyfluthrin's isomers were troublesome as the matrix coextract matter shows an ion at *m*/*z* 163 that interfered with quantititation. Additionally, allethrin's first isomers presented integration difficulties (see **Table 5**). In both cases, it was necessary to quantify on the basis of alternate isomer peaks. However, none of allethrin's isomers were accessible in the 10 μg kg^{-1} fortified low-fat composite samples and, therefore, quantitation of this compound was prohibited at this level. Surrogate recoveries ranged from 63.5 to 83.0%.

Phase 2. A method detection limit (MDL) study (*30*) was performed using three types of detection. These included electron capture, quadrupole MS, and ion trap MS-MS. However, this study did not include an instrumental detection limit comparison as injection volumes for the established methods differed slightly. As noted under Materials and Methods, the GC-μECD utilized a 2 μL injection volume. The GC-quadrupole-MS instrument was equipped with an Apex large-volume injector, and volumes were established at 20 μL. The ion trap

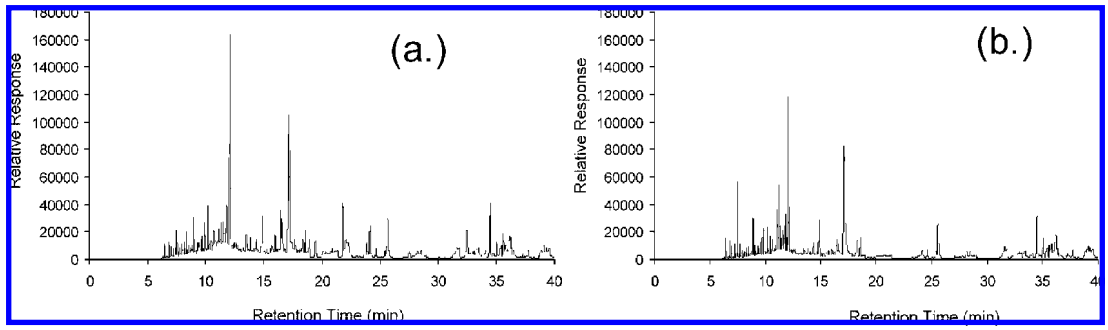

Figure 1. Chromatograms of a composite dietary sample without (**a**) and with lyophilization (**b**). Sample analysis was conducted with an Agilent 6890 GC coupled to an HP 5973 mass spectrometer. Specific conditions are described under Materials and Methods.

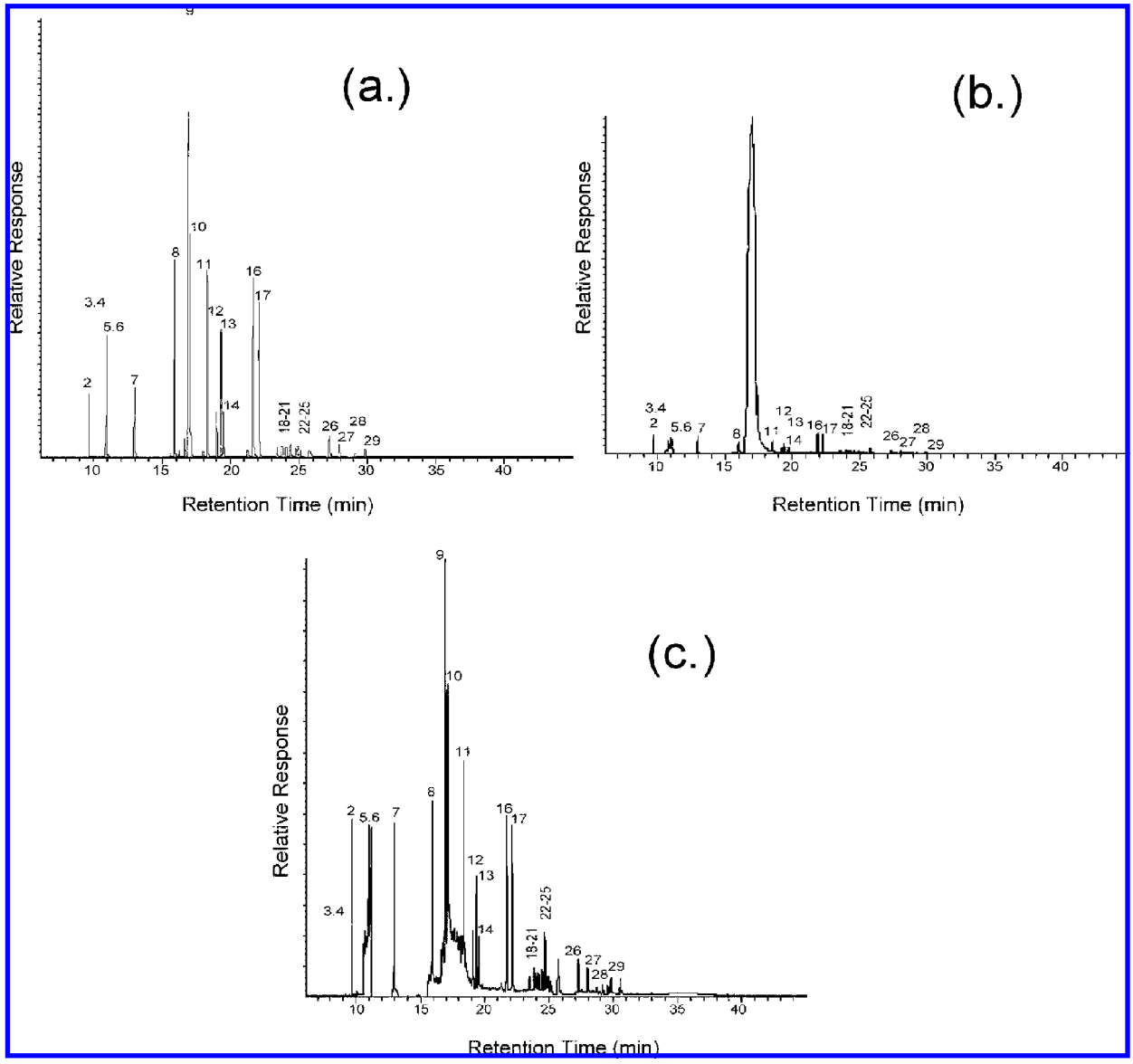

Figure 2. Chromatograms of a pyrethroid standard and composite dietary samples: (**a**) total ion chromatogram (TIC) for the pyrethroid standard mix, 200 μg L^{-1}; (**b**) TIC for field sample 1 fortified at 100 μg L^{-1}; (**c**) TIC for field sample 3 fortified at 100 μg L^{-1}. Sample analysis was conducted with an Agilent 6890 GC coupled to an HP 5973 mass spectrometer. Specific conditions are described under Materials and Methods.

instrument had a cooled injector and contained a carbo-frit injection liner that allowed a 5 μL injection volume.

Seven replicates of the medium-fat composite diet were fortified with 5 μg kg^{-1} of the pyrethroids of interest. The samples were extracted under the newly established protocol and analyzed on each instrument. Three fragment ions were selected for each pyrethroid compound undergoing mass spectrometric analysis (three daughter ions in MS-MS analysis). Standard deviations were calculated; these values were then multiplied by Student's t value for $n = 7$ (3.14) and the results taken as the MDL. Comparative values are given in **Table 6**.

Each instrumental method possessed a different level of selectivity (ion trap in MS-MS mode > quadrupole MS in

Table 5. Recovery of Pyrethroid Pesticides from Standard Composite Diets ($n = 6$)[a]

	% recovery (RSD)					
	HFCD1[b]		MFCD[b]		LFCD[b]	
analyte	10 μg kg^{-1}	50 μg kg^{-1}	10 μg kg^{-1}	50 μg kg^{-1}	10 μg kg^{-1}	50 μg kg^{-1}
allethrin 1a	NR[c]	87.8 (7.9)	NR	92.6 (6.0)	NR	84.2 (1.1)
allethrin 1b	NR	88.1 (10)	NR	85.0 (10)	NR	92.3 (4.2)
allethrin 2a	72.9 (3.5)	88.6 (5.8)	88.9 (3.4)	85.3 (5.7)	NR	88.8 (4.3)
allethrin 2b	85.2 (8.1)	88.7 (17)	101 (13)	90.0 (14)	NR	90.3 (6.8)
resmethrin	65.1 (14)	80.3 (8.4)	50.2 (19)	26.2 (29)	59.7 (11)	70.3 (4.1)
bifenthrin	90.5 (4.8)	96.0 (6.2)	90.3 (11)	91.8 (1.7)	85.0 (9.5)	97.9 (2.3)
tetramethrin	105 (6.5)	93.3 (3.5)	91.3 (8.7)	91.1 (1.3)	91.6 (3.3)	94.6 (1.9)
phenothrin	86.7 (9.0)	88.5 (6.2)	88.9 (10)	89.8 (1.4)	97.1 (27)	90.9 (1.4)
L-cyhalothrin 1	112 (4.2)	95.3 (3.9)	79.6 (8.9)	92.0 (1.8)	79.2 (16)	95.4 (1.6)
mirex (surrogate)	63.5 (18)	79.1 (5.3)	67.1 (18)	79.4 (6.8)	81.9 (2.2)	83.0 (3.0)
L-cyhalothrin 2	89.7 (14)	101 (7.2)	94.0 (10)	92.9 (2.3)	77.0 (8.1)	93.8 (1.8)
cis-permethrin	92.9 (4.2)	90.3 (2.4)	94.5 (11)	92.7 (3.1)	93.5 (2.5)	93.2 (2.3)
trans-permethrin	88.0 (8.5)	91.9 (2.1)	63.9 (9.5)	93.5 (6.2)	93.4 (4.6)	93.8 (2.6)
cyfluthrin 1	80.4 (26)	81.7 (14)	92.0 (16)	96.3 (4.9)	86.7 (5.4)	95.2 (2.1)
cyfluthrin 2	NR	NR	NR	NR	NR	124 (25)
cyfluthrin 3	NR	NR	NR	NR	NR	NR
cyfluthrin 4	NR	NR	NR	97.0 (4.4)	NR	100 (8.5)
cypermethrin 1	104 (6.8)	91.8 (4.5)	103 (11)	93.7 (3.6)	102 (4.9)	92.7 (2.0)
cypermethrin 2	126 (4.8)	92.7 (6.3)	106 (8.6)	91.0 (17)	109 (10)	89.4 (9.8)
cypermethrin 3	104 (9.0)	90.1 (4.8)	103 (5.2)	94.8 (3.8)	105 (8.0)	94.4 (1.7)
cypermethrin 4	96.6 (7.0)	91.6 (3.9)	92.5 (4.9)	93.8 (6.8)	89.0 (8.9)	91.2 (5.5)
fenvalerate	95.1 (16)	91.0 (11)	98.2 (8.7)	92.8 (13)	147 (11)	89.9 (11)
esfenvalerate	85.9 (16)	81.9 (4.8)	68.6 (11)	80.5 (9.7)	90.8 (7.2)	88.2 (8.1)
tralomethrin	84.4 (9.6)	79.0 (5.7)	66.1 (16)	84.5 (14)	88.7 (12)	95.0 (12)
deltamethrin	128 (24)	91.5 (13)	119 (24)	92.1 (17)	99.7 (7.3)	86.3 (12)

[a] Extraction was performed with the final method. Sample analysis was conducted with an Agilent 6890 GC coupled to an HP 5973 mass spectrometer. Specific conditions are described under Materials and Methods. [b] HFCD = high-fat composite diet (10% fat content), MFCD = medium-fat composite diet (5% fat content), LFCD = low-fat composite diet (1% fat content). [c] NR = no result.

Table 6. Method Detection Limits for Pyrethroid Pesticides in Medium-Fat Composite Diet[a]

analyte	GC-quadrupole MS (μg kg^{-1})	GC-μECD (μg kg^{-1})	GC-ion trap-MS-MS (μg kg^{-1})
allethrin 1 (a and b)[b]	ND[c]	3	33
allethrin 2 (a and b)[b]	2.2	2	6
resmethrin	1.0	non-μECD active	1
bifenthrin	0.6	1	1
tetramethrin	0.9	8	4
phenothrin	0.5	non-μECD active	3
L-cyhalothrin 1	1.5	0.5	6
L-cyhalothrin 2	0.7	0.5	3
cis-permethrin	0.9	0.5	1
trans-permethrin	1.0	3	1
cyfluthrin 1	5.5	4	1
cyfluthrin 2	ND[d]	1	1
cyfluthrin 3	ND[d]	0.5	2
cyfluthrin 4	ND[d]	2	3
cypermethrin 1	1.0	0.5	2
cypermethrin 2	1.1	2	2
cypermethrin 3	2.3	0.5	2
cypermethrin 4	1.9	0.5	3
fenvalerate	0.7	1	2
esfenvalerate	1.9	4	2
tralomethrin	2.4	4	22
deltamethrin	1.2	1	16

[a] Extraction was performed with the final method. [b] Allethrin isomers represented by two peaks under the temperature program employed in the GC-μECD and GC-ion trap-MS-MS analysis. [c] Not detected due to matrix interference at m/z 123. [d] Not detected due to matrix interference at m/z 163.

Table 7. Recoveries (Percent) from Composite Dietary Samples Collected in an Exposure-Monitoring Field Study[a]

analyte	sample 1	sample 2	sample 3	sample 4	sample 5
allethrin 1a	NR[b]	89.9	100	90.3	105
allethrin 1b	NR	94.0	117	88.9	92.1
allethrin 2a	97.9	88.1	95.7	87.5	90.9
allethrin 2b	75.9	95.7	98.9	94.0	118
tetramethrin	86.5	80.1	86.9	83.8	94.8
bifenthrin	NR	92.4	96.9	93.3	99.3
tetramethrin	NR	91.9	94.5	96.0	101
phenothrin	109	90.9	96.8	92.7	99.4
L-cyhalothrin 1	113	92.7	97.6	97.2	97.0
mirex (surrogate)	71.2	73.0	80.1	62.4	75.0
L-cyhalothrin 2	108	92.4	94.7	96.4	88.9
cis-permethrin	104	90.6	98.3	95.3	102
trans-permethrin	104	91.0	99.0	96.4	99.2
cyfluthrin 1	111	92.0	98.0	101	NR
cyfluthrin 2	110	92.6	125	97.8	NR
cyfluthrin 3	110	89.6	95.9	NR	NR
cyfluthrin 4	107	94.3	107	88.5	NR
cypermethrin 1	99.9	91.3	97.7	97.1	104
cypermethrin 2	110	91.4	99.4	100	NR
cypermethrin 3	118	92.4	98.5	98.9	NR
cypermethrin 4	107	92.2	84.3	99.2	100
fenvalerate	108	88.0	98.6	95.5	89.6
esfenvalerate	115	88.3	104	98.5	NR
tralomethrin	120	85.6	108	102	96.0
deltamethrin	109	85.7	81.8	97.4	119

[a] Samples were fortified at 50 μg kg^{-1} with each of the pyrethroid compounds. Sample analysis was conducted with an Agilent 6890 GC coupled to an HP 5973 mass spectrometer. Specific conditions are described under Materials and Methods. [b] NR = no result.

SIM mode > μECD). The instrument of highest selectivity would be most desirable in this application; however, the ion trap demonstrated poor sensitivity for the late-eluting pyrethroids. The MDL for tralomethrin was calculated at 22 μg kg^{-1} and that for deltamethrin as 16 μg kg^{-1}. The first isomer of allethrin also showed poor detection. μECD proved to be fairly sensitive; however, two of the pyrethroids of interest were not visible to this detector (resmethrin and phenothrin). Finally, the quadrupole MS equipped with a large volume injector demonstrated detection limits ranging

from 0.6 to 6 μg kg^{-1} for all but one isomer of allethrin and three of the isomers of cyfluthrin due to interferences, forcing the measurement of these analytes solely on a single isomer. As a result, the quadrupole-MS was deemed to possess the best combination of sensitivity and selectivity for the composite dietary matrix, which was not surprising due to the ability to inject a large volume of sample.

The final method was evaluated on composite duplicate diet samples collected in an exposure-monitoring field study. They were fortified at 50 μg kg^{-1} with each of the pyrethroid compounds, and recoveries ranged from 75.9 to 125%. Results for five samples are shown in **Table 7**. Surrogate recoveries ranged from 62.4 to 80.1% for all five samples. **Figure 2** shows the chromatograms of a calibration standard at a concentration of 200 μg L^{-1} and those obtained from fortified field samples 1 and 3. Concentrations for two of the analytes (bifenthrin and tetramethrin) could not be calculated for the first sample and for two of the analytes (cyfluthrin and esfenvalerate) in sample 5. This demonstrates that diversity within the diet may still need to be further addressed.

Conclusions. A method for the determination of select pyrethroids in composite diet samples was developed. The method includes two additional steps that had been shown to be successful by other researchers (*11*, *12*, *15*). First, lyophilization was performed prior to sample preparation. Second, C_{18} was included as another adsorbent in the cleanup phase, and elution of target analytes was achieved with acetonitrile. Extract residue weights were reduced, and the final method demonstrated pyrethroid recoveries were independent of fat content in laboratory-prepared composite diet samples. The final method employed GC-quadrupole-MS for analysis operated under SIM mode with a temperature-programmable preseparation column in the inlet. Method detection limits were very good for most analytes; however, interferences at the 5 μg kg^{-1} fortification level prevented calculation of detection limits for allethrin's first isomer and cyfluthrin's second, third, and fourth isomers. Although recoveries from fortified field samples were within acceptance criteria, field sample data revealed the fact that no two composite diet samples are exactly the same and interferences will often appear for some analytes. Although methods were developed using standard composite diets, in reality, samples collected in the field are as variable as the human diet itself. Thus, it may not always be possible to quantify every analyte in every sample due to interferences arising from different components of the diet.

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