Determining Ten Synthetic Pyrethroids in Lettuce and Ground Meat by Using Ion-Trap Mass Spectrometry and Electron-Capture Gas Chromatography

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Conventional extraction procedures with acetonitrile were compared with supercritical fluid extraction (SFE) with CO_2 for determining 10 pyrethroids in fortified lettuce and meat samples. Because SFE utilized a minimal cleanup procedure, nonvolatiles may accumulate on the capillary column in both the gas chromatography-ion trap mass spectrometry (GC-ITMS) and electron capture (EC) detection modes shortening the lifetime of the columns after multiple injections. The GC-ITMS method proved satisfactory for analysis of lettuce whereas EC-GC proved more suitable for residue analysis of meat samples. Improvements in multiresidue methods for monitoring of synthetic pyrethroids in raw agricultural commodities should aid in registration and reregistration activities.

Keywords: *SFE; insecticides; meat; fat; ion-trap*

INTRODUCTION

The synthetic pyrethroids are effective broad spectrum insecticides with low mammalian toxicity and short-term environmental persistence (Elliott, 1977). They are used at low concentrations compared with other agricultural chemicals on a variety of fruits, field crops, ornamentals, and animals, and for the control of household, industrial, and veterinary pests. Because the established tolerance levels for pyrethroid insecticides in meat are low and metabolism in animals is high, residues exceeding established tolerance levels for meat and other commodities may occur only because of rare accidental or deliberate adulteration.

Various methods of extraction and cleanup of the individual pyrethroids are described in the literature. Electron-capture gas chromatography (EC-GC) and mass spectrometry (MS) have been used for determining bifenthrin residues in pumpkins (Wei, 1991); deltamethrin in milk and butter (Venant et al., 1990); cypermethrin in stored wheat (Joia et al., 1985); permethrin in bovine tissues (Oehler, 1979), grasshoppers, and duck tissue (Reichel et al., 1981); and fenvalerate in milk (Wszolek et al., 1980), in cabbage and lettuce (Lee et al., 1978), and in processed apple and tomato products and byproducts (Spittler et al., 1982, 1984), the latter resulting in the establishment of a specific tolerance for fenvalerate in tomato pomace when used as an animal feed. Braun and Stanek (1982) found permethrin, cypermethrin, and fenvalerate in vegetable and animal tissue, removing much of the water and residual lipids from the acetonitrile extraction solvent simply by freezing the extract. Chapman and Harris (1978) determined four pyrethroids in four vegetables after acetone extraction and hexane partitioning.

The selective extraction of an analyte from lipids with supercritical CO_2 is extremely difficult (Murugaverl et al., 1993). Unfortunately, the efficiency of extraction of lipids with supercritical CO_2 continually increases as the density of CO_2 increases (Hierro and Santa-Maria, 1992; Gere and Derrico, 1994). Carbamate insecticides are more polar than the synthetic lipophilic pyrethroids and were found in meat after a preliminary acetonitrile extraction of the sample to remove triglycerides before supercritical fluid extraction in an effort to simplify earlier methodologies (Argauer et al., 1995). Valverde-Garcia et al. (1996) and Lehotay and Eller (1995) have investigated the use of supercritical fluids for the extraction of vegetables and fruits.

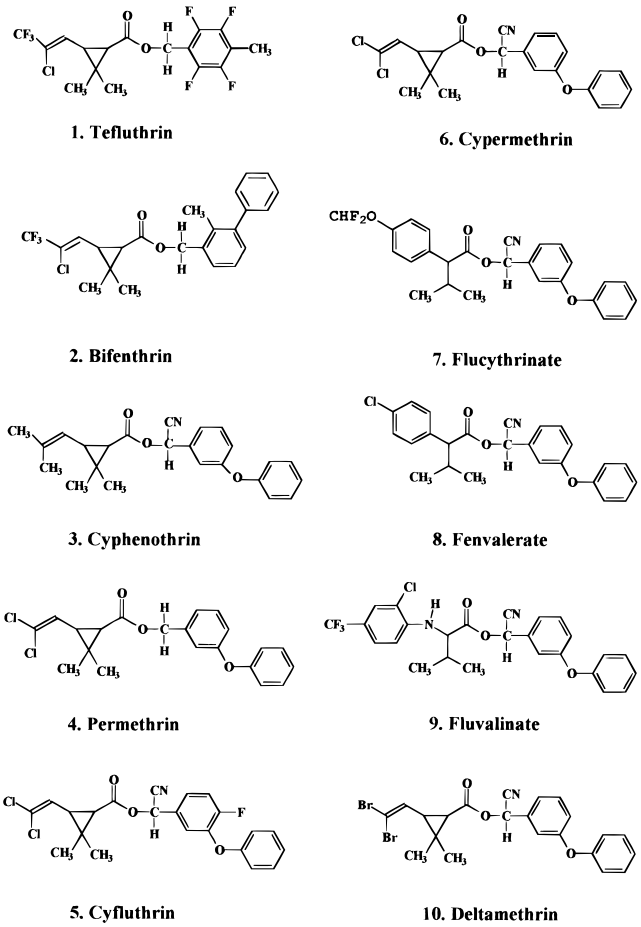

Figure 1. Chemical structures of the 10 synthetic pyrethroids used in this study.

Ten pyrethroid insecticides (Figure 1) were selected as model compounds for this study. Lettuce was used

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as an example of a sample that is high in water and low in lipid content and suitable for supercritical fluid extraction. Samples high in lipid content, such as meat or meat products, required more rigorous conventional extraction and cleanup methodologies.

MATERIALS AND METHODS

Standards. Reference standards were obtained from the Environmental Protection Agency. Analytical standard solutions of 1 mg/mL were prepared in HPLC-grade acetonitrile and stored in the refrigerator. Working standard solutions containing 5- and 10-μg/mL mixtures of each of the pyrethroids were prepared by combining 1 mL of each of the analytical standard solutions and diluting with acetonitrile. Chrysene-d_{12} (Cambridge Isotope Laboratories Woburn, MA) was used as an internal standard at a final concentration of 1 μg/mL for quantification by ion trap mass spectrometry (ITMS). Supercritical fluid extraction (SFE) was performed with chromatographic SFE (SFC/SFE) grade CO_2 (Air Products, Allentown, PA). A lower grade of CO_2 was used for cryogenic cooling of the SFE trap and for the septum-programmable injector on the GC-ITMS apparatus. Chem Elut-Hydromatrix (Celite 566, a pelletized diatomaceous earth) was obtained from Varian Sample Preparation Products Company, Harbor City, CA. Ground beef was obtained through the Meat Science and Dairy Science Laboratories (U.S. Department of Agriculture, Agricultural Research Service, Beltsville, MD) from animals that were not exposed to pyrethroid insecticides and from the meat department of several food stores.

SFE of Pyrethroids from Lettuce. A model 7680T SFE Module (Hewlett-Packard, Avondale, PA) was used that included an automated variable restrictor and a solid phase sorbent trap prepacked with 30 μm of Hypersil ODS into which the carbon dioxide extraction solvent was decompressed during collection. The CO_2 density was 0.85 g/mL at an extraction pressure of 329 bar at 60 °C. The 7-mL extraction thimble was dynamically extracted with CO_2 at a flow rate of 1.6 mL/min following an initial 2-min static extraction for a total of five thimble volumes. The nozzle temperature was 50 °C, and the sample extract was collected on a 1-mL ODS sorbent trap at 9 °C.

Twenty grams of lettuce and 20 g of hydromatrix were mixed in a mortar to form a powder. Five grams of the powder, equivalent to 2.5 g of lettuce, was transferred to each of two extraction thimbles. To the first thimble was added 2.5 μg of pyrethroid standard and the SFE extraction proceeded as described. The extracted sample was eluted from the trap with 1.5 mL of acetonitrile at 0.4 mL/min and a trap temperature of 50 °C and collected in 2-mL glass vials placed in a fraction collector. The octadecylsilane (ODS) trap was regenerated between extractions by rinsing with 2 mL of ethyl acetate followed by 2 mL of acetonitrile at 1 mL/min to waste. The time for the extraction/elution procedure per sample was ~30 min. Pressure and temperature were adjusted to a CO_2 density of 0.85 g/mL for extraction.

After extraction, 2.5 μg of pyrethroid standard was added to the vial containing the 1.5-mL acetonitrile eluate from the SFE trap extract from the second thimble. Then, 15 μL of the internal standard chrysene-d_{12} (100 μg/mL) was added to each glass vial prior to injection of 1 μL for analysis by GC-ITMS. The peak areas of the monitoring ions on the chromatograms were measured, and the concentration of each pesticide was determined from each calibration curve.

GC-MS. A Finnigan MAT model ITS40 ion trap (Finnigan MAT, San Jose, CA) with a Varian 3300/3400 gas chromatograph equipped with a DB-5ms capillary column (J&W Scientific, Folsom, CA); (30 m, 0.32 mm i.d., 0.25 μm film thickness), a 5-m phenyl-methyl deactivated guard column (Restek Corp., Bellefonte, PA; 0.32 mm i.d.), and a CTC A200S autosampler (Finnigan MAT, San Jose, CA) was used. One-microliter aliquots of solutions of standards and samples in acetonitrile were injected into a model 1093 septum programmable injector (Varian, Walnut Creek, CA) with a 3-s needle hold time in port before injection. The injection port was held at 55 °C for 30 s then taken to 230 °C in 1 min. The helium column head pressure was 5 psi. The oven temperature was held at 55 °C for 30 s, then ramped to 125 °C at 50 °C/min and to 250 °C at 3 °C/min and then held at 250 °C for an additional 4 min. The transfer line was heated to 240 °C and the detector manifold was heated to 215 °C. The ion trap was operated in the electron impact mode, with 10 μA filament current, 1850 V electron multiplier tube, 1 ms ion time, and automatic gain control at 20 000. Mass spectra were acquired at 70−550 *m/z* from 3 until 47 min after injection. A Magnum version 2.4 software package and a Gateway 2000 computer (Gateway 2000, N. Sioux City, SD) were used for data acquisition, peak identification, and quantitation.

Recovery of Pyrethroids from Ground Beef Fortified at 1 ppm with 10 Pyrethroids As Determined by EC-GC with Two Extraction Procedures. Fifty grams of ground beef was added to a blender. Five milliliters of a 10-μg/mL standard solution of the pyrethroids in acetonitrile was pipetted directly onto 50 g of ground beef in the blender. After 5 min, 95 mL of acetonitrile was added, and the sample was blended for 3 min and filtered through 9-cm Whatman no. 1 filter paper under gravity, and the filtrate was collected in a 250-mL Erlenmeyer flask.

Procedure 1. An 8-mL aliquot of the collected filtrate was transferred to a 25-mL Erlenmeyer flask, the solvent was evaporated under water aspirator pressure, and the residue was dissolved in 20 mL of hexane with the aid of ultrasonic agitation. One microliter of the dissolved residue was injected into the EC-GC and peak heights were compared with those of a 200-ng/mL standard containing 10 pyrethroids in hexane.

Procedure 2. The Erlenmeyer flask containing the filtrate was placed in a freezer. After 3 h at −29 °C, the acetonitrile solution was decanted from the ice adhering to the walls of the flask, and a 50-mL aliquot was washed with 10 mL of hexane. An 8-mL aliquot of the hexane-washed acetonitrile was evaporated, and the residue was dissolved in 20 mL of hexane with the aid of ultrasonic agitation. Then, 1 μL of the dissolved residue was injected into the EC-GC and peak height were compared with that of the pyrethroid standard injected.

These procedures can be compared with our earlier procedure for apple and tomato (Spittler et al., 1982, 1984) where 100 g of the sample is routinely extracted with 200 mL methylene chloride, filtered, dried over anhydrous Na_2SO_4, and concentrated to near dryness on a Rinco evaporator, and the residue is dissolved in 5.0−100 mL of hexane for analysis by EC-GC.

EC-GC Detection. A Hewlett Packard model 5890 series II gas chromatograph equipped with a ^{63}Ni electron capture detector, a 30 m × 0.32 mm i.d. × 0.25 μm J&W DB-17 fused silica capillary column with helium carrier gas at 3 mL/min and make-up gas at 30 mL/min, an electron-capture detector, a HP model 7673 automatic liquid sampler, and a HP Pascal Chemstation controller-data handling system was used. The column oven temperature was held at 230 °C for 1 min, ramped to 280 °C at 2 °C/min, and held at 280 °C for 15 min. The inlet was 250 °C and the electron-capture detector was at 300 °C. One-microliter injections were used. Peak heights were compared with those obtained for 50-, 100-, and 200-ng/mL standard injections in 1 μL of hexane.

RESULTS AND DISCUSSION

The chromatogram obtained for a 5 ng injection of 10 pyrethroids obtained by GC-ITMS in the total ion mode is shown in Figure 2. Under the specified gas chromatographic conditions, permethrin is resolved into its two pairs of enantiomers, cyfluthrin into four pairs, cypermethrin is partially resolved into two pairs, and fenvalerate is partially resolved into its two pairs of enantiomers. Selective ions were used for quantification and are shown in Table 1. The chromatogram obtained for a 200-pg injection of the 10 pyrethroids obtained by EC-GC under the specified GC conditions is shown in Figure 3. Relative retention

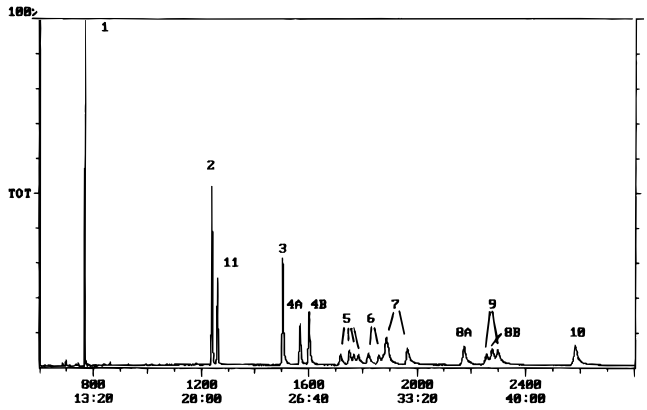

Figure 2. GC-ITMS total ion chromatogram for 10 pyrethroids. Chrysene-d_{12} was used as an internal standard for quantitation. Numbers correspond with structures in Figure 1.

Table 1. Selective Ions Used for Quantitation of the 10 Pyrethroids

no.[a]	pyrethroid	MW	m/z
1	tefluthrin	418.06	177*+197
2	bifenthrin	422.13	165+166+181*
3	cyphenothrin	375.18	123*+181+375
4a	*cis*-permethrin	390.08	183
4b	*trans*-permethrin	390.08	183
5	cyfluthrin	433.06	127+163+199+206*+226
6	cypermethrin	415.07	181*+163
7	flucythrinate	451.48	157+199*+225+451
8a	fenvalerate	419.13	125*+225*+419
8b	esfenvalerate	419.13	125*+225*+419
9	fluvalinate	502.93	181+250*+252+502
10	deltamethrin	505.22	172+181+253*+505
11	chrysene-d_{12} (internal standard)		240

[a] Numbers correspond with chemical structures in Figure 1.

times for fenvalerate and fulvalinate appear to have exchanged between the two different GC columns used.

Pyrethroids in Lettuce. SFE and GC-ITMS were used to determine the recoveries (efficiency of extraction of pesticide from sample) for samples of lettuce fortified with pyrethroids. Recoveries for the 10 pyrethroids ranged between 53 and 68%, much lower than the 90−100% recoveries experience by us using methodology based on ECGC (Spittler et al., 1982, 1984). The signal to noise ratio for the 10 pyrethroids (~1 ng injected into the GC-ITMS) ranged between 4970 and 10 for the representative ion-monitoring chromatograms. These low recoveries were attributed to losses that may have occurred during elution of the pyrethroids from the C-18 trap with acetonitrile. Elution with larger volumes of acetonitrile or elution with other solvents, such as ethyl acetate, was not attempted.

Initial Study of Pyrethroids in Ground Meat by GC-ITMS. If a 3-g sample of ground meat containing 20% fat was extracted as were the lettuce samples by SFE, nearly 600 mg of lipid, mainly as the triglycerides, would be extracted. This amount would completely clog the solid-phase sorbent trap contained within the model 7680T SFE module. Hence, a more conventional extraction procedure followed by a column chromatographic cleanup was used. In brief, 100 g of ground beef (78% lean, 22% fat) fortified with 100 μg of pyrethroid standard was blended with 200 mL of acetonitrile. Then, 150 mL of the filtrate was extracted with 100 mL of hexane, an aliquot of the acetonitrile was evaporated to near dryness, the residue was dissolved in methylene chloride and percolated through a silica gel column, and

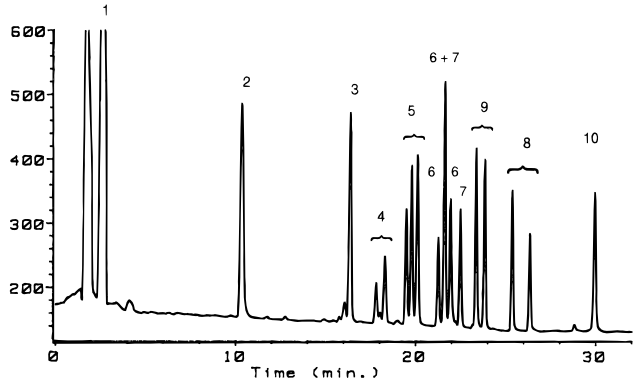

Figure 3. EC-GC chromatograms for 10 pyrethroids. Numbers correspond with structures in Figure 1.

Table 2. Pyrethroids Found in Acetonitrile Layer after Extracting Equal Volumes of Hexane or Isooctane

	pyrethroid (%)			
pyrethroid	EC[a,c]	ITMS[b,c]	EC[a,d]	ITMS[b,d]
tefluthrin	62	68	66	68
bifenthrin	55	65	65	69
cyphenothrin	90	91	90	94
permethrin	71	79	71	77
cyfluthrin	96	96	97	93
cypermethrin	95	94	93	95
flucythrinate	99	99	99	99
fenvalerate	95	96	95	98
fluvalinate	98	98	97	99
deltamethrin	95	93	96	98

[a] Initial concentration of pyrethroid determined by EC was 200 ng/mL in nonpolar phase. [b] Initial concentration of pyrethroid determined by ITMS was 5000 ng/mL in nonpolar phase. [c] Hexane. [d] Isooctane.

the eluate was evaporated, redissolved in acetonitrile and injected into the GC-ITMS. The chromatograms were compared with those from injections of standard pyrethroid (1−5 ng/μL). Poor recoveries (25−60%) of the pyrethroids were found. Both the partitioning step with hexane and the initial extraction step with acetonitrile were next investigated.

Determination of the Partitioning of Pyrethroids between Acetonitrile/Hexane and Acetonitrile/Isooctane. Historically, the partitioning of pesticides between polar and nonpolar solvents aids in separating lipids from the insecticide prior to analysis. The percentage of each pyrethroid in the acetonitrile layers after partitioning with equal volumes of hexane or isooctane was determined by both EC-GC for initial concentrations of pyrethroid at 200 ng/mL and by GC-ITMS for initial pyrethroid concentrations of 5000 ng/mL (Table 2). Large amounts of tefluthrin, bifenthrin, and permethrin were present in the hexane layer. The other seven pyrethroids, each containing a nitrile group in their molecular structure, partitioned nearly 100% into the acetonitrile layer.

Effect of Fat in the Meat Matrix on Extraction Recovery. Two procedures for extracting pyrethroids from ground meat were developed and compared. Recoveries are reported in Table 3. Clearly procedure 2 produces higher recoveries for all of the pyrethroids with the exception of tefluthrin, bifenthrin, and permethrin. In procedure 2, the 8-mL aliquot used represents a more concentrated acetonitrile extract because water was removed by freezing. Procedure 2 also includes a 10-mL hexane partitioning of the 50-mL acetonitrile aliquot, which contributes to the reduced recoveries of tefluthrin, bifenthrin, and permethrin. It is clear from

Table 3. Recoveries of 10 Pyrethroids Determined by EC-GC with Two Different Extraction Procedures

	recovery (%)	
pyrethroid	extraction procedure 1	extraction procedure 2
tefluthrin	43	33
bifenthrin	70	58
cyphenothrin	91	105
permethrin	29	27
cyfluthrin	100	113
cypermethrin	93	89
flucythrinate	93	108
fenvalerate	63	90
fluvalinate	67	81
deltamethrin	38	50

Table 4. Specific Tolerances Established for Residues of Pyrethroids in Cattle Meat and Selected Commodities as of July 1, 1994[a]

CFR part	pesticide	cattle meat tolerance (ppm)	commodity	tolerance (ppm)
180.440	tefluthrin	—	corn	0.06
180.442	bifenthrin	0.1	hops	10.0
	cyphenothrin	—	—	
180.378	permethrin	0.25	lettuce	20.0
			tomatoes	2.0
180.436	cyfluthrin	0.05	hops	4.0
185.1250	cyfluthrin	0.05	all foods	0.05
80.418	cypermethrin	0.05	lettuce	10.0
180.400	flucythrinate	0.1	lettuce	2.0
180.379	fenvalerate	1.5	tomatoes	1.0
185.1300	fenvalerate	—	all foods	0.05
186.1300	fenvalerate	—	tomato pomace	10.0
180.427	fluvalinate	0.01	honey	0.05
180.435	deltamethrin	—	tomatoes	0.2
185.1580	deltamethrin	—	tomato concentrate	1.0

[a] Part 180, raw agricultural commodities; part 185, food additives (crack, crevice, surface treatment); part 186, animal feeds.

the results obtained by procedure 1 that the lipid content of the ground meat sample also affects the recoveries of the pyrethroids. When the recoveries are compared with the 25−60% values obtained in the initial study using GC-ITMS, it is clear that water, present in concentrations of ∼20−30% in the acetonitrile meat extract, drives the pyrethroids into the less polar hexane phase.

It is not known if repetitive injections of meat sample extracts contaminate the capillary column because no detailed study was made to determine the lifetime of the column in the presence of various concentrations of unidentified coextractives.

Pyrethroid Levels in Meat and in Other Commodities. Some of the specific tolerances established for residues of pyrethroids in beef and for selected commodities are summarized in Table 4. No tolerances have been established for several of the synthetic pyrethroids in meat; hence, no residues are permitted (*Code of Federal Regulations*, 1994).

In designing multiresidue procedures, one needs first to choose the absolute level required for detection based on the established specific tolerances found in Table 4. Certainly, under our conditions, EC was more sensitive than ITMS and should be used initially to screen samples for possible insecticide residues. ITMS demonstrates the singular advantage of confirmation with mass identification. ITMS, and with proper adjustments in instrumental signal noise ratios, should prove equally as sensitive. Next, one needs to decide on the size of the aliquot needed to obtain an optimal signal-to-noise ratio for the EC and ITMS detectors. Finally, one needs to choose between using either a more rigorous cleanup step before injection or minimum cleanup, allowing contaminating coextractives to accumulate on the gas chromatographic column, which would be cleaned or replaced with new columns later.

This research serves to assure the consumer a safe food supply by providing more rapid quantitation of samples containing insecticides and increasing the number of samples monitored. The results presented in this paper were not designed to be used by regulators, but by those who wish to continue in their quest to replace conventional solvents with supercritical fluids for extraction. Some of the difficulties encountered when fatty foods, such as meat, are extracted by SFE are presented.

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