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Determining Lipophilic Pyrethroids and Chlorinated Hydrocarbons in Fortified Ground Beef Using Ion-Trap Mass Spectrometry

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A rapid procedure has been developed that is offered as an improvement for the analysis of lipophilic insecticides in meat. The number of physical and chemical manipulations required of previous methodologies to separate pesticide analytes from fat has been minimized, and the number of solvent evaporations has been reduced to one. Removal of fat from the meat extracts was accomplished with an iso-octane/acetonitrile partition and Florisil column cleanup. Recoveries of the organochlorine pesticides, lindane, *p,p'*-DDE, *p,p'*-DDT, heptachlor epoxide, and dieldrin, and the pyrethroids, bifenthrin, permethrin, cypermethrin, fluvalinate, and deltamethrin, from fortified meat samples ranged from 59 to 82% with typical relative standard deviations of 10% except for *p,p'*-DDT, which gave lower, more variable recoveries likely due to degradation. Limits of detection of the pesticides in ground beef ranged from 2 ng/g for bifenthrin and *p,p'*-DDE to 24 ng/g for heptachlor epoxide.

Keywords: *Pesticide residue analysis; meat; ion-trap mass spectrometry*

INTRODUCTION

The extraction of lipophilic pesticide residues from fatty matrices, such as ground beef, necessitates the coextraction of considerable amounts of fats to achieve high recoveries. The use of automated extraction techniques, such as supercritical fluid extraction with CO_2 (Argauer et al., 1995; Hopper, 1997; Murugaverl et al., 1993; Nam and King, 1994; Parks and Maxwell, 1994; Stalling et al., 1992) or accelerated extraction methodologies with heated liquids (Lehotay and Lee, 1997; Richter et al., 1996; Lopez-Avila et al., 1996; Pylypiw et al. 1997), cannot overcome this condition. In-line cleanup to separate the analytes from the lipids has been performed (Hopper, 1997; Murugaverl et al., 1993; Nam and King, 1994; Parks and Maxwell, 1994; Stalling et al., 1992), but automated cleanup techniques are not always cost-effective or ready for use in routine laboratories. Manual, liquid-based extraction and cleanup methods will continue to be used in many laboratories for years to come, and streamlining existing methods is an effective approach to improving laboratory efficiency.

Proven methodologies for sample extraction and cleanup for pesticides in fatty foods were developed over 25 years ago, several of which are listed in the *Pesticide Analytical Manual* (Food and Drug Administration, 1994). Methodologies based on the classical extraction and cleanup procedures have demonstrated their performance in interlaboratory validation studies (Carr, 1971; Sawyer, 1973). Porter (1970) developed a method for the extraction of lipophilic residues from animal tissues with petroleum ether in which anhydrous sodium sulfate was used to remove water and help disperse the sample. For cleanup, the method involves a lengthy liquid chromatographic step that involves fractionation using diethyl ether or methylene chloride.

In this paper, we report an improvement in the analysis of lipophilic insecticides in meat. The number of physical and chemical manipulations required to separate analyte from matrix has been minimized, and the number of solvent evaporation steps was reduced to one. To test the method, five chlorinated insecticides and five pyrethroids were selected on the basis of their historical and present-day use in agriculture and the diversity of their *p* values (Argauer et al., 1996). Analysis was performed using gas chromatography/ion-trap mass spectrometric detection (GC/ITD) which enabled both quantitation and confirmation of pesticide residues at low concentrations in the complex matrix extracts.

MATERIALS AND METHODS

Chemicals. Reference standards were obtained from the U.S. Environmental Protection Agency (EPA). Analytical standard solutions of 1 mg/L were prepared in HPLC grade acetonitrile saturated with iso-octane and stored in the refrigerator. A working standard solution containing 5 μg/mL of each of the 10 insecticides was prepared in acetonitrile saturated with iso-octane. A stock solution containing 20 μg/mL of chrysene-d_{12} and of anthracene-d_{10} (Cambridge Isotope Laboratories, Woburn, MA) in acetone was used as an internal standard for GC/ITD quantitation. Ground beef (22% fat) was obtained through the Meat Science and Dairy Science Laboratories (USDA ARS, Beltsville, MD) from animals that were not exposed to insecticides and from the meat departments of different food stores. Dry Florisil powder, 100–200 mesh (Fisher Scientific, Fair Lawn, NJ), with no furnace reactivation, was used as received. For the cleanup columns, 5 g of Florisil was added to 10 mm i.d. $\times$ 330 mm length glass chromatography columns, each containing a sealed-in coarse fritted disk (Kontes, Vineland, NJ). The packed columns were washed with 25 mL of acetonitrile before use. Vacuum suction was used to speed chromatography, but the columns were not permitted to become dry before use.

Sample Preparation. Ground beef samples, 100 g, were weighed in beakers. The working standard solution containing the 10 insecticides was pipetted onto the ground beef to give 50 and 200 μg/g concentrations (1 and 4 mL, respectively). After 3–4 h was allowed for the solvent to evaporate and the pesticides to interact with the matrix, the sample was transferred to a glass blender jar. Anhydrous sodium sulfate, 150 g, and 300 mL of iso-octane were added, and the sample was then blended for 3 min. The blender contents were filtered

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Table 1. 10 Insecticides Included in the Study, GC Retention Times, and Quantitation Masses Chosen for Analysis by GC/ITD

compound	type	U.S. tolerance (ng/g)	retention time (min:s)	quantitation masses (amu)
lindane	organochlorine	7000[a]	17:05	181 + 183 + 219
anthracene-d_{10}	intern. std.	n/a	17:53	188
heptachlor epoxide	organochlorine	200[b]	23:05	289 + 291
p,p′-DDE	organochlorine	5000[c]	25:18	246 + 316 + 318
dieldrin	organochlorine	300[b]	25:29	263 + 277 + 345
p,p′-DDT	organochlorine	5000[c]	26:58	235
bifenthrin	pyrethroid	100[d]	30:11	165 + 166 + 181
chrysene-d_{12}	intern. std.	n/a	30:30	240
trans-permethrin	pyrethroid	250[d]	35:32	183
cypermethrin	pyrethroid	none	38:59	163 + 165 + 181
fluvalinate	pyrethroid	none	44:34	181 + 250 + 252
deltamethrin	pyrethroid	none	48:14	172 + 181 + 253

[a] Tolerance in cattle fat. [b] Regulatory action level in cattle meat or fat. [c] Regulatory action level for DDT and metabolites in cattle meat or fat. [d] Tolerance in cattle meat.

Table 2. Recoveries of 10 Insecticides with Different Partitioning Procedures and Expected Recoveries Based on *p* Values

	recovery (%)				
pesticide	iso-octane fortified, 4:1 nonpolar/polar, no Florisil cleanup[a]	meat fortified, 4:1 nonpolar/polar, Florisil cleanup[b]	meat fortified, 1:1 nonpolar/polar, Florisil cleanup[c]	*p* value[d]	expected recovery [e] (%)
lindane	72	74	72	0.12 [f]	89
heptachlor epoxide	56	50	67	0.29 [f]	79
p,p′-DDE	22	25	52	0.56 [f]	75
dieldrin	52	46	65	0.33 [f]	78
p,p′-DDT	43	44	77	0.38 [f]	76
bifenthrin	43	40	68	0.35 [g]	77
trans-permethrin	59	61	85	0.21 [g]	83
cypermethrin	78	103	129	0.06 [g]	94
fluvalinate	80	97	100	0.02 [g]	98
deltamethrin	81	92	90	0.07 [g]	93

[a] 100 mL of iso-octane containing 33 ng/mL of each pesticide partitioned with 2 × 25 mL of acetonitrile. [b] 100 mL of ground beef extract in iso-octane containing 33 ng/mL of each pesticide partitioned with 2 × 25 mL of acetonitrile and Florisil cleanup procedure.[c] 25 mL of ground beef extract in iso-octane containing 33 ng/mL of each pesticide partitioned with 2 × 25 mL of acetonitrile and Florisil cleanup procedure. [d] *p* value is the fraction remaining in the nonpolar phase after partitioning. [e] Calculated from *p* values [% recovery = (1 − *p* value + *p* value^2) × 100%]. [f] *p* values from Bowman and Beroza (1965). [g] *p* values calculated from Argauer et al. (1996).

through a 9 cm diameter Whatman No. 1 filter paper disk in a Büchner funnel under gravity, and the filtrate was collected in a 500 mL Erlenmeyer flask. In an initial experiment to determine liquid−liquid partitioning factors, different amounts of spiked iso-octane and meat extracts were partitioned in a separatory funnel with two 25 mL portions of acetonitrile. In the final procedure, 25 mL of the collected filtrate (equivalent to 8.3 g of sample) was transferred to a 60 mL separatory funnel and partitioned with two 25 mL portions of acetonitrile (an experiment was also conducted using acetonitrile presaturated with iso-octane). The acetonitrile layers (lower) were combined and percolated through a Florisil column, and the column was then washed with an additional 25 mL of acetonitrile. Approximately 70 mL of the eluate was collected in a 250 mL Erlenmeyer flask, and the solvent was evaporated just to dryness in a rotary evaporator. Iso-octane, 8.3 mL, and 200 μL of 20 μg/mL internal standard solution were added to all extracts. The 5 μg/mL working standard solution was diluted in blank matrix control extracts to provide calibration standards equivalent to 25, 50, 100, and 200 ng/g in the ground beef.

Analysis. For analysis, a Model ITS40 (Finnigan MAT, San Jose, CA) GC/ITD with a Varian 3300/3400 gas chromatograph was used. The analytical column was a 30 m, 0.25 mm i.d., 0.25 μm film thickness DB-5ms capillary column (J&W Scientific, Folsom, CA), which was placed after a 5 m, 0.25 mm i.d. phenylmethyl deactivated guard column (Restek Corp., Bellefonte, PA). The injection inlet was a Model 1093 (Varian, Walnut Creek, CA) septum programmable injector. Injection volume of the final extract was 1 μL using a CTC A200S autosampler. The injection port was held at 50 °C for 6 s, then taken to 230 °C at 20 °C/min, and held at 230 °C until the end of the run. The oven temperature was held at 50 °C for 6 s, then ramped to 130 °C at 20 °C/min and to 260 °C at 5 °C/min, and held at 260 °C until 50 min total run time. The helium column head pressure was 10 psi. The transfer line was heated to 260 °C, and the detector manifold was heated to 220 °C. The ion trap was operated in the electron ionization mode with 10 μA filament current, 1 ms ion time, and automatic gain control typically at 20 000. Mass spectra were acquired at *m/z* 70−360 from 10 min until 50 min after injection, and Magnum version 2.4 software package was used for data acquisition, peak identification, and quantitation. Table 1 lists the retention times for the pesticides and internal standards and the masses used for quantitation in the final extracts.

RESULTS AND DISCUSSION

Historically, partitioning between polar and nonpolar solvents in a separatory funnel has been used to help separate fats from insecticide analytes prior to chromatography. Table 2 summarizes the percentages of the 10 insecticides recovered in different partitioning experiments and allows comparison of the recoveries with the expected recoveries based on the *p* values for the individual insecticides published previously (Argauer et al., 1996; Bowman and Beroza, 1965). The *p* value is the fraction of pesticide that remains in the nonpolar phase in a liquid−liquid partitioning between immiscible solvents using a 1:1 nonpolar/polar ratio.

As shown in Table 2, the pesticide recoveries varied between 22 and 129% depending on the pesticide, matrix, and iso-octane/acetonitrile ratio. The results for *p,p′*-DDT, *trans*-permethrin, fluvalinate, and deltamethrin agreed closely with expected values when the

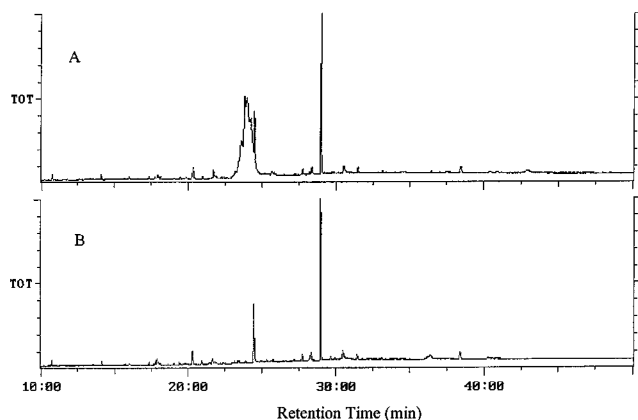

Figure 1. GC/ITD total ion chromatograms of blank ground beef extracts partitioned in (A) 1:1 iso-octane/acetonitrile (neat) and (B) 1:1 iso-octane/acetonitrile (presaturated in iso-octane).

1:1 nonpolar/polar solvent ratio was used. The other pesticides gave lower than expected recoveries by 9–23% except for cypermethrin, which exceeded the plausible 100% recovery due to an unknown error in quantitation. However, the presence of meat coextractives in iso-octane was not the cause of the lower than expected recoveries because, as shown in Table 2, the recovery differences between the pesticides fortified in iso-octane and in meat extracts were not significant. It was curious though that the only pesticides containing nitrile groups (cypermethrin, fluvalinate, and deltamethrin) were the ones that gave the greatest recovery differences between partitioning in neat solvents versus meat extracts at the 4:1 iso-octane/acetonitrile ratio. In a previous study, the compounds containing the nitrile group partitioned nearly 100% into the acetonitrile layer, while larger amounts of pesticides that did not contain nitriles, such as bifenthrin and permethrin, remained in the nonpolar layer (Argauer et al., 1996).

Table 2 also shows that the use of a 1:1 iso-octane/acetonitrile ratio increased the pesticide recoveries in the acetonitrile layer in all cases versus the 4:1 ratio except for lindane, which remained at 72–74%. The recovery of *p,p*′-DDE increased the most dramatically in going from 22–25 to 52% by using the 1:1 nonpolar/polar solvent partitioning ratio. On the basis of these results, the 1:1 iso-octane/acetonitrile ratio was used in the final procedure.

Another interesting observation from these experiments was the effect of using neat acetonitrile versus acetonitrile presaturated with iso-octane in the liquid–liquid partitioning step. If acetonitrile saturated with iso-octane was used, an emulsion formed in the meat extracts which required sonication or other means to break it. No emulsion occurred when neat acetonitrile was used. The partitioning of the pesticides in the solvent layers was unaffected whether neat or presaturated acetonitrile was used, but as Figure 1 shows, a larger amount of matrix coextractives appeared in the final extracts when neat acetonitrile was used. Figure 1 is a comparison of the total ion chromatograms of blank meat extracts when neat acetonitrile or acetonitrile saturated with iso-octane was used in the partitioning step. Despite the presence of the coextracted peak, the procedure using neat acetonitrile was chosen in the final method to avoid the emulsion. Figure 2 demonstrates the ability of the GC/ITD to overcome the potential interferant and still detect the coeluting pesticide, heptachlor epoxide, by selecting quantitation masses 289 + 291 that were not present in the matrix

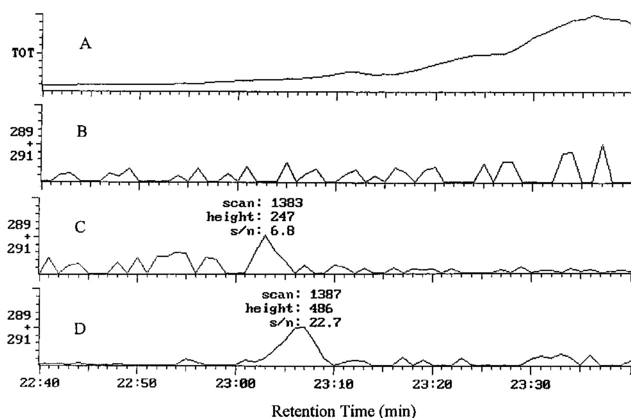

Figure 2. GC/ITD chromatograms of heptachlor epoxide in meat extracts using neat acetonitrile during partitioning: (A) total ion chromatogram of blank meat extract; (B) chromatogram using 289 + 291 quantitation ions for blank; (C) selected ion chromatogram for a 50 ng/g fortified meat sample; (D) selected quantitation ion chromatogram for a 200 ng/g fortified meat sample.

Table 3. Recoveries, Standard Deviations (SD), and Limits of Detection (LOD) of the 10 Insecticides Fortified in Ground Beef Using the Final Method

	% recovery ± SD			
pesticide	for 50 ng/g spike[a]	for 200 ng/g spike[a]	overall[b]	LOD[c] (ng/g)
lindane	72.1 ± 6.3	72.2 ± 5.6	72.1 ± 5.9	3
heptachlor epoxide	62 ± 12	88 ± 16	75 ± 20	24
p,p′-DDE	61.9 ± 5.5	56.3 ± 2.8	59.1 ± 5.2	2
dieldrin	71.7 ± 6.5	69.4 ± 6.0	70.6 ± 6.4	15
p,p′-DDT	48.1 ± 2.6	31.1 ± 4.3	39.6 ± 9.2	21
bifenthrin	71.9 ± 8.6	68.9 ± 4.6	70.4 ± 7.0	2
trans-permethrin	75.4 ± 7.4	71.9 ± 5.8	73.6 ± 6.9	3
cypermethrin	82 ± 12	80.4 ± 6.9	81 ± 10	8
fluvalinate	86 ± 12	79.3 ± 6.6	82 ± 10	10
deltamethrin	83 ± 17	73.5 ± 5.0	78 ± 13	18

[a] $n = 3$. [b] $n = 6$. [c] Concentration in meat at which S/N = 3.

background. The signal/noise (S/N) ratio for heptachlor epoxide was lower in this case, leading to a higher limit of detection (LOD) of 24 ng/g, than when presaturated acetonitrile was used for partitioning (LOD of 11 ng/g), but both values were well below the 200 ng/g regulatory action level.

Table 3 presents the pesticide recoveries and standard deviations for ground beef fortified at 50 and 200 ng/g when using the final method. The overall recoveries in Table 3 were similar to the results from the initial experiment for the 1:1 liquid–liquid partitioning ratio presented in Table 2 except for *p,p*′-DDT. Also, with the exception of *p,p*′-DDT, the recoveries at the 50 ng/g fortification level were similar to those at 200 ng/g. The recoveries of *p,p*′-DDT were 48.1% (5.4% RSD) at 50 ng/g and 31.1% (14% RSD) at 200 ng/g. In practice, *p,p*′-DDT is known to degrade during sample processing or analysis, and the observed discrepancy in its recoveries is believed to be related to analyte degradation.

The average recoveries of heptachlor epoxide increased from 62% (20% RSD) to 88% (19% RSD) when going from 50 to 200 ng/g, but this was not significant due to the relatively variable quantitation. This variability was probably related to the presence of the coeluting matrix component in the meat extracts. The overall percent relative standard deviations (% RSD) for the recoveries varied from 8% for lindane to 27% for heptachlor epoxide. Lindane, *p,p*′-DDE, dieldrin, bifenthrin, and *trans*-permethrin gave reproducibility

<10% RSD, and only heptachlor epoxide and *p,p′*-DDT gave >20% RSD. The overall average variability of the analyses was 14% RSD.

The LOD of the method for each pesticide was determined from the S/N ratio provided by the data acquisition software for each peak (see Figure 2). The concentration at which the S/N = 3 was the LOD for the pesticide. The LODs for each pesticide from the fortified samples were averaged and are listed in Table 3. The variability on the LOD ranged from 9% RSD for *trans*-permethrin to 65% for *p,p′*-DDT, with 20−40% RSD ($n = 6$) being typical for most pesticides. This is very good repeatability considering the low values of the LOD. Also, the S/N ratios were reasonably linear, as in Figure 2 in which the S/N for heptachlor epoxide is approximately 4 times higher for a 4-fold increase in concentration. Furthermore, the LODs already account for the pesticide recoveries of the method, and they are suitably low for most analytical applications.

The final procedure developed in this study can be compared with a previous procedure for apples and tomatoes (Spittler et al., 1982, 1984) in which 100 g samples containing relatively little fat were routinely extracted with 200 mL of methylene chloride. The samples were then filtered and concentrated, and the residue was dissolved in hexane for analysis using GC/electron capture detection. The results in the collaborative efforts compared favorably with those obtained using more extensive cleanup procedures (Spittler et al., 1982, 1984).

In this study, the use of mass spectrometric detection to gain selectivity obviates the need for extensive cleanup prior to analysis. This streamlined procedure for ground meat removes a chromatographic step that requires diethyl ether, a potentially dangerous solvent in the laboratory, and saves time, labor, and material costs. As new improvements in ion-trap monitoring devices are introduced to achieve even higher selectivity and sensitivity through the use of tandem mass spectrometry, it may become possible to further reduce cleanup of complex sample extracts. The ability to quantify and confirm the presence of low-level contaminants with a single injection in GC/MS is also advantageous over traditional methods of analysis using selective detectors that require multiple injections of the same extract.

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