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GAS CHROMATOGRAPHIC DETERMINATION OF DELTAMETHRIN IN BIOLOGICAL SAMPLES*

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SUMMARY

A gas chromatographic method is described for the detection and estimation of deltamethrin in spiked samples of urine, feces, milk, liver, kidney, muscle and subcutaneous fat. It involved extraction with acetone, with the exception of milk and urine samples, liquid–liquid partition with acetonitrile, followed by clean-up on a micro-column using benzene–hexane (1:1) solvent system. Milk and urine samples were extracted with hexane followed by liquid–liquid partition with acetonitrile. Under the gas chromatographic conditions employed, deltamethrin eluted in less than 4 min.

INTRODUCTION

Deltamethrin,[*S*]-α-cyano-3-phenoxybenzyl-(1*R*,*cis*)-2,2-dimethyl-3-(2,2-dibromovinyl)cyclopropane carboxylate, is one of the newly developed synthetic pyrethroids. It is also one of the most potent insecticides currently known and has a high mammalian toxicity[1].

Synthetic pyrethroids including deltamethrin are fast gaining acceptance as agricultural pesticides. Consequently, analytical techniques are needed to detect their presence in food (*e.g.*, milk, animal tissues, etc.) and feed. Various methods, including gas chromatography (GC)[2,3] and high-performance liquid chromatography (HPLC)[4,5], have been reported for the detection and estimation of deltamethrin in vegetable products. However, at present no method is available in the literature to estimate deltamethrin residues in animal tissues. As a part of the on-going research on the metabolism of synthetic pyrethroids in livestock and poultry, analytical procedures for detection and estimation of deltamethrin in urine, feces, milk and animal tissues have been developed and are described in this report.

EXPERIMENTAL

Chemicals

Labeled (^{14}CN) and unlabeled technical-grade deltamethrin were prepared by

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following the published procedure[6]. In this study, the stock solution of deltamethrin also contained small amounts (7–9%) of [*R*]-α-cyano-3-phenoxybenzyl-(1*R*,*cis*)-2,2-dimethyl-3-(2,2-dibromovinyl)cyclopropane carboxylate. A sample of analytical grade deltamethrin was a generous gift from Hoechst Canada Ltd. Pesticide grade solvents were used as received.

Gas chromatographic system

A Perkin-Elmer gas chromatograph Sigma 1, equipped with a ^{63}Ni electron-capture detector, was used. The column used was a glass tube (1 m × 4 mm I.D.) packed with 3% SE-30 on 80–100 mesh Chromosorb W HP. The operating temperatures for injector, column and detector were 280, 260 and 400°C, respectively; methane–argon (5:95) at the rate of 35 ml/min was the carrier gas. The retention time for deltamethrin was *ca.* 3.1 min. Under the GC conditions detailed above, 260 pg of deltamethrin gave 50% full-scale deflection. The peak height method was used for quantitation.

Measurement of radioactivity

Radioactivity measurement of the extracts (2.0 ml) was carried out on a Packard Tri-Carb liquid scintillation spectrometer Model 3320 using Bioscint (12 ml ICN, Chemical and Radioisotope Division, Irving, CA, U.S.A.), a high-efficiency emulsifier liquid scintillation cocktail. Corrections for quenching were made.

Extraction

Biological samples were spiked with radiolabeled deltamethrin (106 μg = $5.2 \cdot 10^5$ dpm) and its recovery was calculated by both GC and radioactivity measurement techniques.

Milk. An aliquot (15 ml) of spiked cow's milk was placed in a Waring blender and blended with 60 ml of hexane for 1 min. The mixture was filtered and the residue was washed with hexane. A known volume of the hexane extract (equivalent to 10 ml of milk) was withdrawn and concentrated to *ca.* 3 ml on a rotary evaporator (*ca.* 35°C). The residue was quantitatively tranferred into a 40-ml centrifuge tube, and the flask was rinsed with hexane (2 × 1 ml). The combined hexane layer was extracted with acetonitrile (3 × 10 ml), and the acetonitrile phase was again shaken with hexane (2 × 10 ml) and the hexane phase was discarded. The acetonitrile extract was dried over sodium sulfate, filtered and evaporated to dryness. The residue was redissolved in 5 ml of benzene–hexane (1:1). An aliquot (2 ml), in duplicate, was used for radioactivity measurement; and 0.5 ml was subjected to column clean-up prior to GC analysis. The details on the column clean-up is described in the *Clean-up technique* section.

Urine. Spiked cow's urine samples (10 ml) were diluted with 10 ml of water and shaken vigorously with 50 ml hexane in a separatory funnel. The hexane layer was collected and the aqueous phase was shaken twice with 25 ml of hexane. The combined hexane layer was dried over anhydrous sodium sulfate, filtered and concentrated on a rotary evaporator. The hexane layer (2 ml in duplicate) after appropriate volume adjustement (5 ml) was radioassayed. A portion (0.5 ml) of the hexane layer was analyzed for deltamethrin directly on GC after necessary dilution.

Feces. An aliquot (10 g) of fresh feces (cow) was transferred into a wide-mouthed

erlenmeyer flask and blended with 10 ml of water and 30 ml of acetone with a Super Dispax homogenizing mill for 3 min. The mixture was filtered, and the residues including filter paper were blended twice with 10 ml water and 25 ml of acetone and re-filtered. The combined extract was transferred into a separatory funnel, and shaken with water (100 ml) and hexane (35 ml) for 3 min. The emulsion was broken by addition of 25 ml of 2% sodium chloride solution. The hexane layer was removed and the aqueous phase was reextracted four times with 25 ml of hexane. The combined hexane extract was dried over anhydrous sodium sulfate, filtered, concentrated and the volume adjusted (5 ml). A 2-ml aliquot, in duplicate, was radioassayed. A portion (0.5 ml) was subjected to column clean-up and analyzed on GC after necessary dilution.

Tissues. Fortified samples (10 g) of liver, kidney, muscle and fat tissues were homogenized with 10 ml of water and 50 ml of acetone in a Waring blender for 5 min (liver pieces were homogenized for 2 min). Prolonged homogenization with fresh liver pieces resulted in a considerable loss of deltamethrin (probably due to metabolic activity of liver enzymes). The homogenate was filtered and the residues were re-homogenized twice with 10 ml of water and 25 ml of acetone and filtered. The combined filtrate was transferred to a separatory funnel and extracted with hexane (100 ml). Occasional emulsions were disturbed by the addition of a sufficient amount of 2% sodium chloride colution. The hexane layer was removed and the acetone–water phase was re-extracted twice with 25 ml of hexane. The hexane extract was dried over anhydrous sodium sulfate, filtered and concentrated to a known volume (5 ml). Radioactivity of a 2-ml aliquot, in duplicate, was determined. A 0.5-ml aliquot was subjected to clean-up on a micro-column and analyzed on a GC, after appropriate dilution.

Fatty tissues, such as subcutaneous fat samples, were difficult to homogenize. Fat samples were mixed with washed sand (1 g of sand per gram of tissue) prior to homogenization. The extraction and clean-up procedures were the same as detailed above.

Clean-up technique

The column, a disposable Pasteur pipet (22.9 cm), was packed with a cotton plug (pre-washed with eluting solvent), 5 cm of deactivated Florisil (10% water) and 3 cm of 25% cellulose in decolorizing charcoal, in that order. The column was washed with 10 ml of benzene–hexane (1:1) just prior to use. A known volume (0.5 ml) of the extract was placed on the column and eluted with benzene–hexane (1:1). The first 3 ml were discarded, and next two 7-ml fractions were collected. GC analyses showed that deltamethrin was completely eluted in the first 7-ml fraction; no tailing in the next 7 ml was observed.

The use of aluminum oxide (neutral) as a column packing material was also investigated. Aluminum oxide tended to retain deltamethrin longer, and polar solvents (*e.g.*, chloroform, dichloromethane, etc.) were required to elute the compound from the column.

Identical extraction and clean-up procedures were carried out for control biological samples and the standard deltamethrin. Deltamethrin standards of known concentration (*i.e.*, known amounts) were applied directly on the extracting solvent, and the procedures detailed above were repeated.

RESULTS AND DISCUSSION

Under the GC conditions detailed above, deltamethrin and [*R*]-α-cyano-3-phenoxybenzyl-(1*R*,*cis*)-2,2-dimethyl-3-(2,2-dibromovinyl)cyclopropane carboxylate had the identical retention time of *ca.* 3.1 min (2.93–3.16). The GC responses of pure deltamethrin and the compound used in this study, which contain 7–9% of [*R*]-α-cyano-3-phenoxybenzyl-(1*R*,*cis*)-2,2-dimethyl-3-(2,2-dibromovinyl)cyclopropane carboxylate, were identical. The response for deltamethrin was linear between 0.03 and 0.35 ng, and the estimated detection limit (twice the height of the noise level) was *ca.* 5 pg. The limit of detection was greatly dependent on the column conditions and the operating temperature of the detector. In order to maintain the column at its optimum performance, it was treated with 50 μl of Silyl 8 after 3 days of continuous use. The detector temperature was kept at 400°C. A lower detector temperature of 325 or 350°C produced inconsistent responses after only a few injections of the biological extracts.

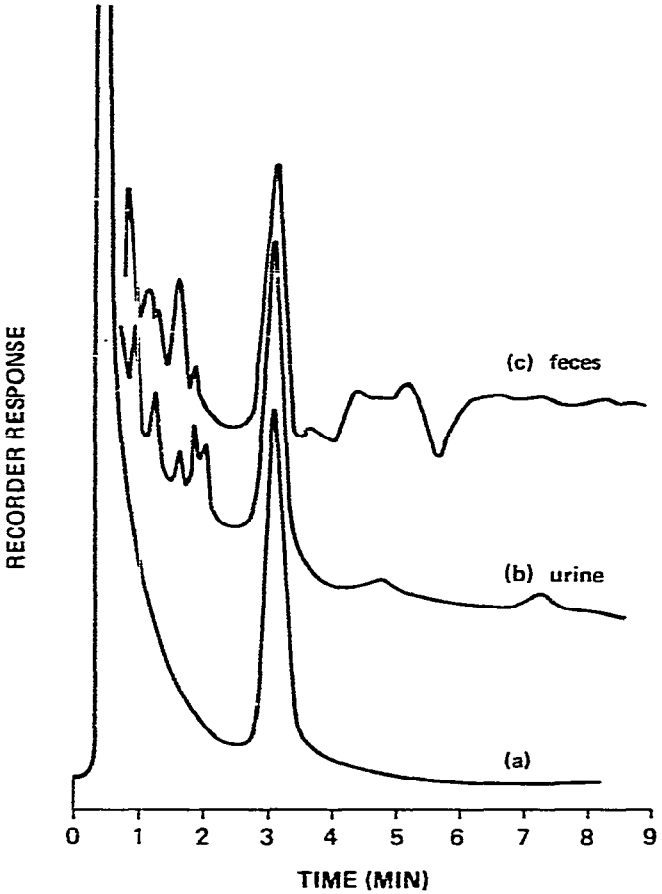

Fig. 1. Chromatograms of extracts of (a) standard deltamethrin after recovery, (b) spiked urine and (c) spiked feces. Studies were carried out at the 0.05-ppm level.

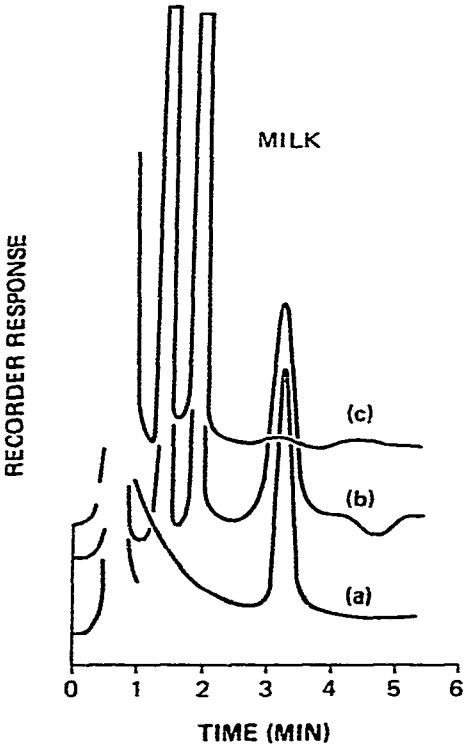

Fig. 2. Chromatograms of extracts of (a) standard deltamethrin after recovery, (b) spiked milk and (c) control milk. Spiking level was 0.05 ppm.

The clean-up procedure utilized in this study makes use of a liquid–liquid partition of the extracts (hexane or acetone) with acetonitrile followed by chromatography on a micro-column (Pasteur pipet) packed with deactivated Florisil (10% water) and 25% cellulose in decolorizing charcoal using benzene–hexane (1:1) as the eluting solvent.

Recovery studies were carried out on both control and spiked biological samples, and deltamethrin standards. A considerable loss (21.4–28.7%) of deltamethrin was recorded when deltamathrin was carried through the entire extraction and clean-up procedures. It is noteworthy that recovery of deltamethrin was excellent (91.5–95%) during the clean-up, as determined by GC analyses, when applied directly on the column.

Chromatograms of the extracts of the biological samples and the deltamethrin are shown in Figs. 1–3. Fig. 1 records the chromatograms of (a) deltamethrin, (b) urine, (c) feces extracts of spiked samples after clean-up. Urine and feces extracts did not exhibit interfering peaks at the retention time of deltamethrin.

Fig. 2 compares the chromatograms of standard deltamethrin (after recovery) with those of extracts from fortified and control milk samples. Again, milk extracts did not show peaks interfering with deltamethrin. However, both control and spiked milk extracts had additional peaks close to the solvent peak. Attempts at further clean-up using hexane were not successful in removing interfering compounds.

Chromatograms of extracts of spiked and control liver, kidney, muscle and subcutaneous fat samples, and standard deltamethrin are presented in Fig. 3. Chromatograms of the control biological samples did not exhibit any peaks that interfered with deltamethrin. This helped the estimation of deltamethrin in the spiked samples.

Recovery of deltamethrin from spiked biological samples are shown in Table I. The data are based on the assumption that both deltamethrin and the other isomer (7–9%) behaved identically during the extraction and clean-up procedures. In addition, data in Table I take into account the losses of deltamethrin during the extraction and clean-up steps. The values in Table I were generated by comparing the recovery values of deltamethrin from biological samples with those obtained without biological samples. In other words, the recovery values of deltamethrin without biological samples have been treated as a standard (100%) and the other values are relative to these values and are corrected values.

Data in Table I are based on both radioactivity measurement and GC analyses of the extracts. Radioactivity data were recorded prior to clean-up, and in most cases were greater than 90%. On the other hand, GC data were obtained after clean-up, with the exception of urine, and the recovery was in the 80% range. The differences between the radioactivity and GC recovery data suggest that a portion of deltamethrin, although extractable, is somehow bound to biological materials and is being retained on the column during the column clean-up.

GC analyses of extracts of spiked liver samples (fresh) show considerable loss of deltamethrin, yet the radioactivity data are still in 90% range. It may be due to transformation of deltamethrin by the action of liver enzyme(s) into product(s) containing ^{14}CN moiety. This phenomenon would reduce the concentration of deltamethrin while maintaining the radioactivity counts. The effect of liver enzyme(s) on the fate of deltamethrin is being studied further.

The method detailed above permits extraction, isolation and quantitation of

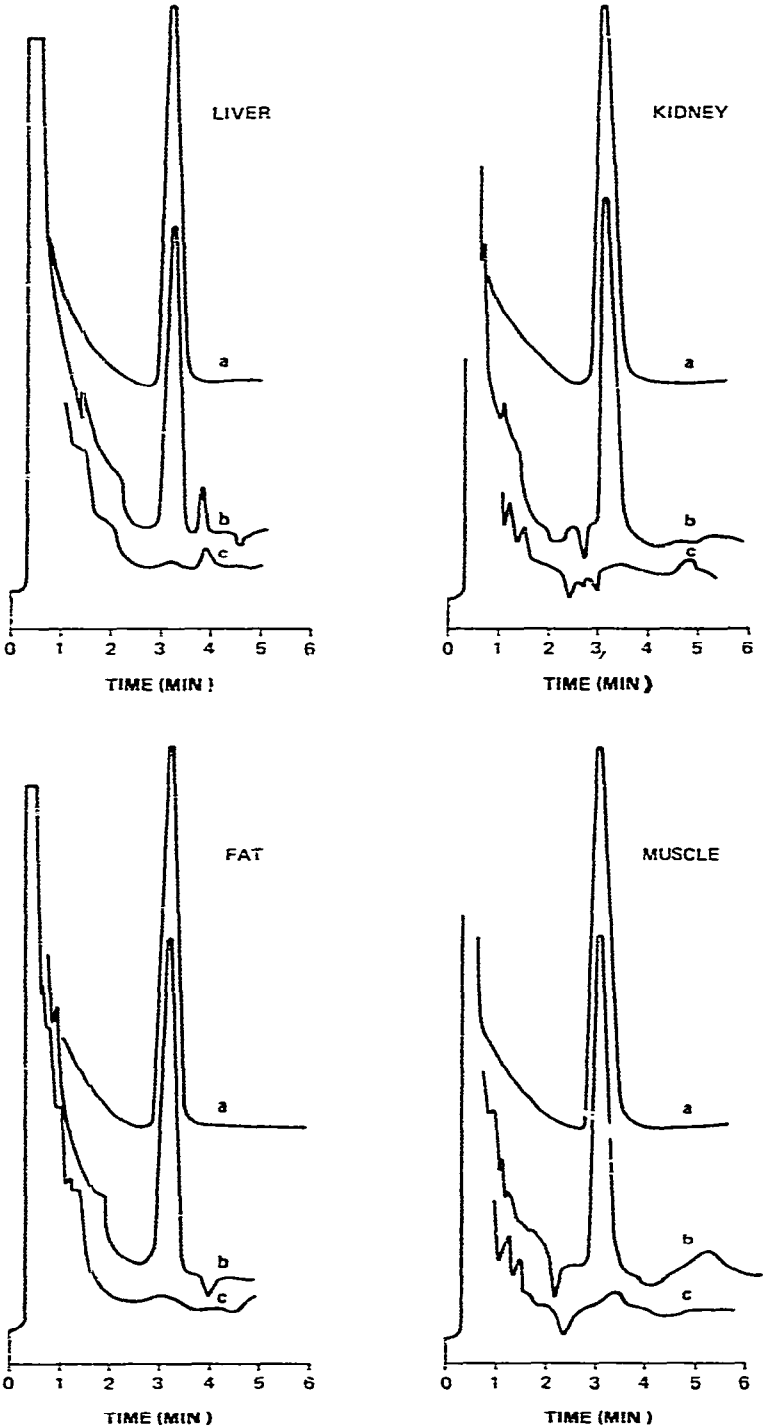

Fig. 3. Chromatograms of extracts. (i) Liver: (a) deltamethrin; (b) spiked liver; (c) control liver. (ii) Kidney: (a) deltamethrin; (b) spiked kidney; (c) control kidney. (iii) Fat: (a) deltamethrin; (b) spiked fat; (c) control fat. (iv) Muscle: (a) deltamethrin; (b) spiked muscle; (c) control sample. Deltamethrin refers to standard deltamethrin after recovery, and all the studies were conducted at the 0.1-ppm level.

TABLE I

RECOVERY OF DELTAMETHRIN FROM BIOLOGICAL SAMPLES

Sample	*Added (ppm)*	*Recovery (%)*·***	
		GC method	*Radioactivity*
Urine	0.1	98–104	99–102
	0.05	81–87	95–97
	0.01	76–81	93–97
Feces	0.1	73–79	95–98
	0.05	70–75	89–93
	0.01	67–72	93–94
Milk	0.1	83–87	99–102
	0.05	80–89	96–97
	0.01	67–72	93–94
Liver	0.1	83–89	92–94
	0.05	67–75	93–95
	0.01	68–73	78–81
Kidney	0.1	82–91	93–102
	0.05	81–87	87–89
	0.01	70–78	93–94
Muscle	0.1	88–95	88–89
	0.01	83–94	93–95
Fat	0.1	80–86	102–104
	0.01	75–79	93–103

* For three determinations each.

** Corrected using deltamethrin recovery values without biological samples as a standard.

detlamethrin from biological samples. The techniques detailed here are being used in *in vitro* and *in vivo* studies of deltamethrin with poultry and farm animals. These procedures are also being extended to include the extract residues of cypermethrin and fenvalerate from biological samples.

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