Simultaneous Determination of Tralomethrin, Deltamethrin, and Related Compounds by HPLC with Radiometric Detection

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Analytical methodologies for the simultaneous determination of the synthetic pyrethroids tralomethrin, deltamethrin, and decamethrinic acid (Br_2CA) in water, sediment, and fish tissue by HPLC with radiometric detection (HPLC-RAM) are described. The separation of the three compounds was achieved using a hexane/dioxane gradient solvent system on a silica column. This solvent system could also be used to resolve α-(*R*)-deltamethrin from *trans*-deltamethrin. Water, sediment, and fish tissue sample preparation involved relatively simple solvent extractions. The quantitation of these pyrethroids was validated at low nanograms per liter (parts per trillion) concentrations for water samples and low nanograms per gram (parts per billion) levels for sediment samples. The objective of this work was to provide a relatively simple, rapid, and sensitive method which permits the simultaneous identification and quantification of radiolabeled tralomethrin, deltamethrin, and their related compounds.

INTRODUCTION

Tralomethrin and deltamethrin are synthetic pyrethroids used to control insect pests in crops. Pyrethroid insecticides are generally very toxic to aquatic organisms (Anderson, 1983; Stephenson, 1982) and usually have extremely low water solubilities. The use of insecticides, oftentimes, is the cause for the contamination of water bodies in agricultural areas. A complete understanding of the environmental fate of these pyrethroids in aquatic ecosystems is critical for ecological risk assessments of these chemicals. To study the environmental fate of these pyrethroids, a selective and very sensitive analytical method is essential.

Tralomethrin has been shown to degrade rapidly to deltamethrin. Deltamethrin, once sprayed on a pond, has been shown to disappear quickly from water with half-lives of less than 24 h (Muir et al., 1985; Maguire et al., 1989). Major degradation or dissipation routes include (a) sorption to suspended solids, plants, and sediment, (b) chemical and photochemical degradation to deltamethrin stereoisomer (Ruzo et al., 1977; Hill, 1983; Hill and Johnson, 1987; Hill and Inaba, 1987), and (c) hydrolysis with subsequent hydrolytic products. One major hydrolytic degradation product, decamethrinic acid (Br_2CA) (Muir et al., 1985), and two isomers of deltamethrin, α-(*R*)-deltamethrin and *trans*-deltamethrin (Maguire et al., 1989), were the focus of this study along with parent tralomethrin and deltamethrin. It should be noted that these degradation products showed significantly less acute toxicity relative to parent deltamethrin (Ruzo et al., 1977).

In this paper, methods for detecting tralomethrin, deltamethrin, and Br_2CA from water, sediment, and fish tissue are described. Because of the extremely low concentration and the complexity of sample matrices, radiolabeled (^{14}C) materials were used. In addition, the HPLC separation also resolved α-(*R*)-deltamethrin and *trans*-deltamethrin from parent deltamethrin, making it possible for a single-run analysis of all five compounds. This method was tested in an aquatic microcosm study with tralomethrin and deltamethrin. In this microcosm study, the degradation of tralomethrin and deltamethrin in water, sediment, and fish tissue was monitored. To determine the kinetic half-lives of tralomethrin and deltamethrin in water and sediment, samples were taken and analyzed at 0.5, 1, 2, 4, 8, 24, 96, and 168 h. This method was proved to be rapid, relatively simple, and effective for this kind of labor-intensive outdoor study.

MATERIALS AND METHODS

Chemicals. All pyrethroids and degradation products were received from Roussel Uclaf, Paris. Radiolabeled (^{14}C, methyl-labeled) tralomethrin [[1(*R*)-[1α(*S**),3α]]-2,2-dimethyl-3-(1,2,2,2-tetrabromoethyl)cyclopropanecarboxylic acid, cyano(3-phenoxyphenyl)methyl ester] was identified to have a specific activity of 60 mCi/mmol and a radiopurity of 97.7%. Radiolabeled (^{14}C, methyl-labeled) deltamethrin [[1(*R*)-[1α*(*S**),3α]]-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropanecarboxylic acid, cyano-(3-phenoxyphenyl)methyl ester] was identified to have a specific activity of 60 mCi/mmol and a radiopurity of 100%. Reference standards of α-(*R*)-deltamethrin [[1(*R*)-[1α(*R**),3α]]-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropanecarboxylic acid, cyano-(3-phenoxyphenyl)methyl ester], *trans*-deltamethrin [[1(*R*)-[1α(*S**),3β]]-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropanecarboxylic acid, cyano(3-phenoxyphenyl)methyl ester], and decamethrinic acid [(1(*R*)-*cis*)-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropanecarboxylic acid] were obtained in an unlabeled form. Radiolabeled Br_2CA was also obtained from Roussel Uclaf. Chemical structures of the above compounds including the position of radiolabels are shown in Figure 1. All other chemicals were of analytical or reagent grade, and organic solvents were of HPLC grade.

Water, Sediment, and Fish. Water and sediment were collected from a freshwater pond located in Wareham, MA. The sediment was dredged from an area approximately 60 ft offshore. This sediment contained 3.2% organic matter. Before use, pond water and pond sediment were stored refrigerated in the laboratory. Fathead minnows (*Pimephales promelas*), ranging in age from 60 to 90 days, were obtained from a culturing unit maintained at Springborn Laboratories.

Sample Preparation. *1. Water.* Water samples were prepared in pond water. They were 1 L in size and fortified with stock solutions of [^{14}C]tralomethrin, [^{14}C]deltamethrin and [^{14}C]-Br_2CA (acetone stock solutions). The fortification levels produced were 12–120 ng/L for tralomethrin, 9.38–93.8 ng/L for deltamethrin, and 10.9–121 ng/L for Br_2CA. Fortified water samples were first acidified with 1 mL of concentrated HCl (pH approximately 2.5–3) and immediately extracted with 200 mL of

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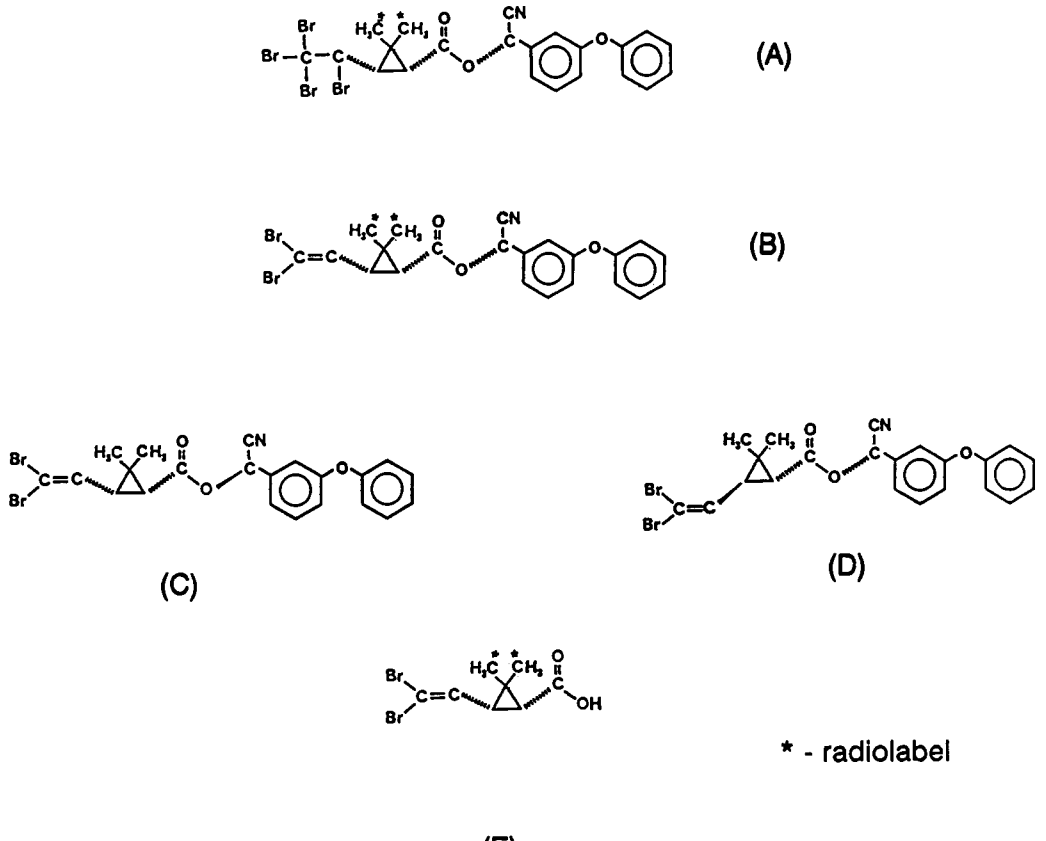

Figure 1. Chemical structures: (A) [^{14}C]tralomethrin, (B) [^{14}C]deltamethrin, (C) α-(*R*)-deltamethrin, (D) *trans*-deltamethrin, and (E) [^{14}C]-Br_2CA.

a hexane/ethyl acetate mixture (1:1). The hexane/ethyl acetate was allowed to separate and was filtered through anhydrous sodium sulfate to remove water. The separatory funnel was rinsed with a small amount of hexane which was also filtered through the sodium sulfate and combined with the other solvent. The hexane/ethyl acetate was rotary evaporated as quickly as possible (approximately 10 min) while a temperature below 30 °C was maintained. The total time for rotary evaporation was kept as short as possible to minimize the possibility of degradation occurring during this step. Before dryness, the residue was transferred to a pear-shaped flask with several rinses of hexane. Just at dryness, the residue was reconstituted with 1 mL of hexane and transferred to a vial for high-performance liquid chromatography with radiometric detection (HPLC-RAM) and liquid scintillation counting (LSC) analyses.

2. *Sediment.* Sediment samples were prepared in refrigerated pond sediment. They were 50 g (wet weight) in size and fortified with stock solutions of [^{14}C]tralomethrin, [^{14}C]deltamethrin and [^{14}C]-Br_2CA (acetone stock solutions). The stock solutions were introduced onto the surface of sediment using syringes. The fortification levels produced were 0.95–9.5 ng/g for tralomethrin, 0.94–9.4 ng/g for deltamethrin, and 0.96–9.6 ng/g for Br_2CA. After fortification, samples were held for approximately 10 min at room temperature before solvent extraction was initiated. The extraction was conducted in a polyethylene centrifuge bottle with 100 mL of a hexane/acetone mixture (8:2). The polyethylene centrifuge bottle was shaken vigorously by hand for approximately 3 min and then placed on an orbital shaker table for an additional 10 min of mechanical shaking. The extract and sediment were separated by centrifugation at approximately 3000 rpm for 5 min. The organic phase was carefully transferred to a 250-mL separatory funnel and the water/acetone phase (lower layer) removed and returned to the centrifuge bottle containing the extracted sediment for further analysis. This lower phase (approximately 10–15 mL) consisted of water and some dissolved acetone. The upper organic phase was filtered through anhydrous sodium sulfate to remove any water contained in the phase. The dried organic phase was transferred to a round-bottom flask and rotary evaporated at 30 °C or below. The rotary evaporation time was kept as short as possible. As soon as the volume was small (approximately 1–2 mL), the residue was rinsed with acetone, tansferred to a pear-shaped flask, and again rotary evaporated to dryness under the same conditions. The residue was reconstituted with exactly 1.0 mL of hexane and transferred to a vial for HPLC-RAM and LSC analyses. To ensure complete recovery of Br_2CA, a consecutive extraction was performed after the remaining sediment was acidified with 0.5 mL of concentrated HCl. Sediment samples were then extracted with 150 mL of methylene chloride. The bottle was hand-shaken for 3 min and placed on an orbital shaker table for 20 min. The bottles were subsequently centrifuged for 5 min at 3000 rpm, after which time the supernatant was poured into a 250-mL separatory funnel. The organic layer was removed and filtered through anhydrous sodium sulfate. The solvent was collected in a round-bottom flask and the methylene chloride rotary evaporated (temperature and time were not important with this sample as it only contained Br_2CA). Just before dryness, the solvent was transferred to a pear-shaped flask and the round-bottom flask rinsed with hexane after the transfer. Following evaporation, the residue was reconstituted with exactly 1.0 mL of hexane and transferred to a vial for HPLC-RAM and LSC analyses. Since the concentrations studied were at nanograms per gram levels, the amount of hexane added (1 mL) was sufficient to dissolve the polar Br_2-CA residues.

3. *Fish.* Fish samples were collected from an aquatic microcosm study with [^{14}C]tralomethrin and [^{14}C]deltamethrin. Fathead minnows (*P. promelas*) were exposed to [^{14}C]tralomethrin and [^{14}C]deltamethrin at 2.9 and 2.2 μg/L, respectively, for 96 h under static conditions. Approximately 100 fish (100–120 g total combined weight) were used. They were first blotted dry and then dissected. The tissue was homogenized in a Waring blender and extracted four times with 100 mL of a hexane/acetone mixture (1:1). The hexane layers were combined and concentrated. The concentrated extract was analyzed directly by HPLC-RAM. The acetone/aqueous phases were combined and extracted twice more with 100 mL of hexane. The remaining acetone/aqueous fraction was acidified to pH 2 with concentrated HCl and extracted twice with 100 mL of methylene chloride to remove

Br_2CA. The methylene chloride extracts were combined with the hexane extracts and analyzed by HPLC-RAM. The remaining acetone/aqueous extract was assayed by LSC for polar residues. Finally, the remaining tissue was also quantified for bound residues by oxidative combustion followed by LSC.

High-Performance Liquid Chromatography (HPLC). HPLC was performed by a gradient normal-phase system with radiometric detection. Instruments included an Autochrom Model 112 CIM gradient controller, a Waters Model 6000A solvent pump, a Waters intelligent sample processor (WISP) Model 710B autosampler, a Kratos Model 757 variable-wavelength detector, and a liquid cell (500 μL) Radiomatic Model A-280 radiochromatography detector with FLO-ONE/Data II software. A Phenomenex Zorbax silica column [5 μm, 250 mm (length) × 4.6 mm (i.d.)] was employed. A gradient solvent system of hexane/dioxane (solvent A, 96/4; solvent B, 50/50) was used with flow rate of 2.0 mL/min. The gradient program was as follows: 0 min, 100% A; 10 min, 100% A; 11 min, 100% B; 20 min, 100% B; 22 min, 100% A. Radiomatic FLO-SCINT A and FLO-SCINT II scintillation cocktails were used for detection of ^{14}C at a flow rate of 4.0 mL/min. A UV detector (220 nm) was placed in-line physically before the RAM detector to monitor nonradioactive reference standards. The injection volume was 200 μL for all sample extracts.

HPLC radiochromatograms of eluted radioactivity were recorded and quantified using the integrated graphics and data acquisition software package previously noted. Radioactive compounds were identified by comparing their retention times to those of nonradioactive reference standards run on the same day and monitored by UV detection (220 nm). The quantitation of [^{14}C]tralomethrin, [^{14}C]deltamethrin, and [^{14}C]-Br_2CA was derived from counts per minute (cpm), the detector counting efficiency (DPM factor), and the specific activity of parent compounds. The following equation was used:

$$\text{analytical result } (\mu g/L \text{ or } \mu g/kg) = \frac{\text{cpm} \times \text{DF}}{\text{SA} \times V \times \text{DPM factor}}$$

In this equation analytical result is the concentration of the compound in a water or sediment sample, SA is the specific activity (dpm/μg) of the compound, V is the HPLC injection volume (μL), DF is a dilution factor, ratio of final sample extract volume (μL) to initial sample volume or weight (L for water and kg for sediment); and DPM factor is the efficiency of the radiometric detector. The quantitation of identified degradation product(s) was carried out in a similar fashion to that described above for parent compounds, except that a molecular weight factor was applied to the calculation. Under the assumption that the parent compound transformed into degradation product(s) in a one to one ratio based upon ^{14}C, the following equation was used to calculate the concentration of degradation product(s):

$$\text{analytical result } (\mu g/L \text{ or } \mu g/kg) = \frac{\text{cpm} \times \text{DF} \times \text{MWF}}{\text{SA} \times V \times \text{DPM factor}}$$

Here MWF is the ratio of molecular weight of the degradation product to the molecular weight of the parent compound.

The background of the RAM detector was determined by injecting a blank sample at the beginning of each analysis set. The determined background cpm multiplied by 1.5 was used as the value for background subtraction. External standardization of the RAM detector was used to obtain DPM factors. This was achieved by injecting a known amount of dpm directly into the detector (without an HPLC column attached) under isocratic HPLC condition (50% solvent A and 50% solvent B). The ratio of the cpm detected by the RAM detector to the nominally applied dpm was defined as the DPM factor. The nominal dpm was obtained by total ^{14}C measurements in the scintillation counter.

Liquid Scintillation Counting (LSC). LSC radioactivity analyses were performed using a Beckman LS 5000TD liquid scintillation counter calibrated by an external quench program employing factory-prepared standards. All samples were counted until a 2σ error at 5% was attained or for 100 min, whichever period was shorter. Sample extracts were mixed with 15 mL of Monophase scintillation cocktail before LSC counting. Background correction (50–60 cpm) was applied on all analyses automatically by the instrument software. Tissue-bound residue was determined by oxidative combustion using a Packard Model 306 sample oxidizer followed by LSC measurement.

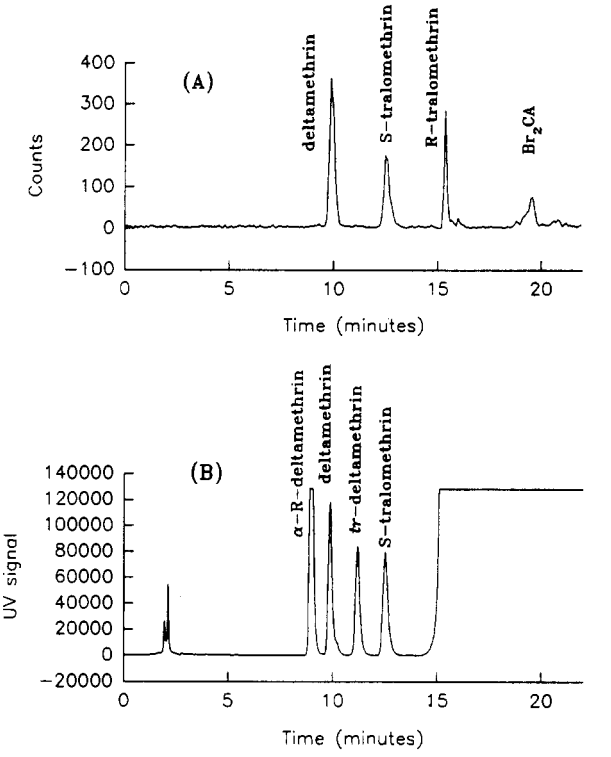

Figure 2. HPLC chromatograms: (A) authentic standards of radiolabeled compounds by radiometric detection, 50-μL injection; (B) authentic standards of nonradiolabeled compounds by UV detection (220 nm), 50-μL injection.

RESULTS AND DISCUSSION

A representative chromatogram of tralomethrin, deltamethrin, and related compounds is shown in Figure 2. Chromatogram A was collected through the radioisotope channel showing the resolution of ^{14}C-labeled tralomethrin (*R* and *S* isomers), deltamethrin, and Br_2CA. Chromatogram B was collected through the UV (220 nm) channel displaying the separation of authentic standards (nonradiolabeled) of α-(*R*)-deltamethrin, deltamethrin, *trans*-deltamethrin, tralomethrin, and Br_2CA. Due to the change in mobile-phase composition (gradient elution), the UV absorbance increased significantly after 15 min and the baseline drifted off-scale. (*R*)-Tralomethrin and Br_2CA were, therefore, not detectable in the UV channel. However, the resolution of these two compounds was clearly demonstrated in the radioisotope channel. One of the advantages of radiometric detection is its consistent baseline that is independent of mobile-phase composition change. This advantage was exhibited in this example. The HPLC separation of various deltamethrin stereoisomers was studied previously (Maguire, 1990) using a chiral DBNPG column. Due to the nature of chiral separations, the assay time was long (100 min) and peaks were relatively broad. As a result, rapid quantifications at low concentration levels (parts per billion to parts per trillion) were difficult to achieve using the chiral separation method. The separation method described in this paper exploited radioisotope detection and enabled rapid signal-run separation and quantification of five related compounds at low concentrations on a routine basis. Br_2CA, a carboxylic acid, behaved poorly on the normal-phase silica column. Its peak was relatively broad, and a decreased signal-to-noise ratio was observed. The method was consequently less sensitive to Br_2CA than to tralo-

Table I. Method Validation Data for Total ^{14}C Analysis of Tralomethrin, Deltamethrin, and Br_2CA from Pond Water As Determined by LSC[a]

compound	concn fortified, ng/L	total dpm fortified	total dpm recovered	total recovery, %	no. of replicates
tralomethrin + Br_2CA	120 + 121	73573	63426 ± 2457	86.2 ± 3.4	3
tralomethrin + Br_2CA	48.0 + 48.4	29429	26092 ± 1230	88.6 ± 4.2	3
tralomethrin + Br_2CA	12.0 + 10.9	6861	6415 ± 234	93.4 ± 3.5	3
tralomethrin + Br_2CA	0.960 + 0.968	589	521 ± 20	88.5 ± 3.4	3
deltamethrin	93.8	24507	18106 ± 249	73.9 ± 1.1	3
deltamethrin	46.9	12254	9197 ± 252	75.1 ± 2.0	3
deltamethrin	9.38	2450	2229 ± 51	90.9 ± 2.1	3
deltamethrin	0.938	245	217 ± 13	88.6 ± 5.3	3

[a] Detection limit for a 1000-mL sample was approximately 0.25 ng/L for each compound.

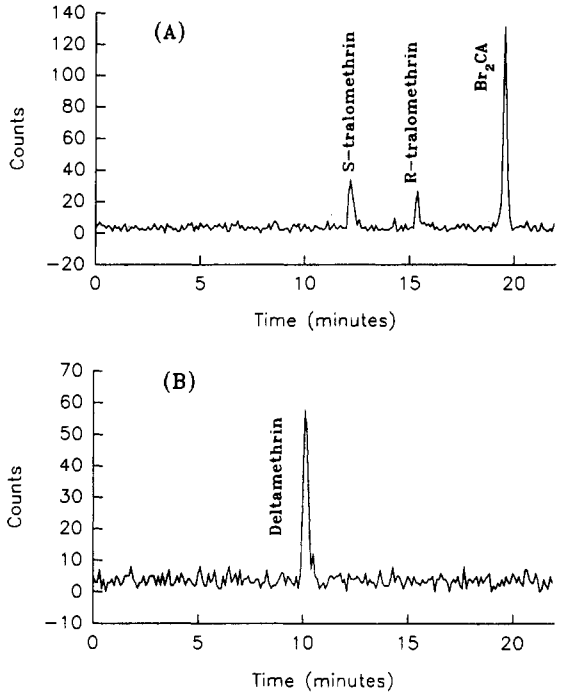

Figure 3. HPLC-RAM chromatograms: (A) [^{14}C]tralomethrin and [^{14}C]-Br_2CA water samples fortified at 48 and 48.4 ng/L, respectively; (B) [^{14}C]deltamethrin water sample fortified at 46.9 ng/L.

methrin or deltamethrin. The separation of tralomethrin and deltamethrin on a reversed-phase C_{18} column with acetonitrile and water as mobile phase was also investigated. Although the separation of tralomethrin, deltamethrin, and Br_2CA was sufficient, the reversed-phase conditions could not resolve deltamethrin isomers. Considering the high solubility of tralomethrin and deltamethrin in hexane, normal-phase separation with a hexane/dioxane mobile phase was chosen. Figure 3 shows representative chromatograms of tralomethrin, deltamethrin, and Br_2CA water extracts using HPLC-RAM and normal-phase chromatography.

Water and sediment method validations were conducted in triplicate at three concentration levels. Table I presents the recovery of tralomethrin, deltamethrin, and Br_2CA from pond water by total ^{14}C residue LSC measurements. Relatively high and consistent recoveries indicated that the extraction scheme was efficient. A single extraction with mixtures of hexane and ethyl acetate proved to be effective at the selected concentrations. The use of ethyl acetate helped to reduce emulsions and increase phase separations during liquid–liquid extractions. Ethyl acetate also improved the extraction efficiency of the more polar Br_2CA. Chemical specific HPLC-RAM analyses of the recovery samples are presented in Table II. Due to the rapid transformation of tralomethrin to deltamethrin, efforts were made to minimize this degradation during

Table II. Method Validation Data for HPLC-RAM Analysis of Tralomethrin, Deltamethrin, and Br_2CA from Pond Water[a]

compound	concn fortified, ng/L	concn recovered, ng/L	recovery, %	no. of replicates
tralomethrin	120	84.3 ± 7.6	70.3 ± 6.4	3
tralomethrin	48.0	31.4 ± 3.5	65.5 ± 7.3	3
tralomethrin	12.0	6.27 ± 1.8	52.3 ± 14.8	2
deltamethrin	93.8	72.2 ± 2.1	76.9 ± 2.2	3
deltamethrin	46.9	41.0 ± 6.0	87.4 ± 12.8	3
deltamethrin	9.38	5.54 ± 1.4	59.1 ± 14.8	3
Br_2CA	121	103 ± 6	85.0 ± 5.1	3
Br_2CA	48.4	38.3 ± 5.1	79.2 ± 10.6	3
Br_2CA	10.9	7.87 ± 3.1	72.4 ± 28	3

[a] Detection limits for a 1000-mL sample were approximately 4 ng/L for tralomethrin, 2 ng/L for deltamethrin, and 2 ng/L for Br_2CA.

sample processing. This included extracting the sample immediately after fortification, minimizing the amount of extraction solvent, minimizing sample processing time, and controlling water bath temperature during rotary evaporation (less than 30 °C) to avoid thermal degradation. The results shown in Table II indicated that essentially no deltamethrin was formed during the laboratory workup. The experimental procedures were less rigid for deltamethrin and Br_2CA since they were much more stable compared to tralomethrin. The acidification of water samples with HCl prior to solvent extraction improved the efficiency for removal of Br_2CA and, consequently, enabled the recovery of all three compounds from a single solvent extraction. Table III presents the recovery of tralomethrin, deltamethrin, and Br_2CA from pond sediment. Tralomethrin was found to degrade very rapidly to deltamethrin in nonrefrigerated sediment. The degradation was significantly reduced when refrigerated sediment was used. These findings suggested the high reactivity of tralomethrin through microbial transformation. Refrigeration of sediment presumably inhibited microbial respiration and consequently reduced microbial transformation of tralomethrin to deltamethrin. It should be noted, however, that the clay matrix, inorganics, or other chemically induced processes also could have contributed to the degradation of tralomethrin. These chemical factors also would be suppressed at lower temperatures. Again, extra efforts were placed on minimizing sample extraction and concentration time. The relatively low recovery (66%) of Br_2CA from sediment suggests that more polar solvents (e.g., acetonitrile, methanol, acetone) are needed to improve the extraction efficiency. The high selectivity of HPLC-RAM permitted the measurement of pyrethroids free from the matrix contamination that would be seen using UV detection.

The detection limits listed in Tables I–III were calculated on the basis of a 2:1 signal-to-noise ratio. True instrumental detection limits are a function of background, peak width, counting efficiency, and radioisotope residence time (Reich and Reich, 1988). Residence time is also a

Table III. Method Validation Data for HPLC-RAM Analysis of Tralomethrin, Deltamethrin, and Br_2CA from Pond Sediment[a]

compound	concn fortified, ng/g	concn recovered, ng/g	recovery, %	degradation, %	no. of replicates
tralomethrin	9.5	8.4 ± 0.7	88 ± 8	2 (deltamethrin)	3
tralomethrin	4.8	4.1 ± 0.7	86 ± 13	2 (deltamethrin)	3
tralomethrin	0.95	0.59 ± 0.1	63 ± 9	21 (deltamethrin)	2
deltamethrin	9.4	9.5 ± 0.4	101 ± 4	NA[b]	3
deltamethrin	4.7	4.3 ± 0.3	91 ± 6	NA	3
deltamethrin	0.93	0.87 ± 0.05	93 ± 5	NA	3
Br_2CA	9.7	7.1 ± 0.2	73 ± 2	NA	3
Br_2CA	4.8	3.4 ± 0.2	70 ± 3	NA	3
Br_2CA	0.96	0.54 ± 0.06	56 ± 6	NA	3

[a] Detection limits for a 50-g sample were approximately 0.1 ng/g for tralomethrin, 0.05 ng/g for deltamethrin, and 0.05 ng/g for Br_2CA. [b] NA, not applicable.

function of flow cell volume and total eluent flow rate (the flow rate of HPLC eluent plus the flow rate of scintillation cocktail). Throughout the study, the detector background was between 30 and 40 cpm and counting efficiency (dpm factor) was consistently between 90 and 96%. A 2:1 ratio of scintillation cocktail flow rate to HPLC flow rate proved to be satisfactory, and increasing the ratio did not improve the detector counting efficiency. Realistic method detection limits were close to half of the low-concentration levels. They were approximately 6 ng/L for tralomethrin, deltamethrin, and Br_2CA in water and 0.5 ng/g for tralomethrin, deltamethrin, and Br_2CA in sediment. At these detection limits, there were approximately 230 dpm/HPLC injection for water extracts and 900 dpm/injection for sediment extracts. If a lower detection limit is required, a larger flow cell (1000–2000 μL) could be used but only at the expense of chromatographic resolution. Time resolved liquid scintillation counting (TR-LSC) techniques could also be used to improve the detection limit. TR-LSC could decrease the background noise (up to 80%), therefore increasing the signal-to-noise ratio.

Approximately 100 g of tissue from fathead minnows exposed to 2.9 μg/L [^{14}C]tralomethrin for 96 h was extracted and assayed by HPLC-RAM. The tissue contained 21.4 ng/g (*S*)-tralomethrin, less than 0.1 ng/g (*R*)-tralomethrin, 111 ng/g deltamethrin, 32.7 ng/g α-(*R*)-deltamethrin, 63.1 ng/g *trans*-deltamethrin, and 14.3 ng/g Br_2CA. Mass balance, calculated for the extraction and analysis of this tissue sample, was 90.4%, with 75.6% removed from the tissue in the hexane/acetone extract, 8.0% removed in the hexane back extraction from the acetone/aqueous extract, 0.4% not able to be removed from the acetone/aqueous extract, and 6.4% nonextractable from the tissue. Approximately 100 g of tissue from fathead minnows exposed to 2.2 μg/L [^{14}C]deltamethrin for 96 h was also extracted and assayed by HPLC-RAM. Assay resulted in tissue concentrations of 69.1 ng/g deltamethrin, 19.5 ng/g α-(*R*)-deltamethrin, 40.4 ng/g *trans*-deltamethrin, and 7.16 ng/g Br_2CA. Mass balance, calculated for the extraction and analysis of this tissue sample, was 91.9%, with 63.2% removed from the tissue in the hexane/acetone extract, 15.4% removed in the hexane back extraction from acetone/aqueous extract, 1.2% not able to be removed from the acetone/aqueous extract, and 12.1% nonextractable from the tissue. Figure 4 presents the chromatograms of hexane fractions from tissue illustrating resolutions of various tissue metabolites. The profiles of metabolite distribution were strikingly similar comparing fish exposed to tralomethrin to fish exposed to deltamethrin. This resemblance was due to the fact that tralomethrin degrades rapidly in water and tissue, with half-lives of significantly less than 96 h.

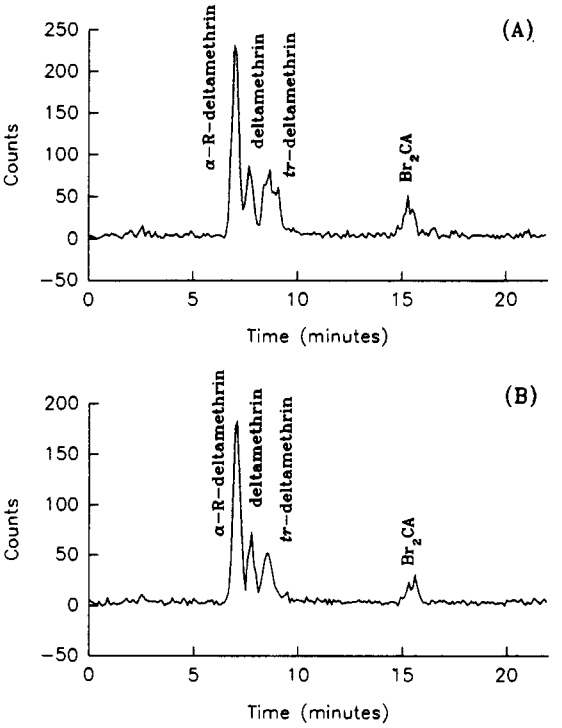

Figure 4. HPLC-RAM chromatograms of fish tissue extracts from a microcosm study: (A) fish exposed to [^{14}C]tralomethrin (2.9 μg/L) for 96 h; (B) fish exposed to [^{14}C]deltamethrin (2.2 μg/L) for 96 h.

CONCLUSION

The HPLC-RAM method described in this paper is an important progression in analysis of pyrethroids at environmentally relevant concentration levels for the following reasons:

(1) relatively simple sample preparation for analysis of water, sediment, and fish tissue samples since typical cleanup steps associated with less specific detections are not necessary,

(2) use of radioisotopes permits detection of pyrethroids at concentrations conventional HPLC detection techniques cannot reach,

(3) single-run normal-phase separation of five related compounds including a polar metabolite (Br_2CA) in a relatively short run time,

(4) single-injection quantification of five related analytes without processing authentic external calibration standards owing to a common detector response for all compounds of interest (^{14}C),

(5) relatively simple detector calibration to quantify radioactivities,

(6) relatively high and consistent detector counting efficiency over a 20-min gradient run owing to normal-

phase solvents (hexane, dioxane), resulting in linear calibration for analytes at the beginning and end of the analysis.

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Received for review September 17, 1992. Revised manuscript received December 23, 1992. Accepted January 25, 1993.