GAS CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF PERMETHRIN RESIDUES IN FORESTRY SUBSTRATES

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ABSTRACT

A simple and reliable EC-GLC method has been developed and reported for permethrin isomers present in forestry substrates. Fortified conifer foliage and fish tissues were extracted by blending with hexane, followed by acetonitrile partition, purification by Florisil® column chromatography, benzene, toluene or dichloromethane elution and analysis by EC-GLC with a glass column (1.2 m x 2 mm ID) packed with 6% QF-1 + 3% DC-200 on 80-100 mesh Chromosorb W, HP.

Recoveries of the *cis* and *trans* isomers were consistent and ranged from 80 to 99% at 1.0 and 0.1 ppm fortification levels. Use of benzene, toluene or dichloromethane as eluting solvents did not show significant differences in recoveries of the chemical from the Florisil® adsorbent column although toluene is preferred over the others. Fortified litter, soil, sediment and water samples also gave consistently good recoveries ranging from 80 to 102%. Use of dichloromethane as eluting solvent for the chemical from the Florisil® adsorbent column instead of the aromatics was found to be advantageous. The method has been evaluated with using different extracts, adsorbents and eluting solvents under different experimental conditions. Best choice of solvent solution and GC experimental conditions for various forestry substrates are evaluated and reported.

INTRODUCTION

Permethrin [3-phenoxybenzyl (±) - cis, trans 3 - (2, 2- dichlorovinyl) -2, 2- dimethylcyclopropanecarboxylate] is a new synthetic pyrethroid insecticide developed by Elliott et al.[1] It is registered as a control product against a wide range of pests in horticulture, agriculture, livestock, public health, stored products, etc.[2] DeBoo[3] found that the insecticide is also effective in controlling various lepidopterous defoliators, especially the larvae of the spruce budworm, *Choristoneura fumiferana* (Clem.), when applied twice several days apart at a dosage of 17.5 g/ha. Permethrin is effective at lower rates and for a longer time than many commonly used insecticides, and it also controls insect strains that have become resistant to a range of organochlorine and organophosphate insecticides.[4]

One of the Canadian registration requirements for any chemical, prior to its large scale use in forestry, is to have sensitive and reliable methods to analyze its residues in various forestry substrates. The purpose of this report is to study and develop the necessary analytical methods for the identification and quantification of permethrin in balsam fir foliage, fish, natural stream water, forest litter, soil, and sediment. The methods consisted of sample preparation, extraction, liquid-liquid partitioning (of the foliage and fish samples), column cleanup and finally gas chromatographic analysis of the permethrin residues.

MATERIALS AND METHODS

Standard Solution

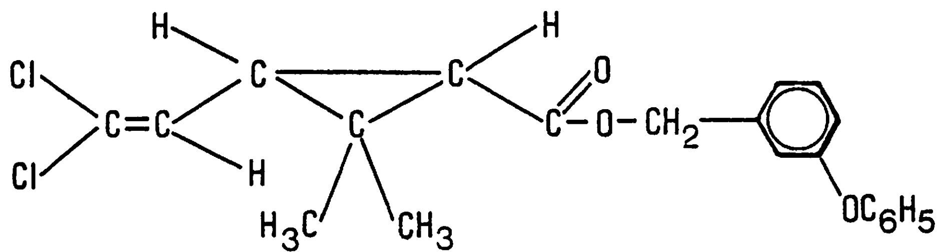
The pyrethroid insecticide used in this study is permethrin (Ambush®; 3-phenoxybenzyl (±)-cis-, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate) (99.0% cis and 99.3% trans, Chipman Chemicals Ltd.) (Fig. 1).

A stock solution of cis and trans-permethrin, in the ratio of 1:1, was prepared at 100 ppm ($\mu g/g$) in hexane. The working solutions used for fortification and gas chromatographic analysis were prepared by appropriate dilution with hexane.

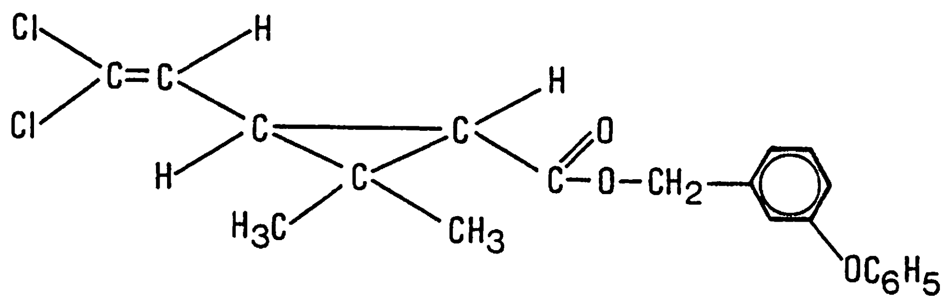
A chromatogram of the permethrin standard solution at the 1.0 parts per million (ppm) level is illustrated in Figure 2.

Apparatus

Homogenizer	- Polytron - Model PT-20 (Brinkman Instruments Canada Ltd.).
	- Omni Mixer (Ivan Sorvall Inc.).
Rotary Evaporator	- with water bath (Buchler).
Evaporation Apparatus	- Meyer N-evap® Model III (Organomation Associates Inc.).
Sample Mixer	- Thermolyne maxi-mix (Fisher Scientific).
Gas Chromatograph (GC)	- Hewett Packard Model HP5730 A equipped with an electron capture detector (ECD) (Ni-63).
Analytical Balance	- (i) Model 2004MP6 Semi-micro Sartorius (Canlab).
	(ii) TP41 (Oerling).



cis-isomer



trans-isomer

Fig. 1. Cis and trans-permethrin.

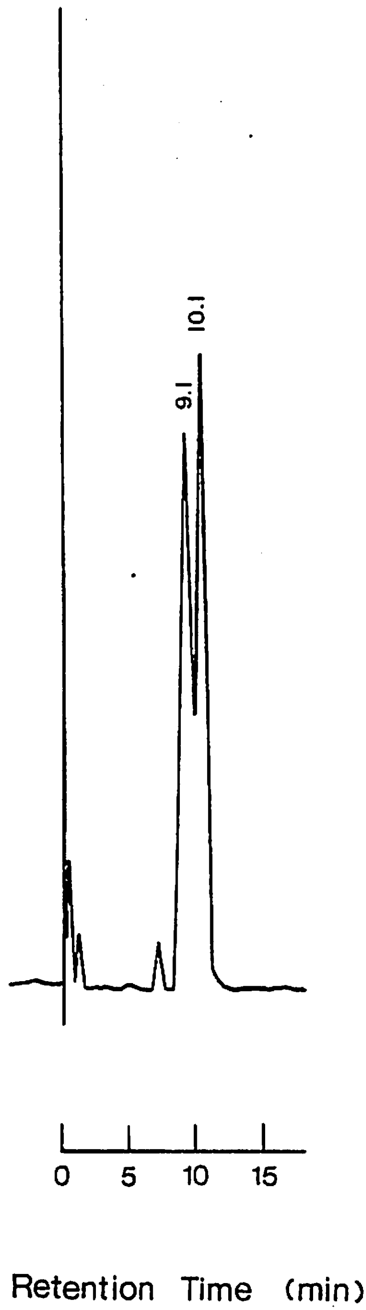


Fig. 2. Chromatogram of permethrin standard – 1.0 µg/ml (cis and trans isomers)

Solvents

Acetonitrile, benzene, dichloromethane, diethyl ether, hexane, and toluene are pesticide grade solvents obtained from Fisher Scientific, J.T. Baker Chemical Co., and Caledon Laboratories Ltd.

Accessories

Filter Paper - Whatman No. 1 Qualitative.

Sodium Sulfate - anhydrous (Fisher Scientific) (oven dried at 250°C for 16 h).

Liquid Chromatographic Columns - 4 ml Pasteur pipet (Fisher Scientific, Cat. No. 13-678-8).

Glass Wool - Pyrex Brand (Owens-Corning Fibre Glass Co.).

Florisil® - 60-100 mesh (Fisher Scientific, Cat. No. F-100).

Pipet - 1000 µl Pipetman (Gilson).

Microsyringe - 10 µl (Hamilton Co.)

GC Column Packing - 6% QF-1 + 3% DC-200 on Chromosorb W, HP, 80-100 mesh (Chromatographic Specialties Ltd.).

Sample Preparation

(i) Coniferous Foliage:

Balsam fir *(Abies balsamea)* foliage was obtained from a forested area surrounding Sault Ste. Marie, Canada. The foliage was clipped with scissors and only the needles were processed and used in the study. The samples were kept frozen (-10°C) prior to processing and were stored at 4°C prior to fortification and analysis.

(ii) Fish:

Brook trout *(Salvelinus fontinalis)* were obtained from the Ontario Ministry of Natural Resources' fish hatchery in Sault Ste. Marie, Canada. The heads and tails of the fish were removed and the body was cut into small (5 mm) sections using a sharp knife. The fish samples were kept frozen and prior to subsequent analysis, they were allowed to thaw and then processed for the study.

(iii) Natural Water:

The natural water samples used in this study were collected from Rainbow Creek, approximately 30 km north of Sault Ste. Marie, Canada. The water samples were stored at 4°C prior to fortification followed by extraction and analysis.

(iv) Litter and Soil:

The forest litter (LHF layer, sandy loam) and soil (mixture of layers A and B (1:1), sandy loam) samples were obtained from a forest area approximately 30 km north of Sault Ste. Marie, Canada. Stones and twigs were removed and each sample was thoroughly mixed by hand, sieved and stored at 4°C prior to extraction, cleanup and analysis.

(v) Sediment:

Sediment (3 cm depth) was collected from Rainbow Creek, approximately 30 km north of Sault Ste. Marie, Canada. All twigs and small stones were removed from the sediment. Excess water was removed by filtering using a large buchner funnel and a Whatman No. 1 filter paper under gentle aspiration. The sediment was stored at 4°C prior to processing and analysis.

Moisture Content

Foliage, litter, soil and sediment:

Duplicate samples (5 g) of thoroughly mixed material were weighed, dried at 120°C in a thermostatic oven for 16 h, and reweighed (Table 1).

Florisil®:

Aliquots of Florisil® were weighed, dried in a thermostatic oven at 120°C for 16 h, and reweighed.

Experimental

Foliage:

(i) Extraction:

Aliquots of balsam fir foliage (20.0 g) and anhydrous sodium sulfate (20 g) were successively homogenized for 3 min. in presence of 60, 50, and 50 ml of hexane using a Polytron PT-20 homogenizer set at a speed of approximately 6,000 rpm. The homogenates were filtered under gentle aspiration through a large buchner funnel containing one Whatman No. 1 filter paper and 3 cm of anhydrous sodium sulfate, which had been prewashed with 25 ml of hexane. The residue in the filtration unit was rinsed with 25 ml of hexane. The extracts and the rinse were pooled, quantitatively transferred to a round bottom flask and evaporated to 10 ml using a rotary evaporator at 30°C. The concentrate was quantitatively transferred to a 50 ml graduated tube and the final volume was adjusted to 20.0 ml with hexane to give a working concentration of 1.0 g of foliage/ml.

Table 1.

Moisture Content of Forest Substrates

Substrate	Moisture Content*
Foliage	48.3% ± 0.2
Litter	36.6% ± 0.7
Soil	17.8% ± 0.4
Sediment (filtered)	10.6% ± 0.2

*each value is the mean of duplicates.

(ii) Fortification:

A 10.0 ml aliquot of crude foliar extract (10.0 g) was spiked with either 10.0 or 1.0 μg of permethrin standard in hexane to yield spiked samples at levels of 1.0 and 0.1 ppm permethrin, respectively.

(iii) Liquid-Liquid Partitioning:

(a) The spiked foliar extract was transferred quantitatively to a separatory funnel using a total of 10 ml of hexane for rinsing. A 60 ml volume of acetonitrile was then added to the extract giving a 3:1 acetonitrile to hexane ratio. The partitioning procedure consisted of shaking the combined phases for 3 minutes and then allowing them to separate into two distinct layers. The hexane layer was discarded while the acetonitrile layer was re-extracted with 20 ml of hexane. The acetonitrile phase was then collected into a round bottom flask and evaporated to dryness at 30°C on a rotary evaporator. The residue was quantitatively transferred to a graduated tube and the final volume was adjusted to 10.0 ml using hexane.

(b) a 10.0 ml aliquot of spiked foliar extract was added to 50 ml of acetonitrile giving a 5:1 acetonitrile to hexane ratio. The extract was then partitioned twice. The hexane phases were pooled and partitioned using 5 ml of acetonitrile. After the final partition, the separating funnel was rinsed with 2 x 5 ml of acetonitrile. All the acetonitrile layers were pooled into a round bottom flask and flash evaporated to dryness at 30°C. Using hexane rinsings, the residue was transferred quantitatively to a graduated tube and the final volume was adjusted to 10.0 ml.

(c) A third partitioning procedure was tried consisting of the same protocol as (b) except that the extract was partitioned three times instead of twice.

(iv) Column Cleanup:

Various microcolumn systems were investigated. Two column assemblies consisted of a 4 ml Pasteur pipet plugged with a small wad of glass wool and containing 5 cm of Florisil®, either 2.3 or 5.0% moisture content, topped with a 1 cm layer of anhydrous sodium sulfate (Fig. 3). A second column assembly was simply a double microcolumn assembly consisting of two of the Florisil® (2.3% moisture content) microcolumns (Fig. 4).

The column assemblies were prewashed with 10 ml of hexane prior to the loading of a 1.0 ml aliquot of extract containing 1.0 g of foliage and either 1.0 or 0.1 μg of permethrin. Soon after the last of the sample entered the column packing, each column was eluted separately with (a) 5% diethyl ether in hexane, (b) 10% diethyl in hexane, (c) 5% benzene in hexane, (d) 10% benzene in hexane, (e) benzene, (f) toluene, (g) 15% dichloromethane in hexane, (h) dichloromethane.

The eluent was then evaporated to dryness under a gentle stream of nitrogen using a N-Evap® and brought up to 1.0 ml in hexane for gas chromatographic analysis.

Fish:

(i) Extraction:

Aliquots of brook trout (50.0 g) and anhydrous sodium sulfate (50 g) were homogenized three times with 150, 100, and 100 ml of hexane for 3 minutes using a polytron homogenizer.

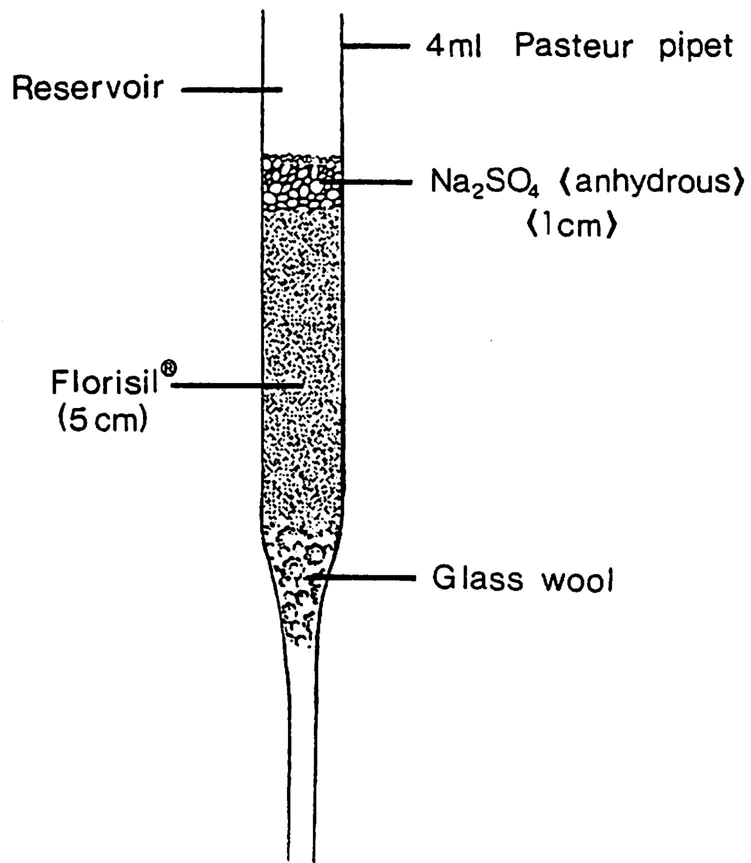


Fig. 3. Single microcolumn assembly.

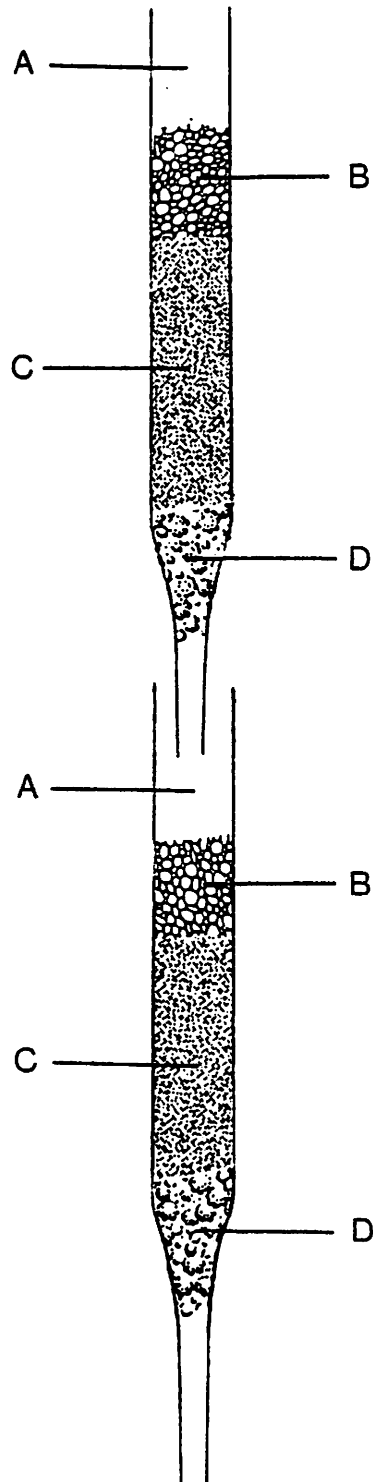


Fig. 4. Double microcolumn assembly:

A - Reservoir

B - Na_2SO_4 - anhydrous (1cm)

C - Florisil® (5cm)

D - Glass wool

A filtration system consisting of a large buchner funnel containing one Whatman No. 1 filter paper and 3 cm of anhydrous sodium sulfate was prepared and prewashed with 25 ml of hexane. The liquid extract from the first two extractions was carefully decanted onto the filtration system. At the end of the final extraction, the whole sample, including tissue fragments, was filtered and then rinsed with 25 ml of hexane.

The pooled extracts were quantitatively transferred to a round bottom flask and evaporated to 10 ml at 30°C on a rotary evaporator. The concentrated extract was then transferred quantitatively to a graduated tube and a final volume of 50.0 ml was made up using hexane, thus yielding 1.0 g of fish/ml of extract.

(ii) Fortification:

Either 10.0 or 1.0 μg of permethrin (in hexane) was added to 10.0 g aliquots of crude fish extract yielding respectively 1.0 and 0.1 ppm spiked fish samples.

(iii) Liquid-Liquid Partitioning:

(a) A 10.0 ml aliquot of spiked fish extract was transferred to a separatory funnel and dissolved in 50 ml of acetonitrile giving a 5:1 acetonitrile to hexane ratio. The sample was shaken for 3 minutes, after which the phases were allowed to separate and then each layer was separately collected in a small flask. The acetonitrile layer was returned to the separatory funnel and re-extracted with 10 ml of hexane. The two hexane phases were then pooled together and extracted with 5 ml of acetonitrile. After this final extraction, the separatory funnel was rinsed with 2 x 5 ml of acetonitrile.

The acetonitrile layers were pooled into a round bottom flask and evaporated just to dryness at 30°C using a rotary evaporator. The residue was then transferred quantitatively to a graduated tube, using hexane rinses, and the final volume was adjusted to 10.0 ml.

(b) A simpler partitioning method was also tried in which the extract consisted of 5:1 acetonitrile to hexane. The extraction was performed twice with only the acetonitrile layers being collected, discarding the hexane phases. The separatory funnel was not rinsed after the final partition as it had been in extraction method (a).

(iv) Column Cleanup:

The microcolumn cleanup system was comprised of a 5 cm layer of Florisil® (oven dried at 120°C for 16 h - with a moisture content of 2.3%) and topped with a 1 cm layer of anhydrous sodium sulfate in a 4 ml Pasteur pipet plugged with a small wad of glass wool at the base.

Prior to the sample loading, the column was washed with 10 ml of hexane. The sample contained 1.0 g of fish extract and either 1.0 or 0.1 ppm permethrin. Soon after the last of the sample entered the column packing, the sample was eluted with either (a) 10% diethyl either in hexane or (b) dichloromethane. The eluent was then evaporated to dryness and brought up to 1.0 ml in hexane for gas chromatographic analysis.

Natural Water

(i) Fortification:

A 50.0 ml aliquot of natural stream water was fortified with either 50.0, 5.0, or 0.5 μg of permethrin (in hexane) yielding 1.0, 0.1 and 0.01 ppm pesticide levels, respectively.

(ii) Extraction:

The spiked water sample was transferred to a separatory funnel followed by 4 x 5 ml hexane rinses of the sample container which was also added to the separating funnel and then gently shaken for 3 minutes. After the phases were allowed to separate, the two layers were collected in separate flasks. The water layer was then returned to the separatory funnel and re-extracted with 20 ml of hexane.

After the final extraction, the water layer was discarded; the hexane phases were pooled, passed through a 3 cm layer of anhydrous sodium sulfate which had been prewashed with 25 ml of hexane, and collected in a round bottom flask. The sodium sulfate was then rinsed with 25 ml of hexane, which was also added to the round bottom flask. The hexane extract was evaporated on a rotary evaporator to 10 ml at 30°C, quantitatively transferred to a graduated tube and made up to a final volume of 50.0 ml in hexane.

(iii) Column Cleanup:

Aliquots of fortified natural stream water extract containing either 1.0, 0.1 or 0.01 ppm permethrin were transferred, using a 1000 μl Pipetman®, to a microcolumn assembly. The assembly, which was prewashed with 10 ml of hexane, consisted of a 4 ml Pasteur pipet plugged with a small wad of glass wool and containing a 5 cm layer of Florisil® (oven dried at 120°C for 16 h) topped with 1 cm of anhydrous sodium sulfate.

Just as the last of the water sample entered the column packing, 15 ml of dichloromethane was used for elution. The eluent was then evaporated to dryness on a N-evap® and brought up to 1.0 ml with hexane for gas chromatographic analysis.

Soil, Sediment, and Litter

(i) Fortification:

Aliquots of soil, sediment, and litter (20.0 g each) were separately fortified with either 20.0 or 2.0 μg of permethrin (in hexane) yielding 1.0 and 0.1 ppm spiked samples.

(ii) Extraction:

The spiked samples (20.0 g) were homogenized three times with 60 ml of either hexane or acetonitrile on a Sorvall Omni mixer at approximately 1500 rpm for 3 minutes. The homogenates were filtered under gentle aspiration through a buchner funnel containing one Whatman No. 1 filter paper and 3 cm of anhydrous sodium sulfate which had been prewashed with 25 ml of hexane. After the entire extract had been filtered, the filtering system was washed with an additional 25 ml of hexane.

The extract was then transferred quantitatively to a round bottom flask, evaporated to 10 ml at 30°C on a rotary evaporator and quantitatively transferred to a graduated tube. The samples were then made up to a final volume of 20.0 ml with the appropriate solvent used in the extraction to yield a 1.0 g/ml sample spiked at either 1.0 or 0.1 ppm.

(iii) Liquid-Liquid Partitioning:

A 10.0 ml aliquot of the acetonitrile extracted sample was placed into a separatory funnel containing 40 ml of acetonitrile and 10

ml of hexane giving an acetonitrile to hexane ratio of 5:1. The sample was gently shaken for 3 minutes. After the phases were allowed to separate, the hexane layer was discarded while the acetonitrile layer was re-extracted with another 10 ml of hexane for 3 minutes.

The acetonitrile phase was then collected in a round bottom flask, flash-evaporated just to dryness at 30°C and transferred quantitatively to a graduated tube. The final volume was adjusted to 10.0 ml using hexane to yield a 1.0 g/ml extract spiked at either 1.0 or 0.1 ppm.

(iv) Column Cleanup:

A microcolumn assembly consisting of a 4 ml Pasteur pipet plugged with a small wad of glass wool contained 5 cm of Florisil® (oven dried at 120°C for 16 h) topped with 1 cm of anhydrous sodium sulfate. The column was prewashed with 10 ml of hexane prior to the loading of a 1.0 ml aliquot of sample containing 1.0 g of substrate and either 1.0 or 0.1 μg of permethrin.

Soon after the last of the sample entered the column packing, the microcolumn was eluted with 15 ml of dichloromethane. The eluent was evaporated to dryness on a N-evap® and then brought up to 1.0 ml in hexane for gas chromatographic analysis.

Gas Chromatographic Determination

A Hewlett Packard 5730A GC/ECD was used for the permethrin residue analysis. The GC conditions were:

Column: 2 mm ID x 1.2 m glass column, packed with 6% QF-1 + 3% DC-200 on Chromosorb W, HP, 80-100 mesh.

Oven Temp: 220°C

Injector Temp: 260°C

Detector Temp: 310°C

Carrier Gas: Argon/methane (95:5) Flow rate: 33.3 ml/min.

Quantitation of permethrin was determined by comparing the sample peak heights to those of injected standard solutions.

Average recoveries of spiked permethrin are expressed in percentages with appropriate standard deviation. Results are given in Tables 2-7.

RESULTS AND DISCUSSION

Recoveries of permethrin insecticide in six different forestry substrates fortified at various levels are given in Tables 2-7. Various cleanup procedures were investigated yielding a wide range of recovery levels.

Tables 2-7 also illustrate that in most cases, the _trans_ isomer has a higher recovery level than the _cis_ isomer. This is probably due to isomerization of some of the _cis_-permethrin isomer to the _trans_-permethrin structure.

During the preliminary investigations, it was observed that the _cis_ isomer was eluted from the microcolumn assembly prior to complete elution of the _trans_ isomer.

Experimentation on balsam fir foliage gave permethrin recoveries ranging from 75 to 96% at the 1.0 ppm fortification level and from 75 to 85% at the 0.1 ppm permethrin level (Table 2). The most acceptable extraction and cleanup method investigated was comprised of the hexane

Table 2.

Recovery of Total Permethrin From Fortified Foliar Extracts at 1.0 and 0.1 ppm Fortification Levels

Original Spiking Level	Extracting Solvent System	Liquid-Liquid Partitioning	Column Cleanup System	Eluting Solvent System	Vol. (ml)	No. of Replicates	% Recovery (cis/trans)	S.D. (±) (cis/trans)	Comments
1.0 ppm	hexane	(1) acetonitrile:hexane (3:1, 80 ml twice)	(i) activated Florisil** (10 cm double column assembly)	10% diethyl ether in hexane	10	3	-	-	interfering co-extractives
			(ii) 5% deactivated Florisil (5 cm)	10% diethyl ether in hexane	10	3	-	-	interfering co-extractives
			(iii) activated Florisil (5cm)	(a) 5% diethyl ether in hexane	10	3	-	-	interfering co-extractives
				(b) 10% diethyl ether in hexane	10	3	-	-	interfering co-extractives
				(c) 5% benzene in hexane	40	3	-	-	inadequate elution volume*
				(d) benzene	15	9	81.9/86.0	3.2/3.3	
				(e) toluene	15	9	78.6/82.8	2.0/2.3	
		(2) acetonitrile:hexane (5:1, 60 ml twice then 1:4, 25 ml once)	activated Florisil (5cm)	(a) benzene	15	4	92.3/94.6	3.7/3.7	
				(b) toluene	15	4	92.2/95.6	3.3/3.1	
				(c) 15% dichloromethane in hexane	35	3	-	-	inadequate elution volume
				(d) dichloromethane	15	4	88.6/90.8	1.1/1.3	
		(3) acetonitrile:hexane (5:1, 60 ml thrice)	activated Florisil (5cm)	(a) benzene	15	4	83.4/85.6	1.9/4.0	
				(b) toluene	15	4	75.5/77.4	3.2/2.6	
				(c) 15% dichloromethane in hexane	35	4	-	-	inadequate elution volume
0.1 ppm	hexane	acetonitrile:hexane (5:1, 60 ml twice then 1:4, 25 ml once)	activated Florisil (5cm)	dichloromethane	15	3	79.9/80.2	1.3/3.9	

* Higher volumes could increase the amount of coextracted impurities in the eluate

** 2.3%

Table 3.

Recovery of Total Permethrin From Fortified Fish Extracts at 1.0 and 0.1 ppm Fortification Levels

Original Spiking Level	Extracting Solvent System	Liquid-Liquid Partitioning	Column Cleanup System	Eluting Solvent System	Vol. (ml)	No. of Replicates	% Recovery (cis/trans)	S.D. (±) (cis/trans)
1.0 ppm	hexane	(1) acetonitrile:hexane (5:1, 60 ml twice)	activated Florisil (5cm)	(a) 10% diethyl ether in hexane	15	6	82.9/95.7	4.1/2.5
				(b) dichloromethane	15	2	86.3/91.1	0.7/1.4
		(2) acetonitrile:hexane (5:1, 60 ml twice then 1:4, 25 ml once)	activated Florisil (5cm)	(a) 10% diethyl ether in hexane	15	1	92.1/93.7	-
				(b) dichloromethane	15	5	96.4/99.0	4.6/3.0
0.1 ppm	hexane	acetonitrile:hexane (5:1, 60 ml twice then 1:4, 25 ml once)	activated Florisil (5cm)	dichloromethane	15	3	91.9/93.9	2.3/0.7

Table 4.

Recovery of Total Permethrin From Fortified Natural Water Extracts at 1.0, 0.1 and 0.01 ppm Fortification Levels

Original Spiking Level	Extracting Solvent System	Column Cleanup System	Eluting Solvent System	Vol. (ml)	No. of Replicates	% Recovery (cis/trans)	S.D. (±) (cis/trans)
1.0 ppm	hexane	activated Florisil (5cm)	dichloromethane	15	5	99.2/100.3	3.4/2.1
0.1 ppm	hexane	activated Florisil (5cm)	dichloromethane	15	4	101.4/102.9	3.3/2.7
0.01 ppm	hexane	activated Florisil (5cm)	dichloromethane	15	5	88.8/97.5	8.8/7.6

Table 5.

Recovery of Total Permethrin From Fortified Forest Litter Extracts at 1.0 and 0.1 ppm Fortification Levels

Original Spiking Level	Extracting Solvent System	Liquid-Liquid Partitioning	Column Cleanup System	Eluting Solvent System	Vol. (ml)	No. of Replicates	% Recovery (cis/trans)	S.D. (±) (cis/trans)
1.0 ppm	hexane	-	activated Florisil (5cm)	dichloromethane	15	4	95.9/96.1	1.8/2.2
	acetonitrile	acetonitrile:hexane (5:1, 60 ml twice)	activated Florisil (5cm)	dichloromethane	15	4	74.6/78.1	2.1/3.0
	acetonitrile	-	activated Florisil (5cm)	dichloromethane	15	2	76.9/80.3	0.7/0.1
0.1 ppm	hexane	-	activated Florisil (5cm)	dichloromethane	15	2	96.9/95.7	2.0/1.2

Table 6.

Recovery of Total Permethrin From Fortified Soil Extracts at 1.0 and 0.1 ppm Fortification Levels

Original Spiking Level	Extracting Solvent System	Liquid-Liquid Partitioning	Column Cleanup System	Eluting Solvent System	Vol. (ml)	No. of Replicates	% Recovery (cis/trans)	S.D. (±) (cis/trans)
1.0 ppm	hexane	-	activated Florisil (5cm)	dichloromethane	15	9	94.6/92.5	2.9/3.9
	acetonitrile	acetonitrile:hexane (5:1, 60 ml twice)	activated Florisil (5cm)	dichloromethane	15	4	85.3/86.6	2.2/1.0
0.1 ppm	hexane	-	activated Florisil (5cm)	dichloromethane	15	4	89.2/86.0	3.2/1.7

Table 7.

Recovery of Total Permethrin From Fortified Sediment Extracts at 1.0 and 0.1 ppm Fortification Levels

Original Spiking Level	Extracting Solvent System	Liquid-Liquid Partitioning	Column Cleanup System	Eluting Solvent System	Vol. (ml)	No. of Replicates	% Recovery (cis/trans)	S.D. (±) (cis/trans)
1.0 ppm	hexane	-	activated Florisil (5cm)	dichloromethane	15	11	92.3/90.4	3.5/5.2
	acetonitrile	acetonitrile:hexane (5:1, 60 ml twice)	activated Florisil (5cm)	dichloromethane	15	3	86.5/88.6	2.9/2.6
0.1 ppm	hexane	-	activated Florisil (5cm)	dichloromethane	15	2	90.2/85.0	3.9/4.2

extraction, acetonitrile and hexane partition (5:1, 60 ml twice then 1:4, 25 ml once), followed by a Florisil® column cleanup using either toluene or dichloromethane as the eluting solvent. This procedure yielded high recoveries as well as a clean chromatogram (Fig.5 (a)).

The toluene elution yielded 93 (± 3.3) and 96 (± 3.1)% recovery of the cis and trans isomers, respectively at 1.0 ppm, while the dichloromethane elution resulted in a 89 (± 1.1) and 91 (± 1.3)% recovery for cis and trans-permethrin respectively at 1.0 ppm. The toluene elution was not investigated at the 0.1 ppm level, however, the dichloromethane elution gave recoveries of 80 (± 1.3) and 80 (± 3.9)% for cis and trans-permethrin at the 0.1 ppm fortification level.

A similar procedure using benzene as the eluting solvent produced recovery levels of 92 (± 3.7) and 95 (± 3.7)% for the cis and trans isomers, respectively, at 1.0 ppm permethrin. Although the benzene elution gave higher recoveries compared to dichloromethane, the latter is preferred due to the higher health hazards associated with benzene. The dichloromethane eluted samples also showed a higher degree of consistency as illustrated with the standard deviations (Table 2).

Many of the procedures investigated were unacceptable due to the presence of interfering co-extractives illustrated in the chromatograms. As an example, when using either a 5 or 10% diethyl ether in hexane solution as eluting solvent the chromatograms showed large peaks close to the permethrin cis and trans isomers which had retension times of 9.2 and 10.2 minutes respectively (Fig. 6).

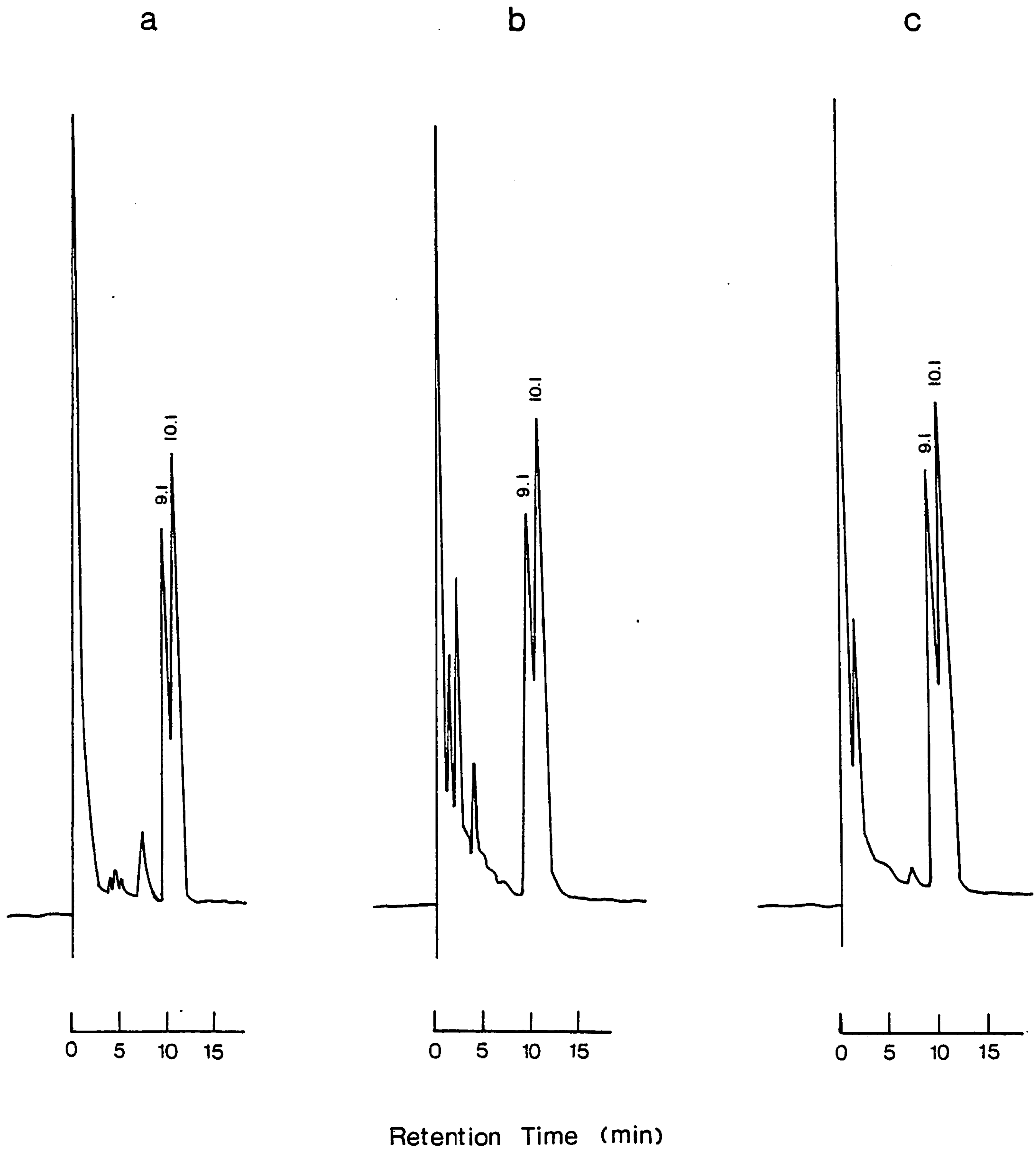


Fig. 5. Chromatograms of fortified forestry substrates (1.0 ppm):
(a) balsam fir foliage,
(b) brook trout,
(c) natural stream water.

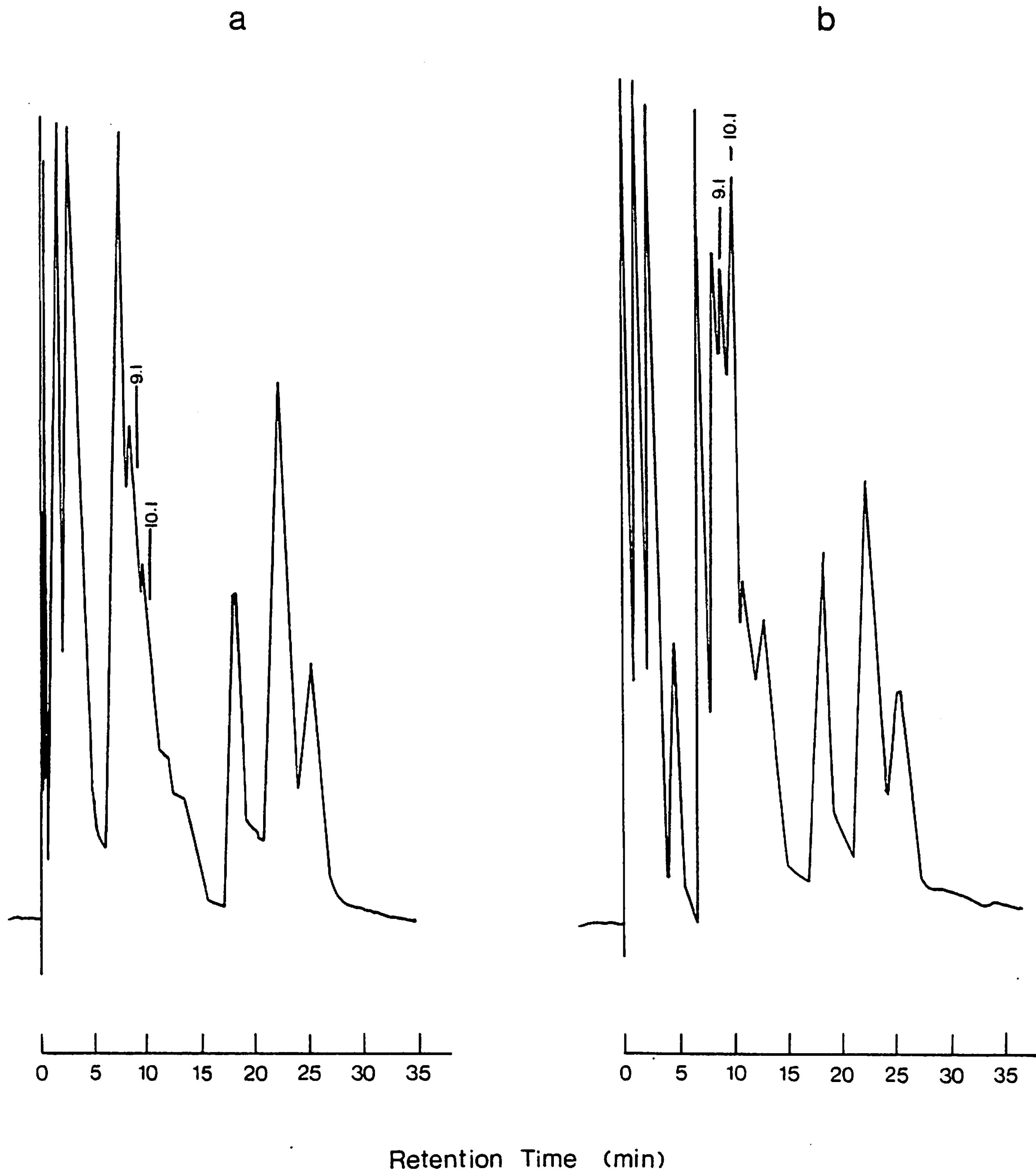


Fig. 6. Chromatograms of the foliar extracts which were eluted with 10% di-ethyl ether in hexane:
(a) non-fortified foliage,
(b) fortified foliage (1.0 ppm)

Another problem encountered in the various column cleanup trials was the large volume of solvent that was required for the elution of the permethrin. For example, 40 ml of a 5% benzene in hexane solution was tried as eluting solvent with < 10% of the permethrin being recovered at the 1.0 ppm fortification level. Even though a higher elution volume may have given a much better recovery, it also would have probably eluted more co-extractives, thus giving a chromatogram with additional and perhaps interfering peaks.

Comparing toluene and dichloromethane as eluting solvents, toluene seems to be the most acceptable for eluting the permethrin isomers, although the standard deviations were slightly higher than that of the dichloromethane. The present study confirms that dichloromethane could serve as a substitute for toluene in permethrin analysis.

Other problems encountered in the methodology development occurred during the liquid-liquid partitioning procedure. By trying different acetonitrile to hexane ratios, it was found that with low acetonitrile ratios, there was excessive loss of permethrin in the non-polar hexane phase. The ratio of acetonitrile: hexane of 5:1 (60 ml) appeared to be the most acceptable for maximum recovery levels.

For the quantification of permethrin in fish, the best extraction and cleanup procedure was found to be hexane extraction, acetonitrile and hexane partition (5:1, 60 ml twice then 1:4, 25 ml once), followed by a Florisil® column cleanup using dichloromethane as eluting solvent. The average recoveries of the cis and trans isomers of permethrin were 96 (± 4.6) and 99 (± 3.0)% respectively, at the 1.0 ppm fortification level, while at the 0.1 ppm level, the recoveries for cis and trans were 92 (± 2.3) and 94 (+ 0.7)% respectively.

Only dichloromethane and a 10% diethyl ether in hexane solution were tested as possible eluting solvents. Both gave good chromatograms with no interference peaks (Fig. 5(b)), however, the dichloromethane eluted samples gave a higher average recovery. The 10% diethyl either in hexane elution only yielded 92 and 93% recovery for the cis and trans isomers at 1.0 ppm compared to the 96 and 99% recovery for cis and trans respectively when using dichloromethane.

As illustrated in Table 3, the hexane phase in the liquid-liquid partitioning stage had to be re-extracted with a small volume (5 ml) of acetonitrile in order to increase the recovery of permethrin. This re-extraction of the hexane phase resulted in a 10% increase in recovery of the cis and trans isomers of permethrin.

Of all the substrates that were studied, the natural stream water was the easiest to extract, cleanup, and analyze, as well, it gave the highest recovery levels.

The only method investigated for the water samples was a hexane extraction followed by a Florisil® column cleanup using dichloromethane as eluting solvent. The average recovery was 99 (± 3.4) and 100 (± 2.1)% for cis and trans-permethrin at the 1.0 ppm level. Recovery values at the 0.1 ppm level were reported to be 101 (± 3.3) and 103 (± 2.7) for cis and trans respectively,while the 0.01 ppm level resulted in recovery, levels of 89 (± 8.8) and 98 (± 7.6) for the cis and trans isomers respectively (Table 4).

The procedure reported for the extraction, cleanup and analysis of the natural stream water proved to be very efficient as illustrated with the high recoveries and clean chromatograms which were free of interfering peaks caused by co-extractives (Fig. 5 (c)).

From the knowledge gained in analyzing the foliage, fish, and natural water samples, the only column cleanup system that was investigated for the soil, litter, and sediment substrates was the Florisil® column using a dichloromethane elution, although toluene could have served a similar purpose. This procedure yielded consistently good recoveries with clean chromatograms (Fig. 7) when hexane was used as the extracting solvent.

The recoveries reported for the forest litter samples, when using this procedure, were 96 (± 1.8) and 96 (± 2.2)% respectively for cis and trans-permethrin at 1.0 ppm and 97 (±2.0) and 96 (± 1.2)% for the cis and trans isomers, respectively at the 0.1 ppm fortification level (Table 5). The soil gave recovery levels of 95 (± 2.9) and 93 (± 3.9)% respectively for the cis and trans isomers at 1.0 ppm while the recoveries at the 0.1 ppm level for cis and trans respectively were 89 (± 3.2) and 86 (± 1.7)% (Table 6). The recoveries of permethrin in sediment at 1.0 ppm were 92 (± 3.5) and 90 (± 5.2)% for the cis and trans isomers respectively. At the 0.1 ppm fortification level, the sediment extractions yielded 90 (± 3.9) and 85 (± 4.2)% respectively for cis and trans permethrin (Table 7).

A procedure using acetonitrile as the extracting solvent was also tested for these three substrates. The results are given in Tables 5, 6 and 7. The acetonitrile extracted sample underwent an acetonitrile and hexane partition (5:1, 60 ml once) followed by Florisil® column cleanup. The partition stage was introduced in order to remove excess lipoidal and other co-extractives.

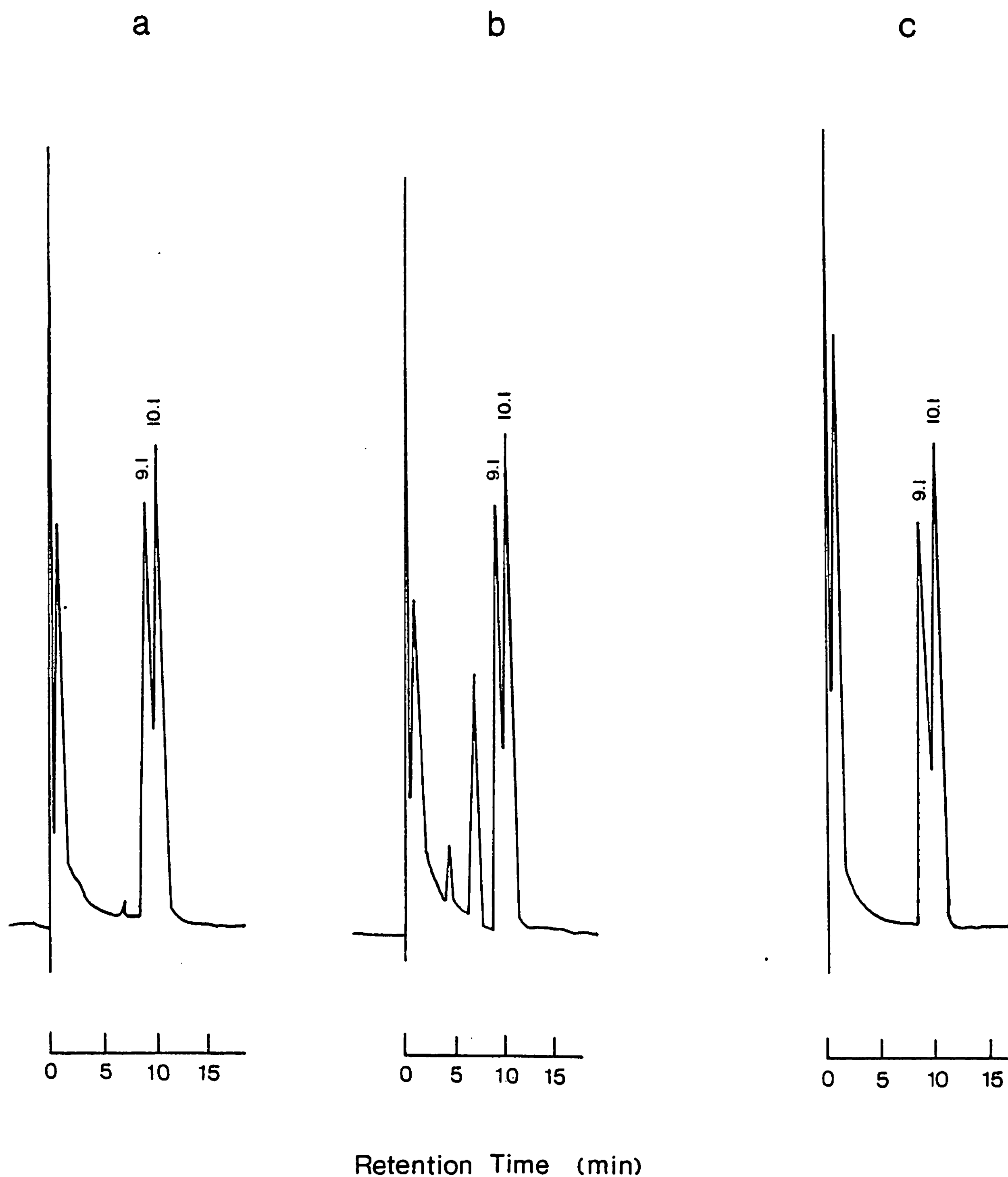


Fig. 7. Chromatograms of fortified forestry substrates (1.0 ppm):
(a) forest litter,
(b) soil,
(c) sediment.

The forest litter samples that were extracted with acetonitrile underwent two separate procedures. One consisted of a liquid-liquid partitioning followed by Florisil® column cleanup while the second one was investigated using only the Florisil® column cleanup.

The litter samples that were only subjected to column cleanup yielded recoveries of 77 (± 0.7) and 80 (± 0.1)% respectively for the *cis* and *trans* isomers at 1.0 ppm permethrin. These recovery values were approximately 2% higher than those obtained when the liquid-liquid partitioning stage was introduced into the cleanup procedure (Table 5).

As compared to the acetonitrile extracted samples, the hexane extractions yielded higher recoveries and also, the extracts were cleaner and hence easier to work with.

SUMMARY AND CONCLUSIONS

A new analytical method has been developed for the extraction and cleanup of permethrin residues present in six forest substrates, namely (1) foliage, (2) fish, (3) natural stream water, (4) forest litter, (5) soil and (6) sediment.

The method developed for the foliage and fish substrates consisted of hexane extraction, acetonitrile and hexane partition, followed by Florisil® column cleanup. The mean percent recovery for the foliage was 89 (± 1.1) and 91 (± 1.3)% respectively for the *cis* and *trans* isomers at 1.0 ppm, while at 0.1 ppm, the *cis* and *trans* recoveries were 80 (± 1.3) and 80 (± 3.9)% respectively. For fish, the recovery was 96 (± 4.6) and 99 (± 3.0)% respectively for *cis* and *trans*-permethrin at 1.0 ppm and 92 (± 2.3) and 94 (± 0.7)% for the *cis* and *trans* isomers, respectively, at the 0.1 ppm fortification level.

The method used for the foliage and fish not only gave good consistent recoveries, but it also yielded clean chromatograms with no interfering peaks.

For natural stream water, forest litter, soil and sediment the method involved hexane extraction followed by Florisil® column cleanup using dichloromethane as eluting solvent. This method provided clean chromatograms as well as high recoveries. The mean percent recovery of permethrin at the 1.0 ppm fortified level varied from 92 to 102%. At the 0.1 ppm level, the recovery was >95% for natural water and litter, whereas the soil and sediment showed a recovery varying from 85 to 90%.

The natural water was also investigated at the 0.01 ppm fortification level. The mean percent recovery for the cis and trans isomers of permethrin at this spiking level were 89 and 97% respectively. The loss of permethrin in the natural water was probably due to the fact that permethrin is hydrophobic and hence it adsorbs onto particulate matter in the water and the container walls.

A comparative study between hexane and acetonitrile in the extraction of permethrin from forest litter, soil and sediment illustrated that hexane produced a much cleaner extract and also, it yielded a higher mean percent recovery.

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