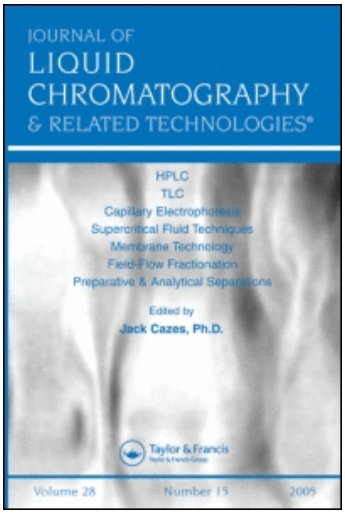

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF PERMETHRIN DEPOSITS IN A FORESTRY SPRAY TRIAL

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ABSTRACT

A convenient and sensitive reversed-phase high performance liquid chromatographic method has been developed for the determination of permethrin [3-phenoxybenzyl (±)- *cis,trans* -3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate] insecticide. Various isocratic and gradient mobile phases, consisting of acetonitrile:water (CH_3CN:H_2O) and methanol:water (CH_3OH:H_2O) solvent systems at two flow rates, were tested to separate and quantify the isomers of permethrin using octadecylsilyl (ODS) (Regis 5 μm) and octylsilyl (OS) (RP-8, 10 μm) bonded columns. The optimal mobile phase for permethrin using the ODS column was 70:30 (v/v) CH_3CN:H_2O mixture at flow rates of either 1.0 or 1.5 mL/min. The measurement was done with a UV detector at 200 nm and 50°C. The OS column gave a less satisfactory separation than the ODS. Gradient elution systems examined did not improve the isomeric separation of permethrin. Using the method developed, deposit levels obtained on various sampling units during a permethrin spray trial were analyzed after elution or extraction followed by necessary column cleanup. Minimum levels of detection for permethrin varied from 1 to 3 ng depending on the nature of the sampler used.

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INTRODUCTION

Permethrin [3-phenoxybenzyl (±)- *cis,trans* -3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate] is one of the new synthetic pyrethroid insecticides 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.

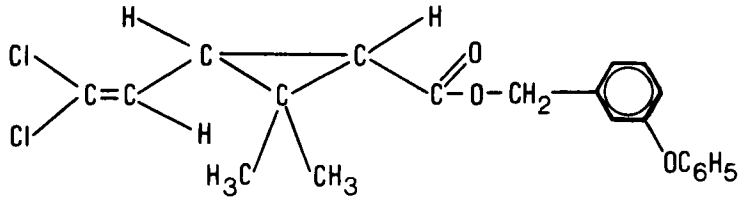
One of the Canadian registration requirements for any chemical, prior to its large scale use in forestry, is to have a convenient and suitable method to analyze its residues in various forestry substrates. Factors that need to be considered are the speed, precision, accuracy, cost and ease of handling for routine analysis of large numbers of samples after forestry spray applications.

Published analytical procedures for the determination of permethrin residues are mainly for agricultural commodities using gas-liquid chromatographic (GLC) methods. Miyamoto (4) reviewed the GLC methods published prior to 1980 for permethrin analysis; since then, additional residue methods, most of them modifications of earlier ones, have been reported (5-15). Reliable GLC analysis of permethrin requires suitable solvent partitions followed by extensive column cleanup procedures and instrument conditioning. Oehler (16), Lam and Grushka (17) and Kikta, Jr. and Shierling (18) have reported high performance liquid chromatographic (HPLC) methods as alternatives to GLC for analyzing permethrin. However, these HPLC methods involved either technical materials or commercial formulations sprayed on agricultural crops. So far, no suitable procedure has been reported for the analysis of permethrin residues in forestry samples after spray application of a formulated end-use product of commercial material. In this paper

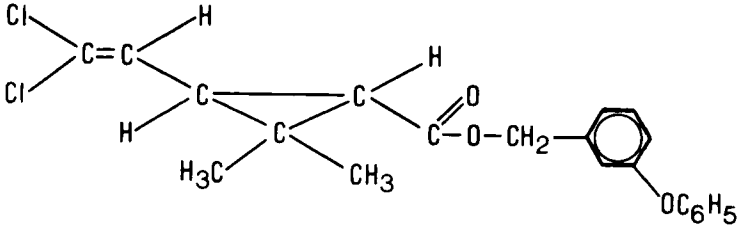
we describe a simple and reliable HPLC technique that can be used to identify and quantify airborne permethrin entrapped in impingers as well as permethrin deposited on water surfaces, glass plates and metal rods used as sampling units during a forestry spray trial.

MATERIALS

Permethrin is a mixture of two geometrical isomers, *cis* and *trans* (Fig. 1). The composition of the two isomers varies with the manufacturing process (19). Analytical grades of the individual isomers and the (±) *cis, trans* mixture used in the present study were supplied by Chipman Inc., Stoney Creek, Ont., Canada.



cis-isomer



trans-isomer

Figure 1. *cis*- and *trans*- Permethrin

The *cis:trans* isomeric composition in the sample received was determined by GLC and HPLC methods and was found to be in the weight ratio of 40 ± 2% and 60 ± 2% respectively.

Acetonitrile, methanol and water solvents used in the study were HPLC grade obtained from Fisher Chemical Co. and were filtered through appropriate Millipore® filters and degassed prior to use. The permethrin samples used as standards were readily soluble in methanol and acetonitrile. The stock solutions were stored at 0°C in darkness. The standard solutions were stable during the entire period (*ca.* 8 weeks) of study. All solutions prepared were filtered prior to injection on the HPLC system.

METHODS

The set up of the HPLC instrument used in this study was similar to one described earlier (20) except for the following variations in operating parameters:

Columns: a) Hewlett-Packard RP-8 (octylsilyl bonded) (OS), 10 μm, 20 cm x 4.6 mm ID

b) Regis Spherisorb Hi-Chrom Rev. ODS-2 Octadecyl II [octadecylsilyl (ODS) bonded] 5 μm, 15 cm x 4.6 mm ID

Column Pressure: 16 - 110 bars (1 bar = 14.5 psig)

Mobile Systems (v/v): a) $CH_3CN:H_2O$
b) $CH_3OH:H_2O$

Flow Rate: a) 1.0 mL/min
b) 1.5 mL/min

Oven Temperature: 50°C

Variable Wavelength: Sample(S):Reference(R)
a) 200:430 nm
b) 220:430 nm

Injection System: Rheodyne (Berkeley, Calif. U.S.A.) Model 7120 syringe loading injector with 20 μL loop size

Sample Size: 20 μL of 1, 10 and 100 μg/mL standard stock solutions

Chart Speed: 0.2 cm/min

Attenuation: 2^4 and 2^7

Slope Sensitivity: 0.2

Processing of Permethrin from Samplers

The toluene samples from the impingers, which were used to trap the airborne droplets of permethrin, were passed through 4 cm Na_2SO_4 columns (1.5 cm ID), flash evaporated to a small volume, and cleaned up by adsorption chromatography using microcolumns (8,21). The residues were taken up in methanol.

The water samples were extracted twice with hexane, dried by passing through Na_2SO_4 columns, concentrated under low pressure and cleaned up as described above. The deposits on the glass plates and metal rods were removed by rinsing repeatedly with hexane and processed according to the procedure used above.

The methanol solution of each sample was first taken into a 10 mL syringe barrel and plunger-pumped through a SEP-PAK® cartridge (C_{18} cartridge from Waters Associates, Inc., Milford, MA) followed by solvent elution and Millipore® filtration and finally concentrated to a suitable volume in an atmosphere of dry nitrogen for HPLC analysis.

The two solvent systems CH_3CN:H_2O and CH_3OH:H_2O and the two reversed phase columns *viz.*, the Regis octadecylsilyl (ODS) 5 μm (C_{18} bonded) and the Hewlett-Packard (HP) RP-8 (octylsilyl) 10 μm (C_8 bonded) were compared using various isocratic (Tables 2-4) and gradient mobile systems (Table 5) for the separation, identification and quantification of permethrin isomers. Samples recovered from the impingers and from the collection units (glass plates, metal rods and water) used in the field trial were also tested on both the Regis and HP columns.

The solutions of mixed permethrin standards and the extracts from spray trials were injected several times to obtain consistently reproducible results. The chromatograms obtained were well defined, having sharp peaks. Permethrin concentrations in field samples were computed from the peak area by comparison with those obtained from the standard solutions. Deviation in retention time (R.T.) for each injection was $<1\%$. The minimum detection limit (MDL) and the detector linear range (LR) found with both the columns, using individual *cis* and *trans* permethrin standards are given in Table 1.

TABLE 1

Minimum Detection Limit (MDL) and Linear Ranges (LR) for the Two Permethrin Isomers in the Two Columns Used*

Column		HP, RP-8, 10 μm	Regis, ODS-2, 5 μm
MDL	*cis*	2 ng (0.1 μg/mL)	1 ng (0.05 μg/mL)
	trans	2 ng (0.1 μg/mL)	1 ng (0.05 μg/mL)
LR	*cis*	0.51 to 10.12 μg/mL	0.10 to 10.12 μg/mL
	trans	0.54 to 10.88 μg/mL	0.11 to 10.88 μg/mL

* Mobile phase $CH_3CN:H_2O$, 70:30, λ = 200 nm.

RESULTS AND DISCUSSION

The retention times of permethrin isomers with the different isocratic solvent systems are given in Tables 2-4. Table 2 contains the R.T.'s obtained using the C_{18} bonded (Regis ODS-2, 5 μm) column with different proportions of $CH_3CN:H_2O$ in the mobile phase at two flow rates, *viz.* 1.0 and 1.5 mL/min. Increased flow rate and higher organic phase content yielded lower R.T.'s for the isomers. Increase of applied pressure, lower viscosity of the organic phase compared to water (0.37 cP *vs* 1.00 cP at 20°C) and higher solute-mobile phase attraction could have contributed to the decrease in R.T. The *trans* isomer eluted before the *cis* as shown by the chromatograms (Fig. 2) obtained for the mobile phase, $CH_3CN:H_2O$ (70:30) at the two flow rates. The peaks are sharp and well separated indicating that for a nonpolar solute such as permethrin, the use of a C_{18} bonded column is suitable for identification and quantification of the isomers. A complete isomer separation and subsequent analysis took 20 min. The other mobile phase compositions used were amenable for the intended purpose, but because of the small differences in R.T.'s observed between the isomers at higher levels of CH_3CN (Table 2), the resolutions were poor, and so mobile phases containing more than 70% of CH_3CN were not favoured. The critical factors in the resolution of

isomers appear to be the $CH_3CN:H_2O$ ratio and the flow rate. If too much polar organic phase was present, poor resolution of the isomers resulted and if the flow rate was low, longer analysis time was required because of increased R.T.

Reverse phase studies for permethrin isomers using a C_8 bonded column (RP-8, 10 μm) with different ratios of $CH_3CN:H_2O$ and $CH_3OH:H_2O$ are reported in Tables 3 and 4 respectively. Similar trends in R.T. changes with variations in organic phase content and flow rates were also observed in these studies. Using the $CH_3CN:H_2O$ (70:30) mobile phase at a flow rate of 1 mL/min, the R.T.'s of the isomers obtained in the C_8 bonded column were shorter (*cis* 8.21 min; *trans* 7.67 min) (Table 3) compared to those obtained with the C_{18} bonded column (*cis* 15.54 min; *trans* 13.43 min) (Table 2). This is due to the large particle size (10 μm) of the OS column compared to the ODS column (5 μm). The analysis times were correspondingly reduced. Despite this apparent advantage, the peaks were not resolved properly and in addition, upward baseline drift and noise were apparent (Fig. 3). Such a shift in baseline and noise would adversely affect the detection limits of the chemical during routine analysis, especially when the residue levels are low.

The use of the $CH_3OH:H_2O$ mobile phase in reverse-phase chromatographic separation of permethrin isomers (Table 4) was not encouraging because of the poor quality of separation. It should be remembered that a direct comparison of the instrument response to both the solvent systems was not possible because the two solvents had different UV cutoff values ($CH_3CN:H_2O$ 200 nm; $CH_3OH:H_2O$ 220 nm). The use of CH_3OH generally resulted in peak broadening and upward baseline drift, which eventually caused poor resolution of the isomers (Fig. 4). In addition, the analysis time was extended unnecessarily when the composition of the aqueous phase was increased.

The separation and quantification of the isomers of permethrin were carried out by using the gradient mobile phase systems listed in Table 5. It is apparent that the percent of organic

TABLE 2

Retention Times (R.T.) (min) of Permethrin Isomers at 50°C and 200 nm Obtained Isocratically by Using Different Ratios of CH_3CN and H_2O at Two Flow Rates with Regis ODS-2, 5 μm Column

	Flow Rate: 1.0 mL/min CH_3CN:H_2O Ratio			Flow Rate: 1.5 mL/min CH_3CN:H_2O Ratio		
	80:20	75:25	70:30	80:20	75:25	70:30
cis	6.71	10.04	15.54	4.61	6.89	10.77
trans	5.97	8.78	13.43	4.11	6.03	9.31

TABLE 3

Retention Times (R.T.) (min) of Permethrin Isomers at 50°C and 200 nm Obtained Isocratically by Using Different Ratios of CH_3CN and H_2O at Two Flow Rates With RP-8 10 μm Column

	Flow Rate: 1.0 mL/min CH_2CN:H_2O Ratio				Flow Rate: 1.5 mL/min CH_3CN:H_2O Ratio		
	80:20	75:25	70:30	60:40	70:30	65:35	60:40
cis	5.40	7.27	8.21	18.03	6.80	7.65	11.99
trans	5.12	6.27	7.67	16.49	6.27	7.08	10.95

TABLE 4

Retention Times (R.T.) (min) of Permethrin Isomers at 50°C and 220 nm Obtained Isocratically by Using Different Ratios of CH_3OH and H_2O at Two Flow Rates With RP-8, 10 μm column

	Flow Rate: 1.0 mL/min CH_3OH:H_2O Ratio			Flow Rate: 1.5 mL/min CH_3OH:H_2O Ratio
	80:20	75:25	70:30	70:30
cis	7.32	11.58	20.04	13.85
trans	6.74	10.39	17.61	12.21

Figure 2. Separation of *cis*- and *trans*- Permethrin by HPLC.
Column: Regis ODS-2, 5 μm
Solvent System: $CH_3CN:H_2O$ = 70:30
a) Flow Rate: 1.0 mL/min
b) Flow Rate: 1.5 mL/min

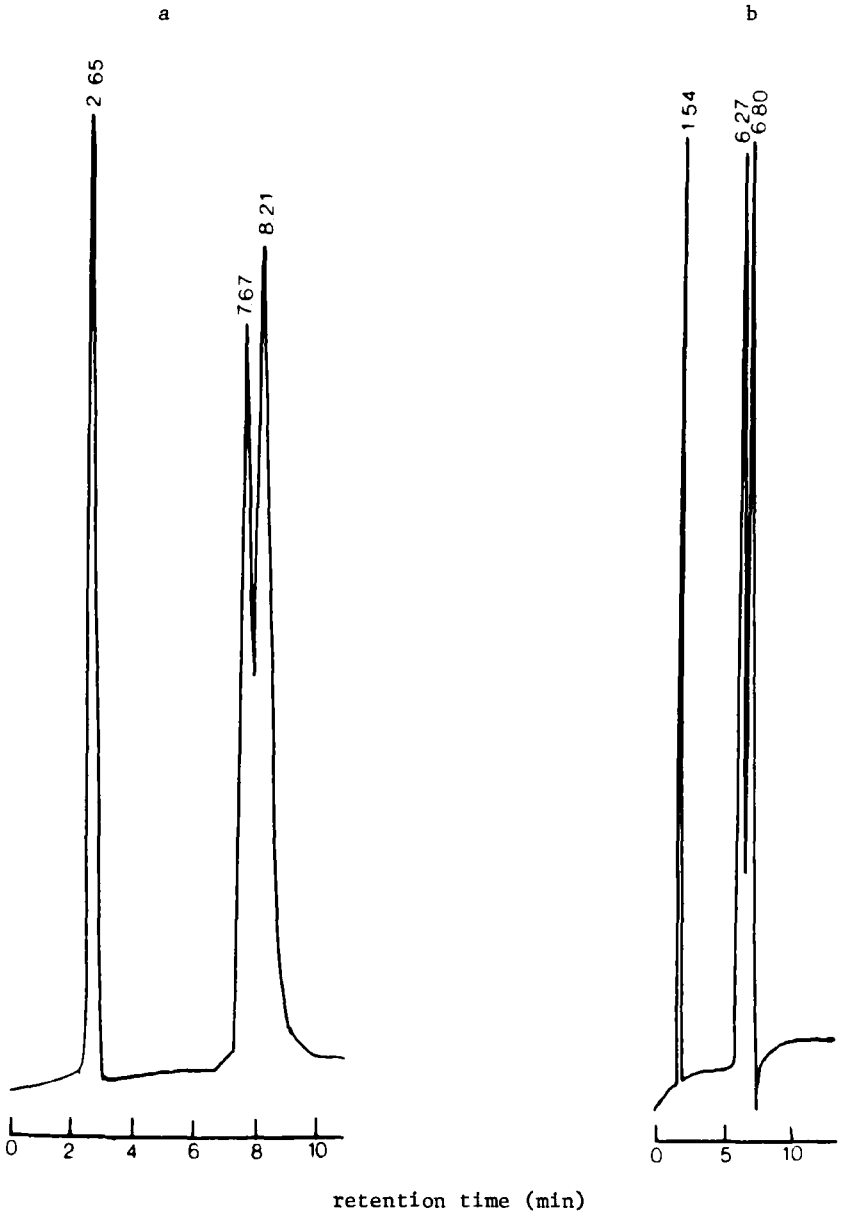


Figure 3. Separation of *cis*- and *trans*- Permethrin by HPLC.
Column: RP-8, 10 μm
Solvent System: $CH_3CN:H_2O$ = 70:30
a) Flow Rate: 1.0 mL/min, Chart Speed: 0.5 cm/min
b) Flow Rate: 1.5 mL/min, Chart Speed: 0.2 cm/min

TABLE 5

Solvent Systems Used in the Gradient Elution of Permethrin Isomers at 50°C and at a Flow Rate of 1.5 mL/min and the Corresponding Retention Times

Gradient system	Mobile Phase A	Mobile Phase B	Column	Time (min)	Mobile Phase Comp. (%) A	Mobile Phase Comp. (%) B	Detector (nm)	R.T. (min) *cis*	R.T. (min) *trans*
a	CH_3CN	H_2O	Regis, 5 μm	0.5	70	30	200	7.86	6.46
				1.0	80	20			
				2.5	80	20			
				3.0	70	30			
b	CH_3CN	H_2O	Regis, 5 μm	0.5	65	35	200	9.63	7.30
				1.0	80	20			
				3.0	80	20			
				4.0	65	35			
c	CH_3CN	H_2O	Regis, 5 μm	1.0	55	45	200	43.16	34.72
				2.0	75	25			
				3.0	75	25			
				4.0	55	45			
d	CH_3CN	H_2O	RP-8, 10 μm	1.0	55	45	200	17.01	14.29
				2.0	75	25			
				3.0	75	25			
				4.0	55	45			
e	CH_3CN	H_2O	RP-8, 10 μm	0.5	65	35	200	15.74	14.83
				1.5	30	70			
				5.0	30	70			
				6.0	65	35			
f	CH_3OH	H_2O	RP-8, 10 μm	0.5	70	30	220	17.16	15.70
				1.0	30	70			
				4.0	30	70			
				4.5	70	30			

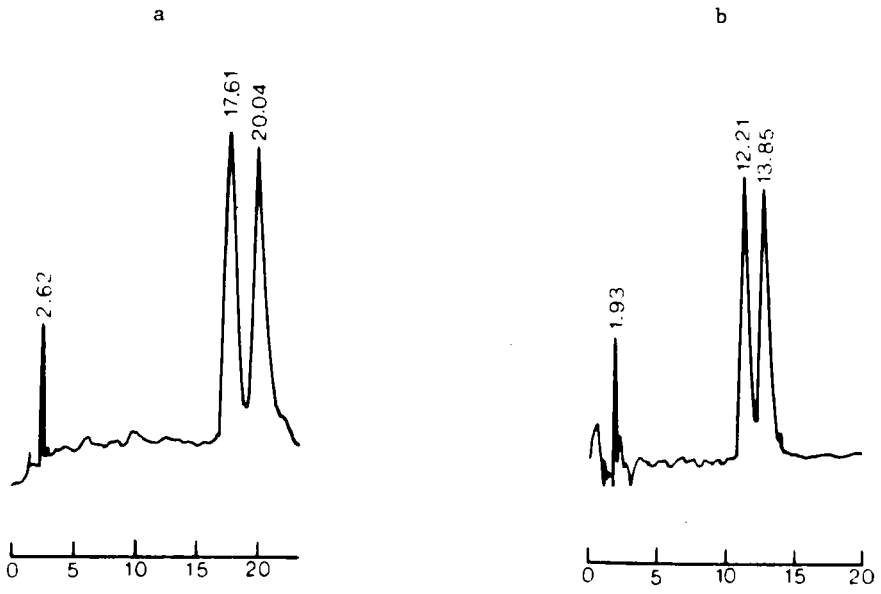


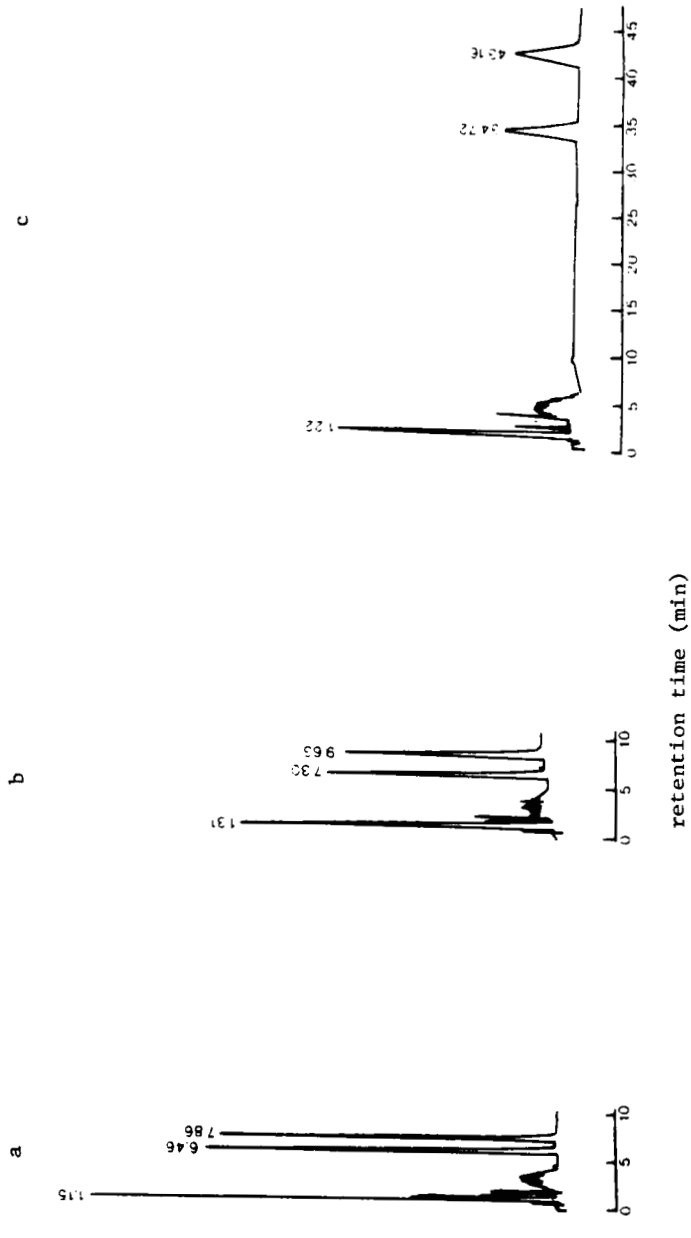
Figure 4. Separation of *cis*- and *trans*- Permethrin by HPLC.
Column: RP-8, 10 μm
Solvent System: $CH_3OH:H_2O$ = 70:30
a) Flow Rate: 1.0 mL/min
b) Flow Rate: 1.5 mL/min

phase constituents in the mobile phase systems did vary compared to the isocratic systems listed in Tables 2 to 4. Consequently, some slight improvements were observed in the analysis time and in resolution, especially when using Regis 5 μm column. The column was usually found to give a good isomeric separation and consistency in sample-to-sample reproducibility in the gradient system. In spite of these advantages, column sensitivity was considerably less than the isocratic system (Fig. 5). Among the eluent combinations studied, system b (Table 5) was found to be useful; the others gave either a very close R.T. (system a) or a longer run

time (system c). Therefore, for reliable analysis at residue levels, best results are achieved by using a C_{18} bonded column isocratically.

The separation of the isomers in the analyte by RP-8 column using the gradient system (either $CH_3CN:H_2O$ or $CH_3OH:H_2O$ system) was not satisfactory. The eluent combinations yielded either a poor differentiation of the isomers or a longer run time with reduced sensitivity and occasional peak broadening (Fig. 5d, 5e, 5f). Efforts to develop and establish a gradient solvent system for better isomeric separation and quantification of permethrin were not successful. The isocratic system reported earlier was adequate to meet these requirements.

Analysis of permethrin residues deposited on the ground samplers used during the spray trial was attempted with the $CH_3CN:H_2O$ (70:30) isocratic system. Both the C_{18} (Regis 5 μm) and C_8 (RP-8, 10 μm) bonded columns were tried. The results are given in Table 6. Analysis was possible without the microcolumn cleanup for some samples, especially for the eluates from metal rods, but the overall sensitivity was low and varied due to interferences from coextracted impurities. Between the columns, as noticed earlier, the C_{18} bonded column gave consistently reproducible residue data for replicate injections of each sample. With this system, the impurities eluted earlier, the peaks were sharp and well defined and drift from the base line was minimal (Fig. 6). The minimum detection limit *(cis + trans)*, ranged from 3 ng (air) to 1 ng (metal rods) depending on the type of sample analysed. For the same sample, at the same injection volume, the RP-8 column usually gave poor resolution for the isomers yielding broader and shorter peaks with considerable base line drift (Fig. 6). Consequently the results were inconsistent with appreciable deviations and in general, were higher for the *cis*-isomer than with the C_{18} column (Table 6). The analysis time was also about 2.5 times longer *(ca.* 20 min *vs* 8 min), because the impurities eluted after the *cis, trans* isomers.



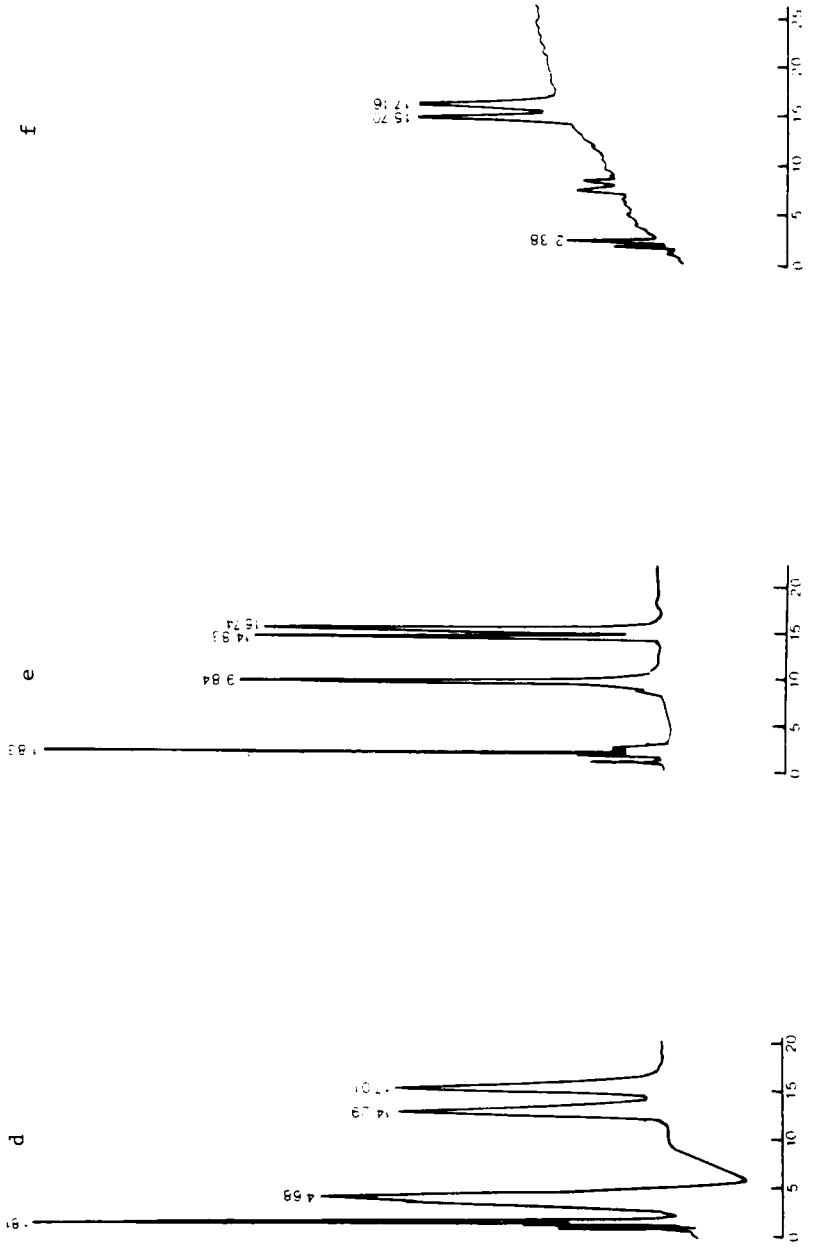


Figure 5. Separation of *cis*- and *trans*- Permethrin by Gradient Elution.
Flow Rate: 1.5 mL/min Columns: a to c Regis, 5 μm
d to f RP-8, 10 μm
Solvent Systems: see Table 5

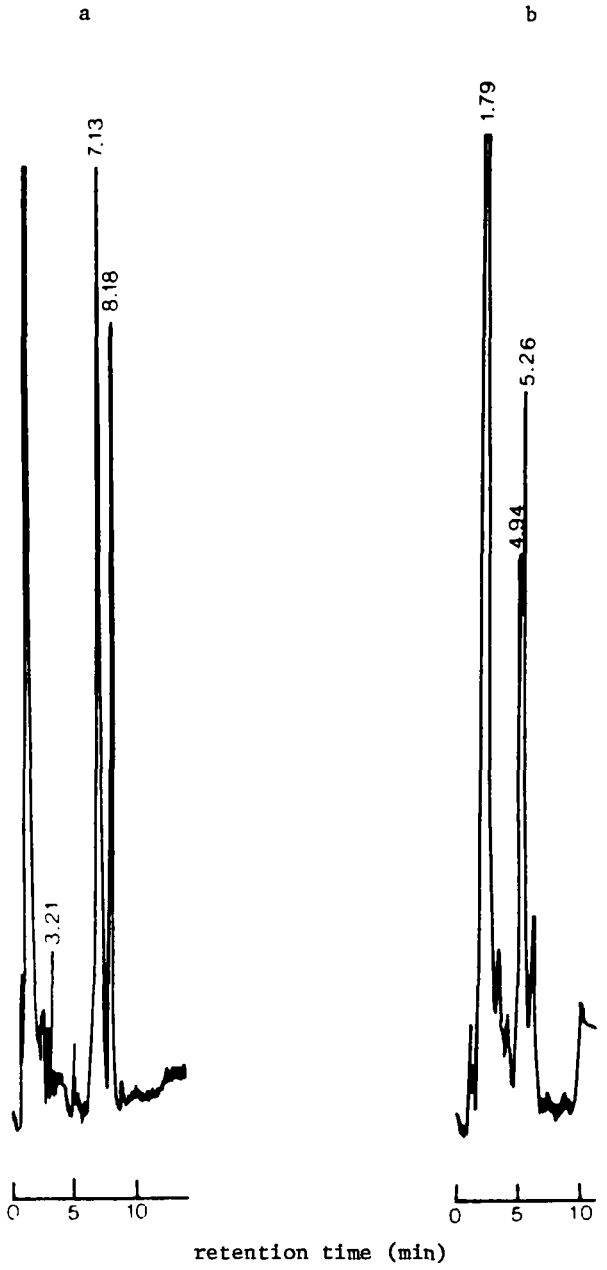


Figure 6. Permethrin from Air Sample after a Spray Trial
Column: a) Regis, 5 μm
b) RP-8, 10 μm
Flow Rate: 1.5 mL/min
Solvent System: $CH_3CN:H_2O$ = 70:30
Quantities of *cis*- and *trans*- Permethrin are given in Table 6.

TABLE 6

Comparison of Deposit Levels of Permethrin Obtained from Spray Trial Using Regis 5 µm and RP-8, 10 µm Columns with Isocratic Solvent System $CH_3CN:H_2O$ (70:30) at λ = 200 nm and Temp. 50°C

Samplers Used for Permethrin Deposit	Permethrin Collected (ng)*					
	Regis 5 µm Column			RP-8, 10 µm Column		
	cis	*trans*	Total	*cis*	*trans*	Total
Impingers	1.99	2.90	4.89	2.40	2.08	4.48
Water	5.59	8.27	13.86	9.36	6.97	16.33
Metal Rod	3.30	5.31	8.61	4.38	5.08	9.46
Glass Plate	6.95	10.85	17.80	6.63	11.60	18.23

*Average from four sampling units.

Methods development research is currently in progress in our laboratories using HPLC and GLC-EC techniques to quantify permethrin from various forestry substrates. Our present experience is that while analyzing environmental samples for permethrin at residue levels, HPLC requires less effort, is operationally easier, and provides more reliable information than GLC-EC analysis.

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