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DETERMINATION OF FENITROTHION RESIDUES IN HONEYBEES AND THEIR PRODUCTS

by K.M.S. Sundaram

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K. M. S. Sundaram

25 Pickering Place

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K1A 0W3

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INTRODUCTION

Fenitrothion, 0,0-dimethyl-0-(4-nitro-m-tolyl) phosphorothioate, has been used extensively since 1969 for the control of spruce budworm in Canadian forests. Aerial application of the insecticide at a dosage of 2 to 4 ozs AI/acre when budworm were in 3rd, 4th and 5th instar stages, prevented defoliation without serious hazard to forest plants, animals, birds and fish (Fettes 1968). The increasing utilization of the insecticide is primarily due to its high biological activity (Miyamoto 1969, Martin 1971), good performance against a broad spectrum of insects (Nigam 1970), low mammalian toxicity (Miyamoto 1969), short persistence (Yule and Duffy 1972), and ready degradability (Sundaram 1973) in forest environments. Current interest at the Chemical Control Research Institute (CCRI) centers on studying possible long term effect and significance of fenitrothion residues arising from its repeated use, on various nontarget species of fauna inhabiting the forest and exposed to the toxicant. To this end, it became necessary to develop sensitive analytical methods or to modify the existing ones for studying the insecticide and its breakdown products in various biological components of the forest. Despite the large number of methods described for quantifying fenitrothion residue in agricultural samples (for a review see Bowman and Beroza 1969), few can be used without modification, because of the high sensitivity, specificity and minimum interferences required in analysing biological samples of forest origin. The

present report describes the development of a gas-liquid chromatographic (GLC) method of analysis of the parent insecticide, its oxon and the cresol hydrolysis product in honeybees (Apis mellifera L.) pollen, beeswax and honey from forested areas which had been treated with fenitrothion applied by aircraft.

MATERIALS AND METHODS

Samples of honeybees and their products

Samples of honeybees and their products were collected from treated and untreated areas of Larose Forest (near Ottawa) during the course of experiments being carried out on effects of operational use of fenitrothion for spruce budworm (Choristoneura fumiferana Clemens) control on environmental flora and fauna. A portion of samples collected from the untreated plot were fortified with known amounts of fenitrothion, fenitrooxon and cresol and used for the development of analytical methods. The samples from the treated areas were analysed for fenitrothion residues according to the developed procedure.

Analytical Methods

Extraction from Bees

Samples of collected dead bees at hives (maximum 2.0 g) were homogenized in a Sorvall Omni-Mixer with Na_2SO_4 (10 g) and acetonitrile (50 ml) for 5 min at a speed setting of 7. The macerate was filtered under suction using a fritted glass funnel.

The residues was washed with additional acetonitrile (10 ml), and the washings combined. The extract was equilibrated twice with hexane (30 ml). The nonpolar phase and the "cuff" were discarded. The acetonitrile phase was flash evaporated to ca 5 ml under reduced pressure. The cleanup was achieved by passing the acetonitrile concentrate through a column (2 x 30 cm) containing charcoal-Celite (10 g 3:2 by weight) sandwiched between Na_2SO_4 (10 g) and prewashed with benzene (Getz 1962, Yule and Duffy 1972). The column was eluted with 25% benzene in ethyl acetate (100 ml) followed by benzene (100 ml). The eluate was flash evaporated to ca 2 ml.

Additional cleanup and separation of fenitrothion and its metabolites (fenitrooxon and 4-nitrocresol) were accomplished by passing the sample through a column of silica gel (10 g containing 20% water) sandwiched between anhydrous sodium sulphate (10 g) and prewashed with benzene (50 ml) (Bowman and Beroza 1969, Yule and Duffy 1972). The elution began with benzene (60 ml) to remove the parent fenitrothion from the adsorbent column, then with more benzene (120 ml) to remove the cresol and finally with acetone (75 ml) to remove the oxon from the column. The eluates were flashed separately to small volumes for GLC analysis.

Extraction from Pollen

The procedure adopted was very similar to that for bees and the amount of pollen used in the extraction was 1.8 to 5.0 g.

Extraction from Beeswax

Samples (10 g) of finely divided beeswax were homogenized for 3 min with acetonitrile (150 ml). The homogenate was filtered through a thin pad of Celite. Soluble fatty materials present in the wax were removed by partitioning the acetonitrile with hexane (50 ml) 3 times. The acetonitrile was flash evaporated to a small volume (10 ml) and transferred quantitatively to a seperatory funnel. Water (300 ml). 5% sodium chloride solution (10 ml) and hexane (50 ml) were added. The mixture was equilibrated and the layers separated. The aqueous layer was re-extracted twice with hexane (50 ml). The combined hexane phases were concentrated (ca 5 ml) and subjected to charcoal and silica cleanups as described under bees.

Extraction from Honey

Fifty-gram samples of honey were mixed with water (100 ml) in a 2-liter separatory funnel. Acetonitrile (200 ml) was added and the funnel was shaken vigorously for 2 min. Hexane (100 ml) was added and the contents shaken vigorously for another 2 min. The sample was allowed to stand until the phases separated. Water (600 ml) was added, followed by 5% sodium chloride solution (10 ml) and shaken for 2 min. The aqueous phase was discarded. The organic layer was washed with two 25 ml portions of water, concentrated to about 2 ml and cleaned up by charcoal and silica columns as described above for bees.

Schematic representations of extraction, cleanup and separation procedures for fenitrothion residues in bees, pollen, beeswax and honey are given in Figs 1-3.

Gas Chromatographic Analysis

Gas chromatographic analysis of fenitrothion and its oxon metabolite in bee, wax, pollen and honey samples, after complete cleanup and separation, were carried out using a Hewlett-Packard F & M model 810 gas chromatograph equipped with a Tracor flame photometric detector. This specific P or S detector also permitted discriminative and confirmative identification and measurement of the parent and oxon materials (see Yule and Duffy 1972).

Operating conditions:

Columns	:	glass, 4 ft. x 0.25 in.
		loadings:
		1) 5% OV1;
		2) 3.8% SE 30; on Chromosorb W. 60/80 mesh, AW-DMCS
Temperatures (^{o}C):		injection ports 200
		column oven 185
		transfer line 190
		detector 160
Gas flow (ml./min):		N_2 60
		H_2 150
		O_2 20
		Air 50
FPD filters (mμ)	:	P 526
		S 394

Bee and bee products containing the cresol were analysed (Bowman and Beroza 1969) with a Hewlett - Packard Model 5750 gas

chromatograph fitted with a 63 Ni electron capture detector. Operating parameters were as follows:

Column	:	180 cm. x 4 mm Pyrex glass packed with 20% (w/w) OV-101 on 80-100 mesh Gas Chrom Q, preconditioned over-night at 280° C.
Temperatures (°C):		injection ports 210-220 column oven 190 detector 280
Carrier gas	:	argon/methane (95/5%) pressure of 50 psi and flow rate of 50 ml/min.
Instrument settings:		Attenuation and range, 16 x 10; pulse rate 50; electrometer 4 x 10^9 amp. full scale with 1 mv recorder.

The gas chromatographs were standardized on the same day as the samples were analyzed by injecting aliquots (1-5 µl) of freshly prepared standard solutions of fenitrothion, fenitrooxon and 4-nitrocresol in benzene, (analytical grades supplied by Sumitomo Chemical Company of Japan), measuring the peak heights, and preparing a calibration curve by plotting peak heights vs concentrations. The extracts of bees and their products were either diluted with benzene or concentrated to the desired concentrations for GC analysis. Quantitative results of the extracted samples were obtained by measuring each of the peak heights after injection (2 to 4 µl), under the same operating conditions, and reading the concentrations from its calibration curves.

All organic solvents used were either pesticide grade chemicals or freshly distilled in glass. The anhydrous sodium sulphate used was of reagent grade, heated at 150° C overnight and stored in a glass-stoppered bottle.

Laboratory sources (chemicals, glassware, filterpapers, absorbents, etc.) of contamination were found to be negligible.

TABLE I

Recovery of Fenitrothion, Fenitrooxon and Nitrocresol* Added to Bees and Bee Products**

Fortification Level of Fenitrothion, Fenitrooxon and Nitrocresol (µg/g)	Percent Recovery+											
	Bees			Pollen			Beeswax			Honey		
	Feni-trothion	Feni-trooxon	Cresol	Feni-trothion	Feni-trooxon	Cresol	Feni-trothion	Feni-trooxon	Cresol	Feni-trothion	Feni-trooxon	Cresol
10.0	89	80	81	94	89	79	87	88	82	94	89	90
5.0	84	78	75	89	83	81	84	82	81	93	82	88
2.0	79	81	79	92	83	80	84	83	79	91	87	84

* 4-nitro-m-cresol

** 2 g of bees, 2 g of pollen, 8 g of wax and 40 g of honey were used in the recovery studies.

+ Each value represents the average of three determinations.

TABLE II

Fenitrothion Residues† in Honeybees and their Products Collected from Sprayed Areas

Sample No.	Bees				Pollen				Beeswax				Honey			
	Mass (g)	Fen (ppm)	Fenox (ppm)	Cresol (ppm)	Mass (g)	Fen (ppm)	Fenox (ppm)	Cresol (ppm)	Mass (g)	Fen (ppm)	Fenox (ppm)	Cresol (ppm)	Mass (g)	Fen (ppm)	Fenox (ppm)	Cresol (ppm)
1	5.38††	2.080	N.D.	N.D.	1.90††	0.005	N.D.	N.D.	4.80*	0.055	N.D.	N.D.	9.70**	T	N.D.	N.D.
2	10.74††	0.405	N.D.	N.D.	10.00††	0.005	N.D.	N.D.	10.80**	0.005	N.D.	N.D.	17.80**	T	N.D.	N.D.

† Fenitrothion concn. is expressed in ppm "as sampled". Results are averages of duplicate analyses.

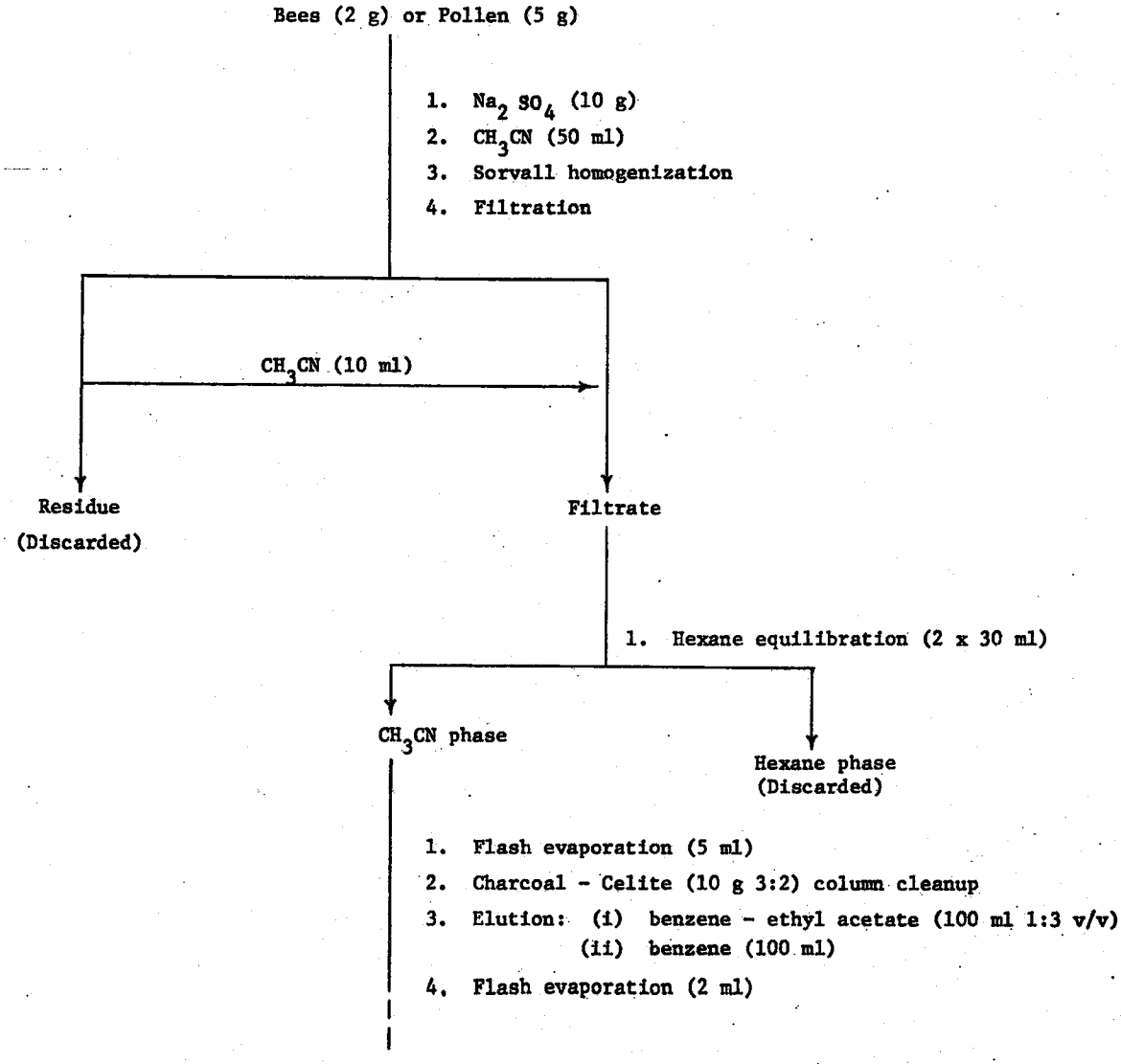
†† 1 Hour postpsray sample

* 29 Days " "

** 49 " " "

T Traces (< 0.005 ppm)

N.D. Not detected


Bees (2 g) or Pollen (5 g)
1. $Na_2 SO_4$ (10 g)
2. CH_3CN (50 ml)
3. Sorvall homogenization
4. Filtration
CH_3CN (10 ml)
Residue
(Discarded)
Filtrate
1. Hexane equilibration (2 x 30 ml)
CH_3CN phase
Hexane phase
(Discarded)
1. Flash evaporation (5 ml)
2. Charcoal - Celite (10 g 3:2) column cleanup
3. Elution: (i) benzene - ethyl acetate (100 ml 1:3 v/v)
(ii) benzene (100 ml)
4. Flash evaporation (2 ml)

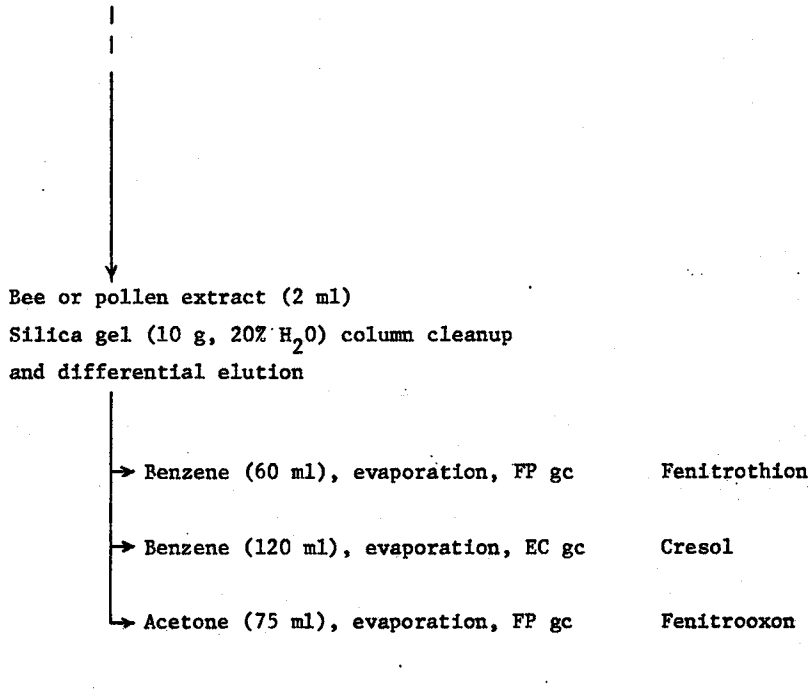
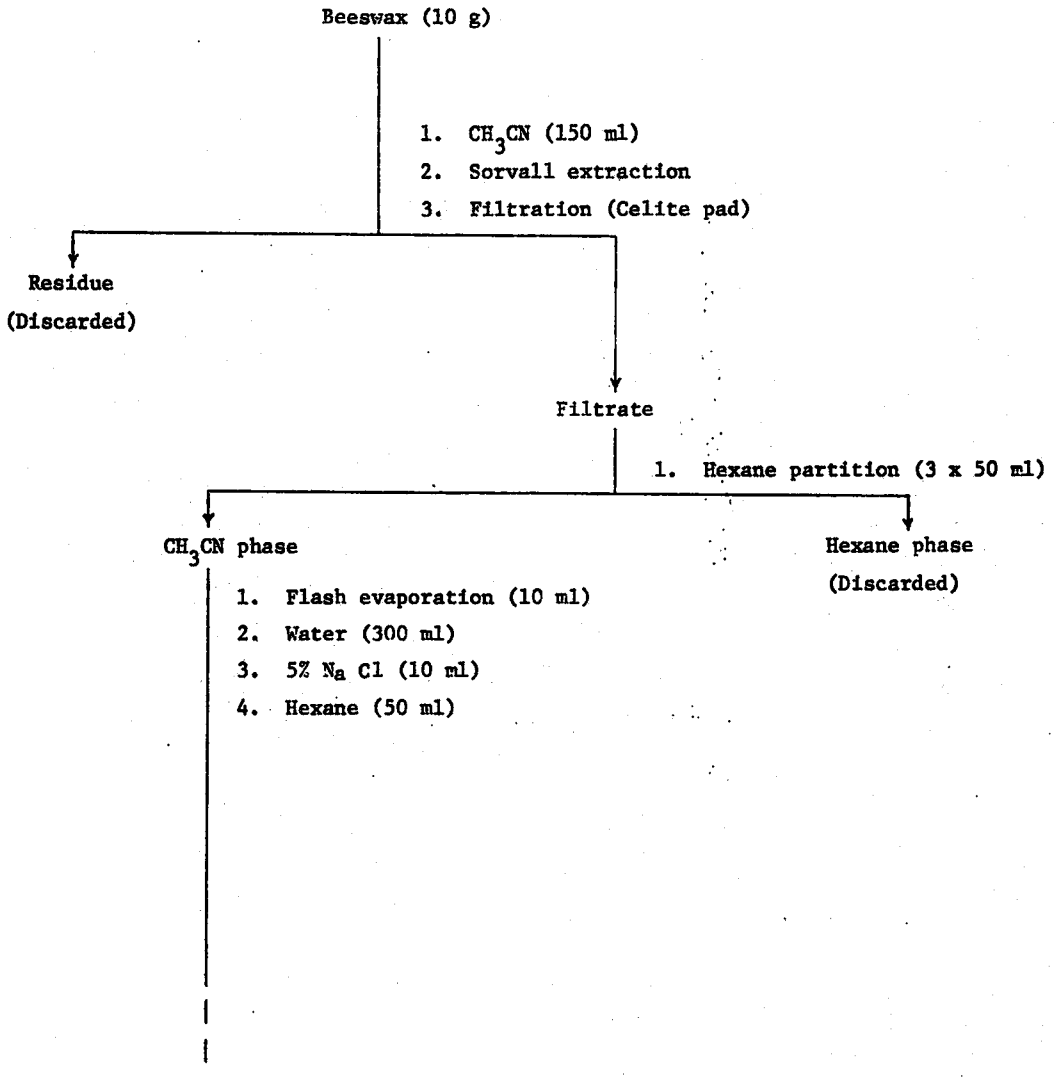


Fig. 1. Schematic representation of extraction, cleanup and separation procedures for fenitrothion residues in bees and pollen.


Beeswax (10 g)
1. CH_3CN (150 ml)
2. Sorvall extraction
3. Filtration (Celite pad)
Residue
(Discarded)
Filtrate
1. Hexane partition (3 x 50 ml)
CH_3CN phase
Hexane phase
(Discarded)
1. Flash evaporation (10 ml)
2. Water (300 ml)
3. 5% N_a Cl (10 ml)
4. Hexane (50 ml)

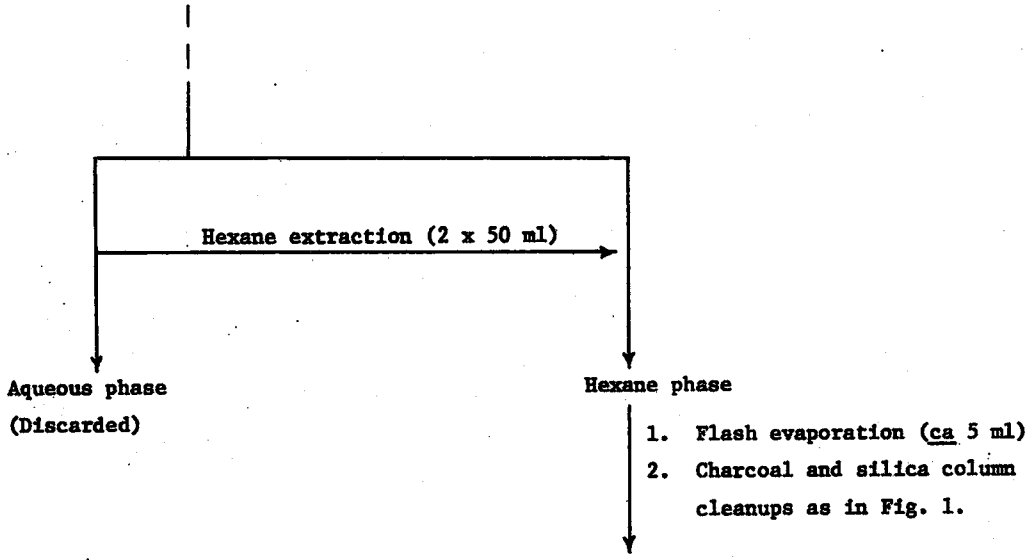


Fig. 2. Schematic representation of extraction procedure for fenitrothion residues in beeswax.

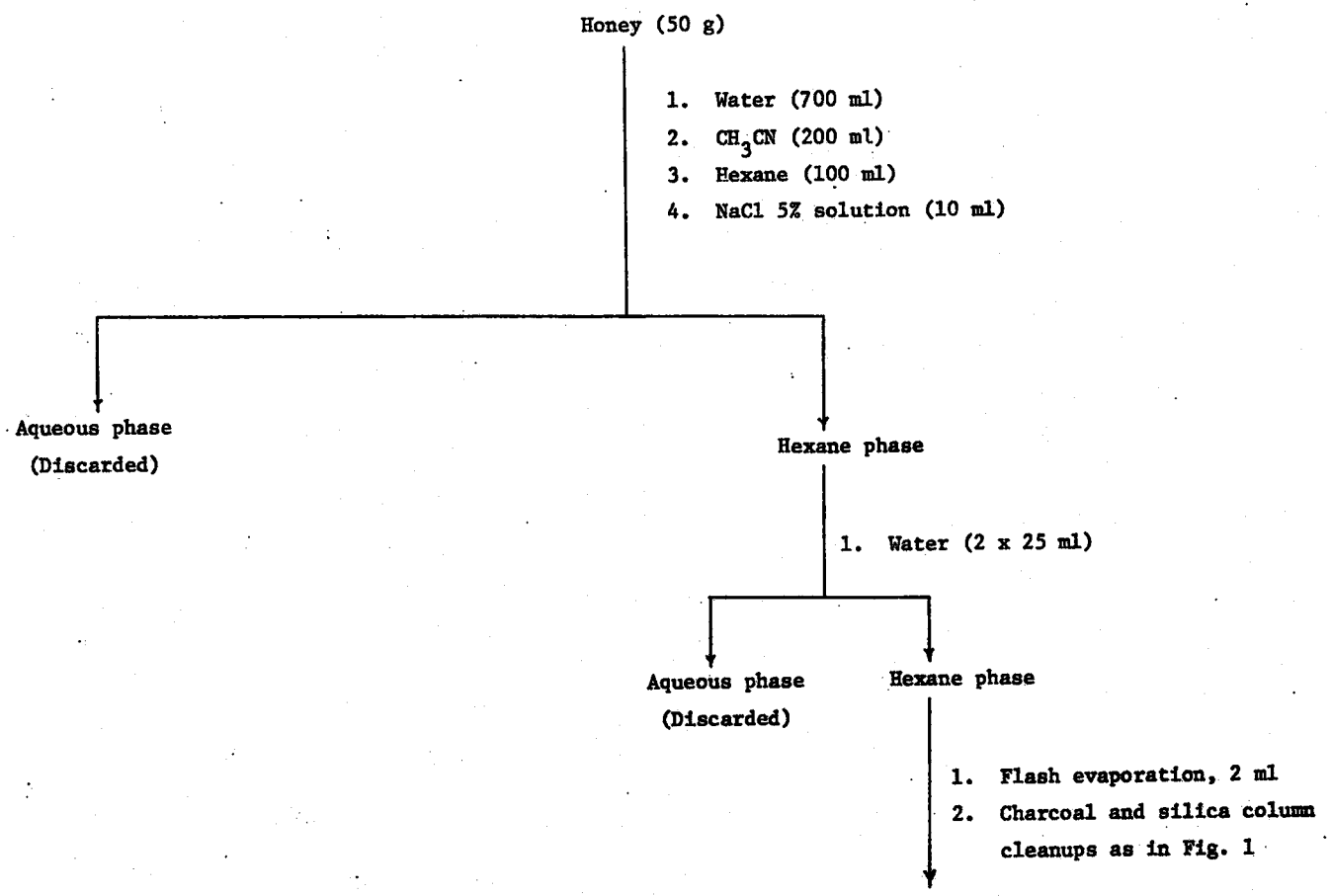


Fig. 3. Schematic representation of extraction procedure for fenitrothion residues in honey.

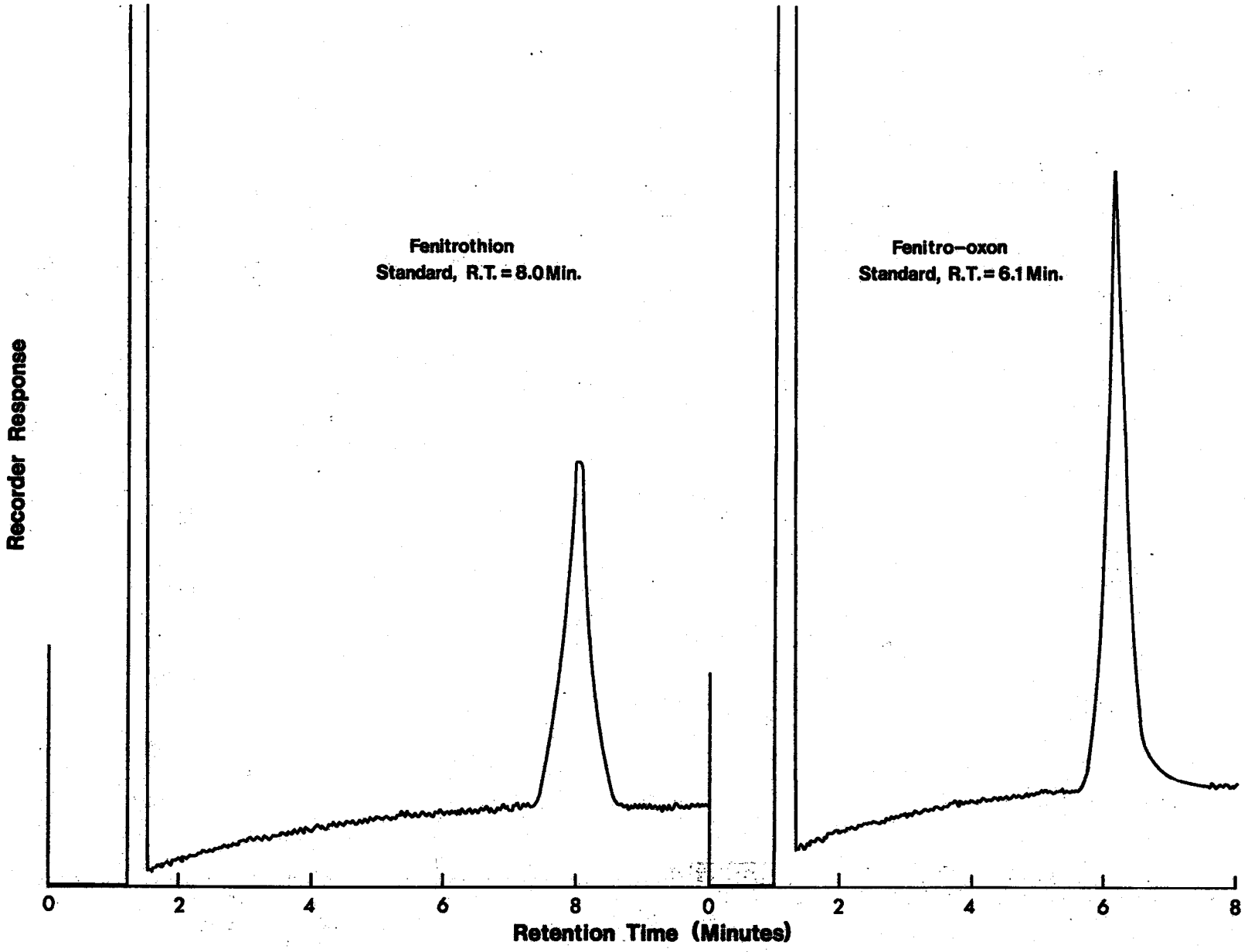


Fig. 4. Typical chromatograms of fenitrothion and fenitrooxon standards

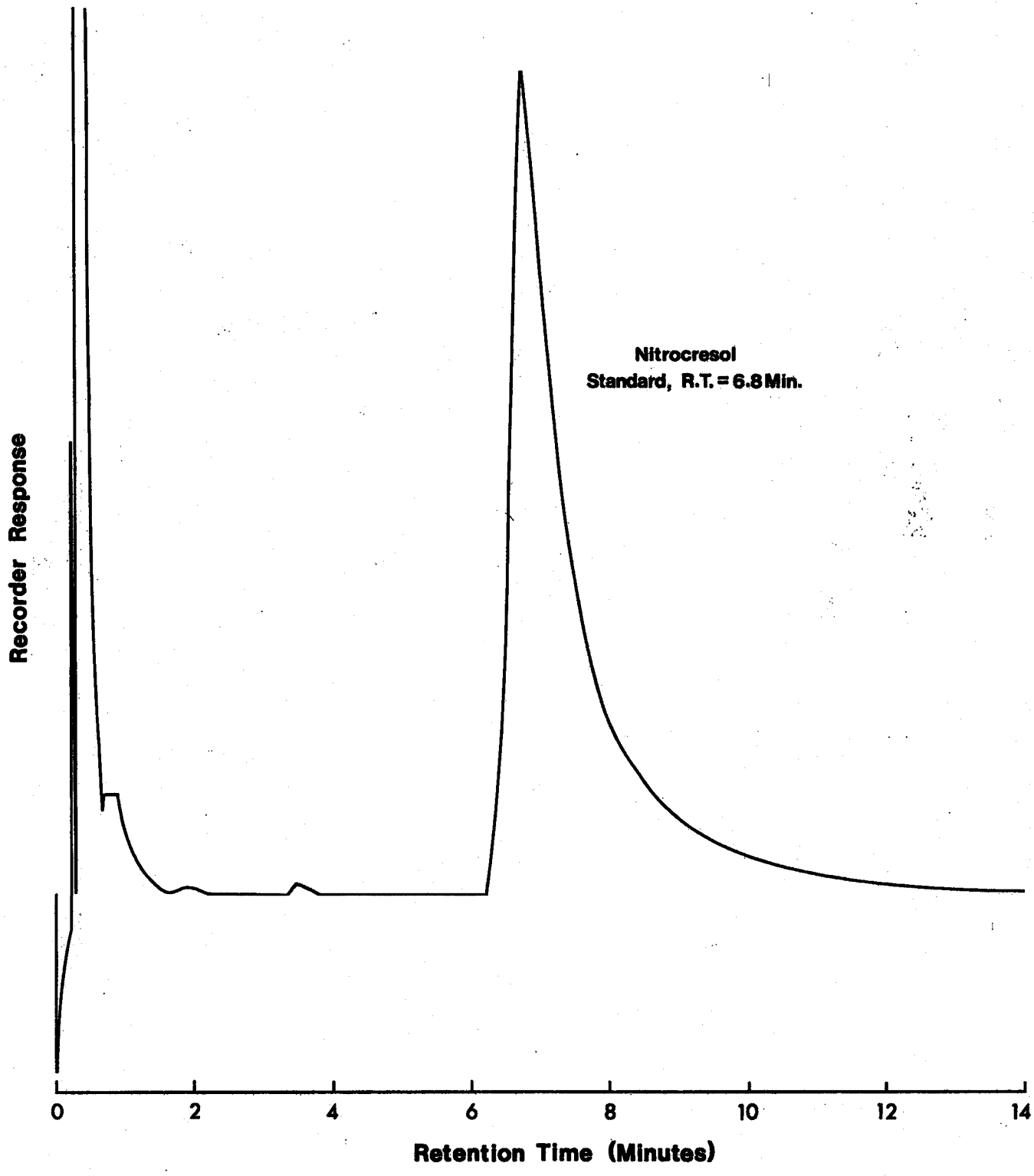


Fig. 5. Chromatogram of 4-nitrocresol standard

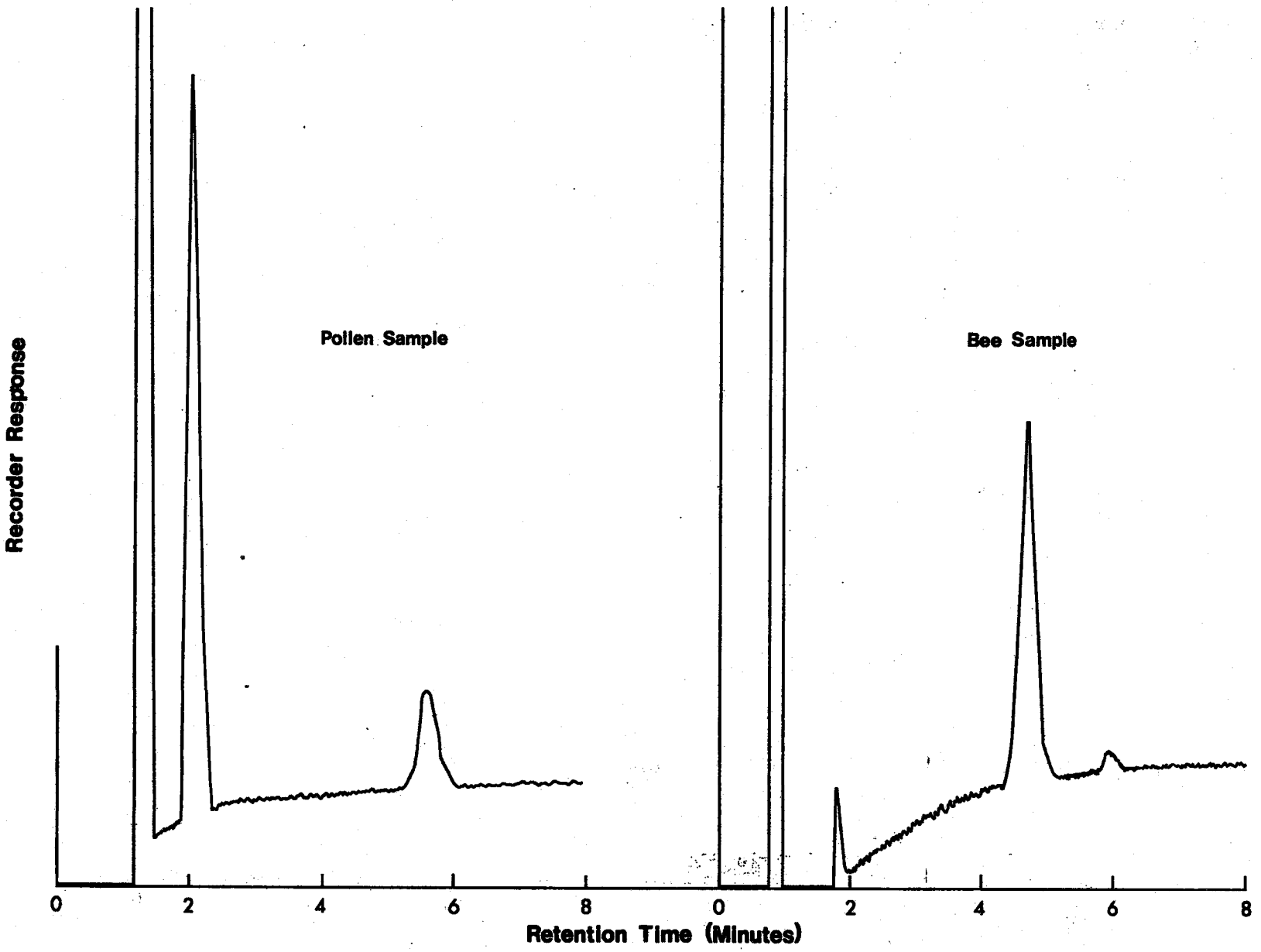


Fig. 6. Chromatograms of pollen and tree extracts after cleanup

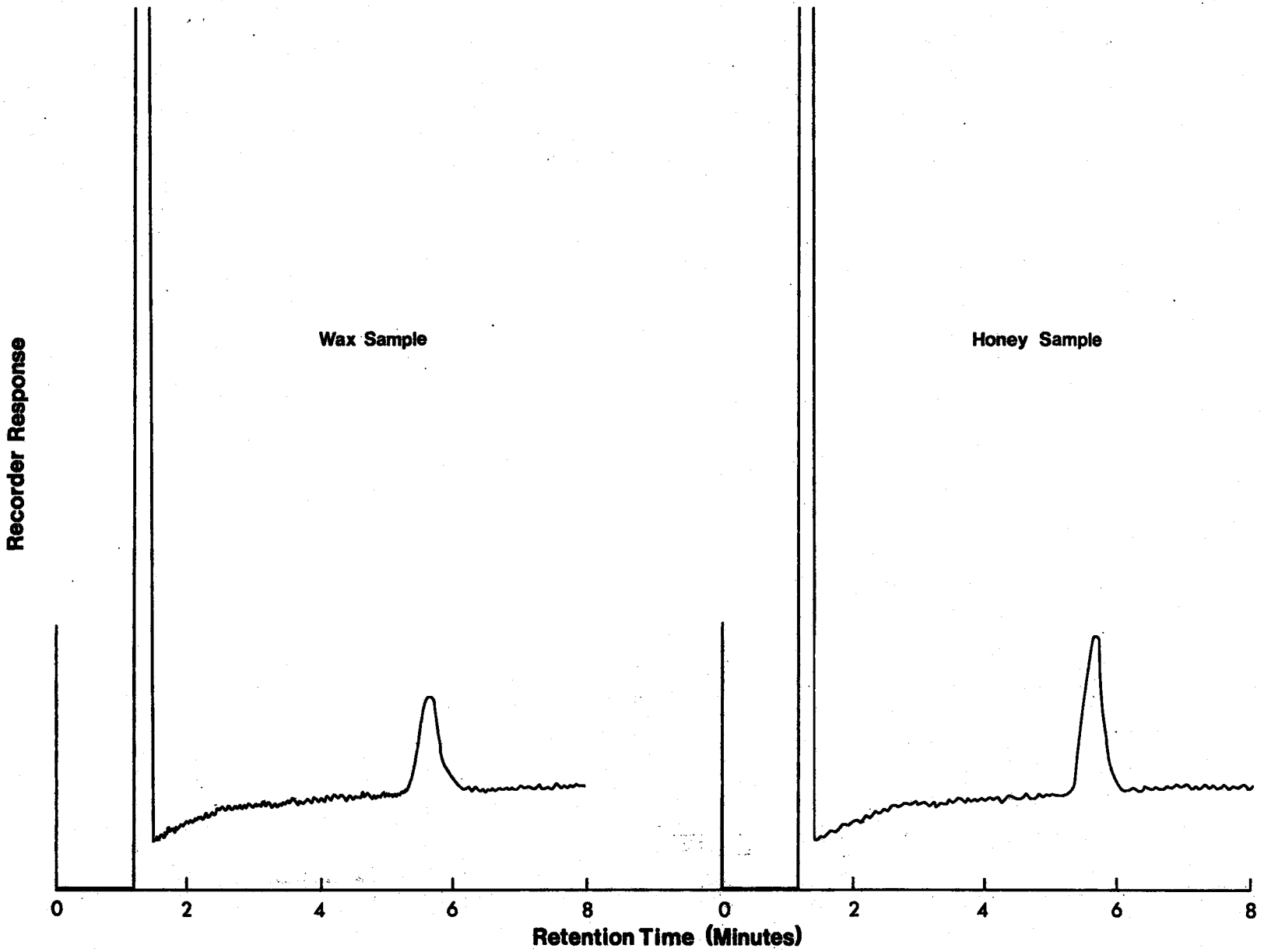


Fig. 7. Chromatograms of wax and honey samples after cleanup

RESULTS AND DISCUSSION

Development of Analytical Method

Recoveries of fenitrothion, its oxygen analog and the 4-nitro-m-cresol in honey bees and their products fortified with 2 to 10 µg/g are given in Table I. Average recovery for the insecticide ranged from 84% in bees to 93% in honey. The recovery of the oxon metabolite ranged from 80% in bees to 86% in honey and for the cresol the results were 78 and 87% respectively. Usually the recoveries of the three residues were high in honey (86 to 93%, average 89%) and comparatively low in bees (78 to 84%, average 81%). The low recoveries may be due to losses in the partition (hexane) and cleanup steps. Usually such losses become more evident at the lower fortification (2 µg/g) level. The GLC responses to fenitrothion, its oxon and the cresol standards are shown in Figs. 4 and 5. The retention times (RT) (min.) for the compounds were found to be 8.0 for the parent molecule, 6.1 for the oxon and 6.9 for the cresol. Similarly, chromatographic profiles for the extracts from bees and their products are illustrated in Figs. 6 and 7. The retention times are lower for fenitrothion (4.6 for bees and 5.8 for bee products) because of a variation (oven temp. increased) in the operating conditions of GC. The background interference in the chromatograms (Fig. 6) was small, showing that the extraction, separation and cleanup operations were adequate. An additional peak (RT 1.8 min.) in the final concentrate after the silica column cleanup appeared in some of the bees and pollen extracts (Fig. 6), the coextractive impurity causing this was eluted well in advance of the fenitrothion range, and did not interfere in

the analysis. An examination of the recovery data presented in Table I and the GLC responses illustrated in Fig. 6 indicates that the analytical method described here is accurate, reproducible and sensitive, suitable for estimating fenitrothion in bees and bee products. The lower limit of detection for fenitrothion in bees was 0.005 ppm showing that the method is sensitive enough to analyse for the low levels of the insecticide usually found in samples collected from the forest environment after spraying.

Good GLC responses (narrow symmetrical peaks) were also obtained for the insecticide and its oxon when aliquots of the concentrates of bee and pollen extracts after charcoal-Celite column cleanup were injected into the gas chromatograph fitted with 20% OV 101 column and FP detector. Under the GLC conditions specified, the background interferences were reasonably low and the reproductibility of peaks was satisfactory. If the cresol hydrolysis product was absent, the silica column cleanup was found to be unnecessary, thereby simplifying the analysis.

Fenitrothion Residues in Bees and their Products from Treated Area.

The GLC methods described here for the analysis of fenitrothion residues in spiked samples of bees and their products were used for analyzing similar samples received from sprayed areas of Larose Forest. The results of the analysis are shown in Table II. The concentration of the samples is expressed in units of ppm "as sampled" including moisture content as a variable. The chromatographic profiles of bees and pollen also contained the additional peak that was observed in some of the spiked samples of bees and

pollen. It did not interfere in the analysis of fenitrothion residues. Two determinations were made on each sample. Good precision was obtained on both analyses with low deviation from the mean (Table II). The parent compound was present, but no oxon or cresol derivatives were found in any of the substrate samples analysed from the treated plot.

The GLC methods used here for the analysis of fenitrothion residues in honeybees and their products are simple, rapid and reasonably precise and can readily be extended to residues of this insecticide in other insect materials.

SUMMARY

Bee, pollen, honey and beeswax samples were collected from control and treated plots from Larose Forest after spraying with fenitrothion for budworm control. Residues of the insecticide, its oxygen analog and its cresol hydrolysis product were determined in the collected samples from the control plot after spiking and extracting with acetonitrile followed by hexane partition and charcoal-Celite column cleanup. The parent compound and the two metabolites were separated by differential elution using a deactivated silica-gel column. Fenitrothion and its oxon, were determined by GLC using a flame photometric detector sensitive to phosphorus and the cresol by a Ni 63 electron-capture detector. Recoveries of the insecticide and its two metabolites from spiked substrates averaged over 80% and residues may be determined at levels as low as 0.005 ppm. No oxon or

cresol derivatives of the parent compound were found in any of the substrate samples analysed. The GLC methods described here for the analysis of fenitrothion residues in honeybees and their products were found to be practical, rapid and precise.

ACKNOWLEDGEMENTS

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