GAS CHROMATOGRAPHIC DETERMINATION OF PHOSPHAMIDON ISOMERS IN FOLIAGE, SOIL AND WATER

By

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INTRODUCTION

Phosphamidon ($C_{10}H_{19}ClNO_5P$; 2-chloro-N,N-diethyl-3-hydroxycrotonamide dimethyl phosphate) is a broad-spectrum systemic insecticide comprising a mixture of cis-(or β) and trans-(or α) isomers in the proportion 73:27. The major isomer, cis-phosphamidon is more toxic (strong cholinesterase inhibitor) to insects than the trans-isomer. The introduction and use of this insecticide for spruce budworm, Choristoneura fumiferana (Clemens), control in Canadian forests has resulted in the need for a rapid and sensitive analytical method to determine the toxicant in soil, water and foliage [spruce, Picea spp. A. Dietr] samples collected from sprayed forest regions. The present report describes a modified residue method using gas-liquid chromatography (GLC) similar to that used for fenitrothion [0,0-dimethyl-0-(4-nitro-m-totyl) phosphorothioate] analysis (Bowman and Beroza 1969, Yule and Duffy 1972, Sundaram 1974). The procedure is easy to follow, practical, has minimum interference, and is more precise and rapid than the earlier gas chromatographic methods reported in literature (Voss et al 1971, Westlake et al 1973) which involved laborious extraction and cleanup processes. The new method can be used readily in analysing large numbers of foliage, soil and water samples of forest origin that are usually received during widespread control operations.

MATERIALS AND METHODS

Experimental Design

The present study was undertaken specifically to develop a simple, rapid and sensitive analytical method to determine phosphamidon

in foliage, soil and water. Samples of spruce foliage and pond water collected from Larose Forest and soil (pH 4.50, moisture content 48%) from the Priceville area of New Brunswick were fortified with known amounts of chemically pure phosphamidon and used during the development of analytical methods. The insecticide was extracted from the spiked foliage and soil samples and determined according to the procedures developed by Voss et al (loc. cit.) (method B) and Westlake et al (loc. cit.) (method C). These results were used as the standard for comparison with the newly developed methods (method A) with regard to sensitivity, specificity, reliability and ease of operation. Phosphamidon was determined quantitatively from fortified water samples after extracting with chloroform, dichloromethane and toluene and the extraction efficiency of these three solvents was also compared.

Extraction Procedures

1. Spruce foliage

Foliage samples were processed according to the procedures described earlier (Sundaram 1973). In method 'A', the extraction and cleanup procedures were essentially the same as described for the analysis of fenitrothion residues from conifer foliage (Yule and Duffy 1972, Sundaram 1974).

Spiked samples were prepared by placing 20-gram lots of shredded foliage (Hobart vegetable shredder) in a Sorvall Omni-Mixer, 10 & 20 µg of analytical grade phosphamidon added and the lot homogenized for 5 min. at a speed setting 6 using 100 ml of ethyl acetate as solvent. The macerate was filtered under suction using Schleicher

and Schuell "Sharkskin" filter paper, rinsed with 25 ml of the solvent; the residue was then re-extracted as before. The combined extracts were filtered through a pad of anhydrous sodium sulphate (ca 75 g) and flash-evaporated to 5 ml. The residue was dissolved in 50 ml of acetonitrile and partitioned twice with 25 ml of hexane to separate the insecticide isomers from lipohilic plant constituents. The nonpolar layers were discarded after re-extraction with 25 ml of acetonitrile and the combined polar phase flash-evaporated to 10 ml; the concentrate was passed through a chromatographic column (2 x 30 cm) containing activated 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, Sundaram 1974). The column was eluted with 100 ml of 25% benzene in ethyl acetate (V/V) followed by 100 ml of benzene. The colourless eluate was flash-evaporated to a small volume and concentrated to 0.5 ml using a gentle stream of air and made up to a known volume with benzene for GLC analysis.

Since phosphamidon is completely miscible with solvents of high polarity, in one set of experiments acetonitrile was used for extraction instead of ethyl acetate for comparison (see the comments in Table II).

In experiment B, spiked foliage samples were extracted as described by Voss et al (1971) using acetonitrile for maceration. The insecticide isomers were transferred to aqueous phase by evaporation after adding water and partitioned thrice with chloroform. The organic phase was dehydrated with Na_2SO_4, evaporated repeatedly in hexane medium under reduced pressure and cleaned up by silica column chromatography. Instead of the recommended Woelm - grade I silica gel, 2 g of activated (150-160^{o}C) Fisher 3405 (80-200 mesh) silica was used (Table II). The

adsorbent column was first rinsed with 30 ml of 50% ethyl acetate in hexane (V/V) (discarded) and later eluted with 50 ml of ethyl acetate. The coloured eluate was flash evaporated to a small volume and concentrated to 0.5 ml using a gentle stream of air and made up to a known volume with benzene for GLC analysis.

Trial experiments varying the adsorbent (SiO_2) quantity and grade were carried out to study cleanup comparison, elution efficacy and recovery and the results are recorded in Table I.

The extraction and cleanup procedures used in method C are described by Westlake et al (1973). The foliage (20 g) samples were extracted twice in an Omni-Mixer with 100 ml of acetonitrile and 20 g of $Na_2 SO_4$. The combined extractives were flash evaporated to a small volume in presence of hexane followed by water. The aqueous residue was partitioned with hexane and extracted with chloroform. The extract was dried by passing through a column of anhydrous sodium sulphate and concentrated to a small volume. The concentrate was cleaned from interfering plant materials by passing through a chromatographic column containing alumina (8 cm length) (Fisher A 540, 80-200 mesh, activity grade II containing ca 5 wt % H_2O) (see the comments in Table II, column 4) and eluted with chloroform. The coloured solvent was flash evaporated carefully to dryness and dissolved in a small volume of ethyl acetate - hexane mixture for GLC analysis.

As in experiment B, studies involving the variation of quantity and activity of the alumina adsorbent were conducted to evaluate the overall efficacy of cleanup techniques.

2. Soils

For residue analysis of soils, 20 g of spiked samples were extracted and processed according to the methods of A, B and C as discussed under foliage and analysed for phosphamidon residues by GLC.

3. Water Samples

Two hundred millilitre volumes of distilled water samples were taken in 0.5 ℓ separatory funnels and spiked with 10 and 20 µg of phosphamidon to give concentrations of 50 and 100 ppb of the insecticide in the water. After adding 10 ml of saturated sodium sulphate solution, the samples were extracted by shaking vigorously for 3 min with 3 x 100 ml of chloroform, toluene and dichloromethane. The organic layers were collected after equilibration and dried by passing through columns of Na_2SO_4. The extracts were flash evaporated to small volumes and concentrated further by stream of air. The phosphamidon isomers were estimated by GLC without any cleanup due to the absence of noticeable background interferences.

Gas Chromatography

The gas chromatographic measurements were carried out with a Hewlett-Packard F and M 810 Research chromatograph equipped with a Melpar Flame photometric detector containing a 526 mµ optical filter for the detection of phosphorous and was operated at 160°C. Columns were made of borosilicate glass, 1200 x 3.5 mm (I D), packed with 20 percent OV-101 silicone fluid on DMCS treated, acid washed Chromosorb W diatomite support. The temperatures of the injection port, column and transfer line were 220, 200, 210°C respectively. Nitrogen was used as carrier gas at the rate of 60 ml/min (3.5 on rotometer). Hydrogen, oxygen and

air flow were 150, 20 and 50 ml/min respectively. Under the conditions of use, this column completely separated the cis and trans-isomers of phosphamidon which are present in the ratio of 73:27.

The gas chromatograph was standardized on the same day as the samples were analysed by injecting aliquots (1-6 μl) of freshly prepared standard solutions of phosphamidon (analytical grade supplied by Mr. B.J. Watt, Ciba-Geigy Canada Ltd.) in benzene, measuring the peak heights of the two isomers which were separated from each other under the GLC conditions applied, and preparing a calibration curve by plotting peak heights against ng insecticide on a log-log scale. Quantitation of the individual phosphamidon isomers extracted from foliage, soil and water were obtained by measuring each of the peak heights after injection of aliquots of extracts, under the same operating conditions, and reading the concentrations from their calibration curves. Extraction efficiencies of the two isomers are based on the total phosphamidon spiked and are expressed in percentages, and their average values representing the extraction efficiency, are reported as the total phosphamidon residues recovered from each foliage, soil and water samples analysed.

All organic solvents used in the recovery studies were either pesticide grade or freshly distilled in glass. The chemicals and glassware used were free from any detectable insecticide contamination.

TABLE I

Recovery* Percentages of Phosphamidon and Coefficient of Variations+

from Spiked Spruce Foliage Using Three Different Methods

Method	A				B				C			
Fortification Level (µg/20g)	Trans	Cis	Average	C.V.	Trans	Cis	Average	C.V.	Trans.	Cis	Average	C.V.
10	92	85	89	6	59	56	58	9	89	87	88	5
20	84	82	83	8	76	71	74	12	89	85	87	6

* Each value represents the average of three analytical replicates.

\+ C.V. = Coefficient of variation. [Standard deviation expressed as percentage of the mean].

TABLE II

Summary of the Three Methods Used for Recovery of Phosphamidon Residues from Spruce Foliage

Method / Steps	A	B	C
Extractant	Ethyl acetate	Acetonitrile	Acetonitrile
Partition	CH_3CN - hexane	Water - $CHCl_3$	Water - hexane
Cleanup column	Charcoal - Celite	Silica	Alumina
Elution	Benzene (25% V/V) in ethyl acetate (100 ml) followed by benzene (100ml)	Ethyl acetate (50%, V/V) in hexane (30 ml) followed by ethyl acetate (50 ml) [Volume adjusted according to the amount of adsorbent].	Chloroform (25 ml) [Volume adjusted according to the amount of Al_2O_3 used in the cleanup procedure]
Limit of detection	0.01 ppm	0.01 ppm	0.01 ppm
Recovery (%)	83-89	58-74	87-88
Comments	1) Acetonitrile as extractant gave the same result but the extracts were highly coloured. 2) Elution with $CHCl_3$ (200 ml) yielded a coloured solution. 3) Method is rapid and sensitive enough for routine analysis of large number of samples.	1) Flash-evaporation of acetonitrile from the aqueous phase was slow and time consuming, due to excess bumping of the boiling solution. 2) Re-extraction of the residues from aqueous phase using chloroform produced stable emulsions. Chloroform layer was highly coloured. 3) Two to ten gram samples of adsorbent silica was not sufficient to remove the coloured pigments and co-extractive impurities. Even 12 g of SiO_2 did not remove all the colour.	1) Strong emulsion formation and poor separation of phases were noted while re-extracting phosphamidon residues from the aqueous phase using chloroform 2) Recommend 3 cm Al_2O_3 (activity grade V) was not sufficient to remove all the co-extractive impurities in the spruce foliage. Eluate was highly coloured. Lower activity grade (Grade II, containing ca 5% H_2O) and higher amounts (8 cm column) were used in the cleanup. Eluate was coloured yellow. Chloroform being polar, brought down all the coloured pigments, but did not interfere in the end determination.

TABLE II - (Cont'd)

Summary of the Three Methods Used in the Analysis of Phosphamidon Residues in Spruce Foliage

Method / Steps	A	B	C
		4) Different eluting solvents (ethyl acetate, hexane, benzene and their mixtures) were used but none was satisfactory. 5) Final recovery of phosphamidon isomers was low 6) Method is complicated, laborious and slow compared to A.	3) Method is tedious and time-consuming.

TABLE III

Percent Recovery Values of Phosphamidon from Spiked Soil Samples Using Three Different Extraction and Cleanup Methods*

Method	A				B				C			
Levels of fortification (µg/20g)	Trans	Cis	Average	C.V.	Trans	Cis	Average	C.V.	Trans	Cis	Average	C.V.
10	71	69	70	8	74	70	72	9	76	78	77	7
20	74	75	75	10	71	66	69	11	74	70	72	9

* See the footnotes in Table I.

TABLE IV

Percentages Recovery of Phosphamidon

from Water Using Different Solvents*

Solvent	Chloroform				Toluene				Dichloromethane			
Fortification Level (µg/200 ml)	Trans	Cis	Average	C.V.	Trans	Cis	Average	C.V.	Trans	Cis	Average	C.V.
10	126	113	120	6	119	114	117	9	76	89	83	18
20	112	110	111	8	118	107	113	10	99	102	101	16

* See the footnotes in Table I.

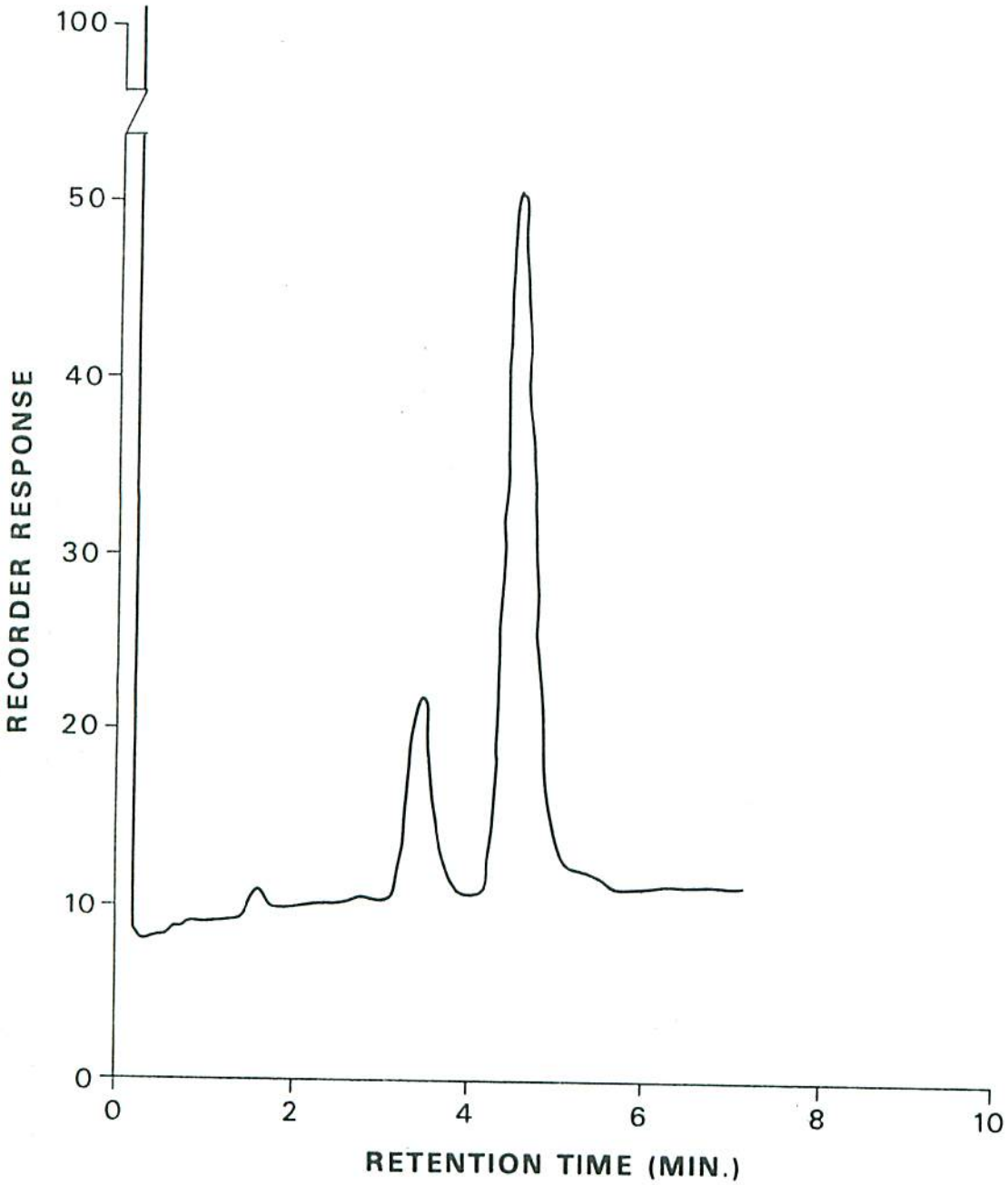

Fig. 1. Gas chromatogram of 15 ng of phosphamidon standard. Trans-isomer: R.T. 3.30 min, peak height 2.8 cm , cis-isomer: R.T. 4.64 min , peak height 10.0 cm. Relative retention times: cis 1.00, trans 0.71.

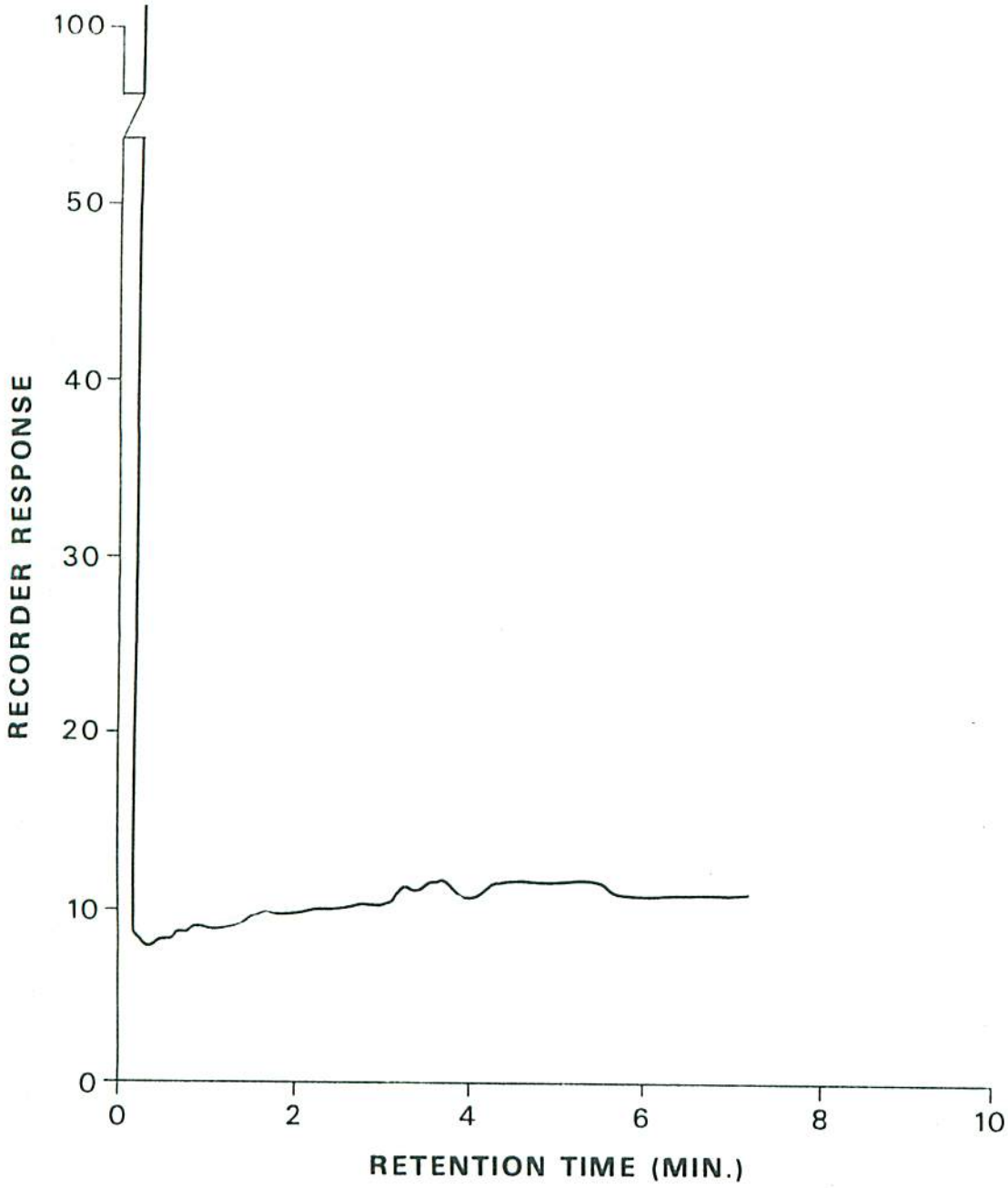

Fig. 2. Gas chromatogram of an extract of untreated spruce foliage after charcoal-Celite column cleanup.

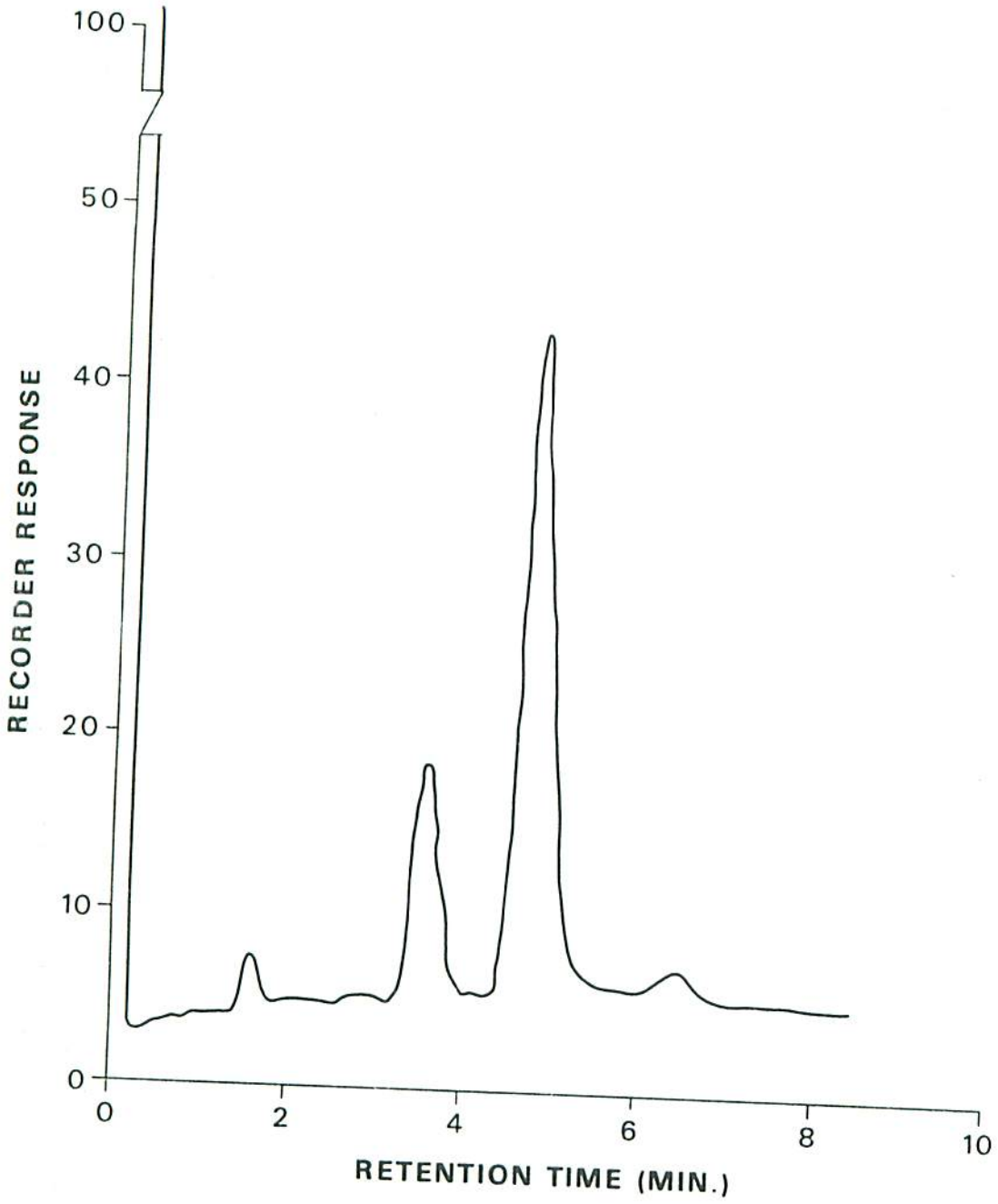

Fig. 3. Gas chromatogram of an extract of spruce foliage fortified with phosphamidon before extraction at 0.5 ppm level: charcoal-Celite column cleanup.

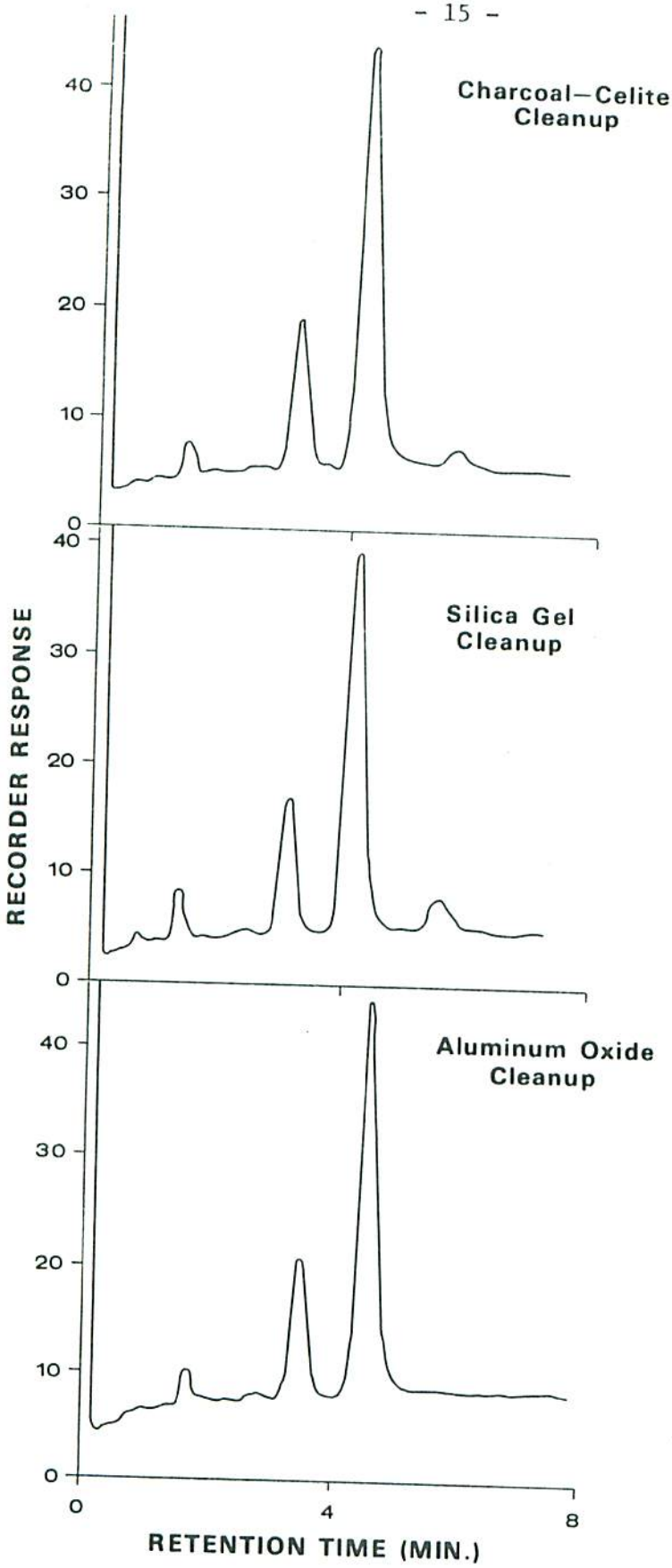

Fig. 4. Chromatograms of foliar extracts of spruce fortified with phosphamidon at 0.5 ppm level after different column cleanups.

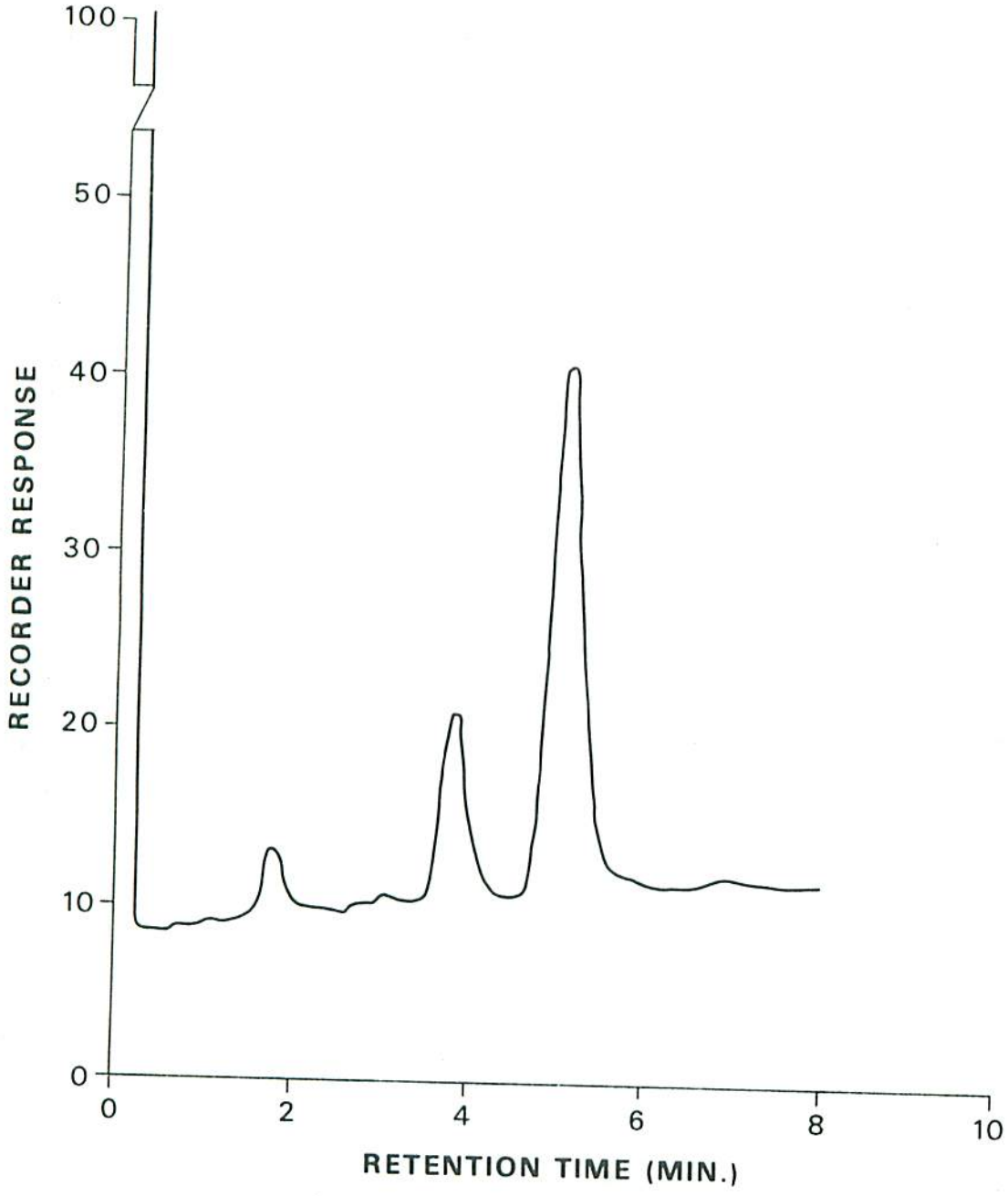

Fig. 5. Gas chromatogram of an extract of soil sample fortified with phosphamidon after charcoal-Celite column cleanup.

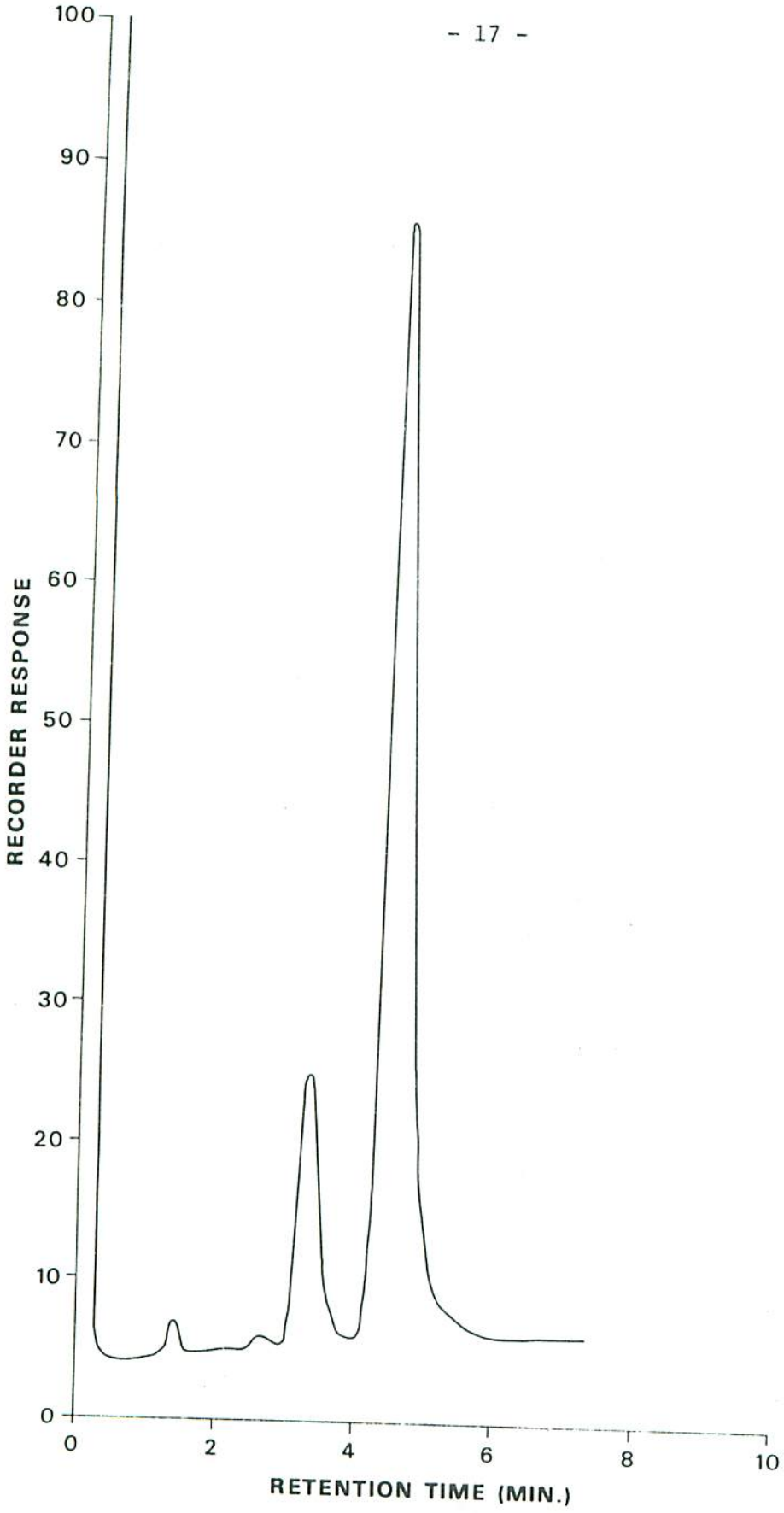

Fig. 6. Gas chromatogram of chloroform extract of water fortified with phosphamidon.

RESULTS AND DISCUSSION

Percent recoveries of the phosphamidon isomers from fortified spruce foliage, using the three methods described above are given in Table I. The three methods of analysis employed are summarized in Table II along with the comments on sensitivity, reliability and ease of operation. The average recoveries of the insecticide isomers were more than 85% with coefficients of variation ranging from 5 to 8 in methods A and C; method B gave recovery of 66% with maximum coefficient of variation (12). The low reliability and recoveries obtained by the method B may have been due to the unavoidable substitution of non-standard silica for recommended Woelm activity grade I in the column cleanup process. Currently the recommended silica gel for adsorption chromatography is available and the experiments will be repeated. Procedure C is specific and sensitive showing an average recovery of 88% with a limit of detection of 0.01 ppm of phosphamidon in spruce foliage. Unfortunately, the method is by far the most time-consuming and laborious and cannot be used in practice for routine analysis of large numbers of samples usually received during forest spray operations.

The gas chromatographic method using the charcoal-Celite column cleanup appears to be quite promosing for qualitative and quantitative determinations of phosphamidon isomers. The method is comparatively simple, accurate and sensitive, suitable for routine analysis of large numbers of samples. The average recoveries from spiked spruce foliage ranged from 83 to 89%, (mean 86%) with coefficient of variations between 6 to 8 (Table I) and the limit of detection of 0.01 ppm in the

concentration range 0.5 to 0.0 ppm, i.e. 10 to 20 µg of phosphamidon per 20 g foliage.

Phosphamidon is a systemic insecticide, so it is likely that a fraction of the spiked material would have penetrated into subcuticular layers through dissolution in aqueous and lipophilic constituents present in spruce foliage. Extraction using a homogenizer such as an Omni-Mixer in the presence of a polar extractant such as ethyl acetate or acetonitrile is necessary to recover the maximum insecticide residues from the foliar tissues. Acetonitrile is more polar than ethyl acetate, and thus extracts more readily the pigments, waxes and other hydrophilic polar plant constituents in addition to the insecticide residues. This causes additional problems such as column poisoning in cleanup operations. Consequently, the choice of ethyl acetate is recommended over acetonitrile as the extractant for phosphamidon isomers from conifer foliage samples to minimize co-extractive impurities such as terpenes and pigments present in the substrate. The acetonitrile-hexane partitioning step removed the bulk of the plant waxes and non-polar impurities in the hexane phase leaving the insecticide residues in the polar phase. Carbon-Celite column adsorption chromatography provided efficient cleanup and removed nearly all the interfering impurities present in the foliar extract. This method of column cleanup was used successfully by Brewerton (1963) prior to spectro-photometric identification of phosphamidon residues in apples. A mixture of benzene in ethyl acetate was used for elution, followed by benzene whereas Voss et al (1971) used ethyl acetate in hexane followed by ethyl acetate in their method using a silica column.

Several different column packings have been reported (Voss et al 1971, Carlstrom 1972) for measuring phosphamidon by GLC. Only one ill-defined and broad peak was detected by a phosphorus detector using 5% OV-1 column whereas the OV-101 silicone fluid as the stationary phase coated on Chromosorb W gave a quantitative separation of both the isomers (Fig. 1) and was used in the present investigation. The GLC response to phosphamidon isomers is shown in Fig. 1. The retention times for the trans and cis isomers were : 3.30 and 4.64 min respectively; the relative retention time taking the cis-isomer as 1 was 1.00:0.71 and the ratio of peak heights assuming the cis-isomer as 1 was 1.00:0.28. During the analysis it was noticed that fenitrothion and its oxon had nearly similar retention times as phosphamidon isomers and could not be determined simultaneously under the gas chromatographic conditions used. In such situations, a simple hexane-water partition is recommended leaving fenitrothion and its oxygen analog in the hexane phase and the isomers of phosphamidon in the aqueous phase. Chromatographic profiles for the unspiked and spiked foliar extracts are illustrated in Figs. 2 and 3. The background interference in the chromatograms was small, showing that the extraction, separation and cleanup operations used in method A (Table 2) were adequate. A comparison of the chromatograms obtained by the three different column cleanups used during the methodology development is given in Fig. 4. The charcoal-Celite column cleanup is comparable to the other two in removing the co-extractive impurities that respond to the phosphorus detector thereby giving minimum background interference.

The three methods used for the extraction, cleanup and quantitation of phosphamidon isomers present in spiked spruce foliage samples were extended to forest soils and the results are summarized in Table III. The average recovery values obtained are 73 ± 9% for method A, 71 ± 10% for B and 75 ± 8% for C. Although the column cleanups were satisfactory with minimum GLC interferences (Fig. 5), the methods have uncertain reliability for phosphamidon isomers due to the low recovery values (ca 73%). The consistent low values obtained in all three methods could be attributed to the strong adsorptive interactions between the soil matrix and the polar amido group of the insecticide molecule as well as to its possible metabolic breakdown in the soil substrate. Extension of the procedures to soils demonstrated the need for the introduction of modifications to the present experimental conditions to increase the recovery levels of the insecticide from soil samples.

Phosphamidon isomers found in spiked water samples are recorded in Table IV. Chloroform and toluene were found to be good extractants for the insecticide from water giving quantitative recoveries. Chloroform is more polar and is the better solvent for extraction of phosphamidon from water, in spite of emulsion formation, and is recommended for future use as an extractant. Good GLC response (Fig. 6) were also obtained for chloroform extracts and further cleanup was found to be unnecessary due to low background interferences. Dichloromethane, although easy to handle and work with (no emulsion problems, distinct and rapid phase separations, etc.), gave an average recovery of only 94% with maximum (17%) coefficient of variation. Compared to the 100% recoveries in chloroform and toluene, the dichloromethane

values are low perhaps due to its solubility (ca 2%) in water.

SUMMARY

A simple and sensitive GLC method involving solvent extraction, partitioning, charcoal-Celite column cleanup and final determination is described for phosphamidon isomers in conifer foliage. The insecticide was determined by a flame photometric (phosphorus mode) GLC using a column containing 20% OV-101 silicone resin coated on Chromosorb W. The new method is compared with two other procedures reported recently with regard to sensitivity, specificity and ease of operation. Recovery values were 86 ± 7% with a sensitivity of 0.01 ppm at the residue level of 0.5 to 1.0 ppm. Because of its ease of operation and sensitivity, the method is recommended for quantitative determination of phosphamidon isomers present in conifer foliage. Extension of the procedure to soil samples gave recoveries slightly over 70% and needed modifications in the experimental method. Recovery studies of phosphamidon using fortified water samples showed that chloroform was preferable to toluene and dichloromethane.

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