

A NEW COLUMN CLEANUP TECHNIQUE FOR
THE ESTIMATION OF PHOSPHAMIDON RESIDUES
IN FOREST ENVIRONMENTAL SAMPLES

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ABSTRACT

A new cleanup technique has been developed for the extraction of phosphamidon from forest foliage and soil samples. The technique involves the use of Norit[®] (S.G. Extra) decolorizing carbon - Celite as the adsorbent and a mixture of benzene in ethyl acetate as the eluting solvent. The mean percent recoveries of the insecticide from fortified foliage and soil samples were quantitative. The technique is suitable for substrates which give highly coloured and pigmented extracts.

RESUME

Une colonne de Norit[®] (S.G. Extra) - Celite est utilisée pour le nettoyage d'extraits de phosphamidon dans les feuillages et le sol. L'éluant est un mélange de benzène et d'acétate d'éthyle. Le pourcentage de recouvrement est quantitatif. La technique est utile pour des substrats très colorés et pigmentés.

INTRODUCTION

Among the important organophosphorus insecticides effective against spruce budworm, *Choristoneura fumiferana* (Clemens), phosphamidon [2-Chloro-3-(diethylamino)-1-methyl-3-oxo-1-propenyl dimethyl phosphate] has been widely used in recent years in forest spray programs in Canada (Sundaram 1975) because of its wide spectrum of biological activity and rapid degradation in the environment (Dever and Davis 1960, Martin and Worthing 1974). The compound exists as a *cis* (or β) and *trans* (or α) pair in the proportion 73:27 and the major isomer is more toxic (strong cholinesterase inhibitor) to target species than the *trans*-isomer. Its chemical, physical, biological and analytical properties have been described by Gunther and Gunther (1971). The cleanup techniques recommended by previous workers (Voss *et al* 1971, Westlake *et al* 1973) for the estimation of phosphamidon residues present in plant and animal materials are commonly employed. While analysing for the insecticide residues from forest environmental samples after phosphamidon spraying, the author found that the cleanup techniques recommended for the insecticide isomers were not only cumbersome and time consuming but also gave inconsistent recoveries in extracts of coniferous and deciduous foliage. Moreover, the cost of adsorbents such as alumina (neutral, activity grade V) and silica gel (Woelm, activity grade I) and different solvents used in these techniques is high especially with the large numbers of foliage and soil samples usually received during widespread insect control operations. In view of the above, a rapid and improved cleanup procedure requiring comparatively less expensive adsorbent and solvent system has been developed.

METHOD

Apparatus and Reagents

- | | |
|--------------------------------|---|
| Grinder and Mixer | - Hobart Food Chopper and Sorvall Omni-Mixer. |
| Evaporative Concentrator | - Buchler Flash-Evaporator. |
| Liquid Chromatographic Columns | - 2 x 30 cm Shell type Pyrex glass column with reservoir, sealed-in coarse fritted disc to support column packing and teflon stopcock for column control. |
| Decolorizing Carbon | - J.T. Baker Norit [®] SG Extra (Used as received, without treatment). |
| Sodium sulphate (anhydrous) | - Fisher S-421, heated overnight to <i>ca</i> 200°C, cooled and stored in air-tight bottles. |
| Celite | - Fisher 545, manufactured by Johns Manville. |
| Glass syringe | - Hamilton syringes, 10 ul size. |
| Solvents | - Acetonitrile, benzene and ethyl acetate are pesticide grade solvents obtained from Caledon Laboratories Ltd. |
| Gas Chromatograph | - Hewlett-Packard model F and M 810 equipped with a flame photometric detector containing 526 mμ optical |

filter for phosphorus.

Preparation of Extract

1. Foliage

Coniferous and deciduous foliage samples were removed from twigs by scissors and chopped in a Hobart machine. By quartering, a 10 g representative sample in triplicate was taken, known amounts of analytical grade phosphamidon were added and homogenized with 100 ml of ethyl acetate in a Sorvall Omni-Mixer for 5 min. at speed 6. The macerate was filtered under suction using Schleicher and Schuell "Sharkskin" filter paper and the residue washed with 25 ml of the extractant. The extract was filtered through a column of anhydrous sodium sulphate (ca 25 g) to remove the water present, the column rinsed with 10 ml of the solvent and flash-evaporated to ca 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 lipoid and terpenoid materials present in foliage. The hexane layers were discarded after re-extraction with 25 ml of acetonitrile and the combined acetonitrile phase was flash-evaporated to ca 10 ml for column cleanup.

2. Soil

The bulk forest soil samples (ca 3 kg), was mixed thoroughly by hand after removing large stones and debris by sifting. A composite sample of ca 200 g was sieved (# 8 mesh, 2.4 mm opening) and 10 g aliquots were used in phosphamidon recovery studies. The Sorvall extraction, partition and concentration steps used were similar to the foliage samples discussed above.

Column Cleanup

The liquid chromatographic column was prepared by placing a glass wool plug in the bottom of the column followed by 10 g of anhydrous sodium sulphate. An intimate mixture of 10 g of charcoal and Celite (3:2 by weight) was added slowly to the column under suction and it was topped finally with 10 g of Na_2SO_4 . The column was rinsed with 50 ml of benzene and allowed to drain slowly to the top of the column. The foliar and soil extracts were transferred quantitatively to the column and eluted with 200 ml of 25% benzene in ethyl acetate. The eluate was flash-evaporated to a small volume and concentrated using a gentle stream of dry air and made up to a known volume with benzene for gas chromatographic analysis.

Gas Chromatographic Determination

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 nm 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 (2-4 μ l) of freshly prepared standard solutions of phosphamidon (analytical grade supplied by Mr B.J. Watt, Ciba-Geigy Canada Limited) 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 fortified foliage and soil samples 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 different foliage and soil samples analysed. Results are given in Tables 1 and 2.

Table 1

Recovery of total phosphamidon from fortified foliar extracts*

| Foliar Type | Phosphamidon Concn. (ppm) | | Recovery (%) | Average Recovery (%) |
|--|---------------------------|----------------------|-------------------|----------------------|
| | Added | Found | | |
| Balsam fir [<i>Abies balsamea</i> (L.) Mill] | 0.50 1.00 1.50 | 0.45 0.94 1.36 | 90 94 91 | 92 |
| Eastern white pine [<i>Pinus strobus</i> L.] | 0.50 1.00 1.50 | 0.46 0.91 1.40 | 92 91 93 | |
| Red pine [<i>Pinus resinosa</i> Ait.] | 0.50 1.00 1.50 | 0.44 0.90 1.38 | 88 90 92 | |
| White spruce [<i>Picea glauca</i> (Moench) Voss] | 0.50 1.00 1.50 | 0.44 0.91 1.41 | 88 91 94 | 91 |
| Eastern white cedar [<i>Thuja occidentalis</i> L.] | 0.50 1.00 1.50 | 0.45 0.91 1.40 | 90 91 93 | |
| Red maple [<i>Acer rubrum</i> L.] | 0.50 1.00 1.50 | 0.43 0.87 1.28 | 86 87 85 | |
| Sugar maple [<i>Acer saccharum</i> Marsh] | 0.50 1.00 1.50 | 0.40 0.84 1.30 | 80 84 87 | 84 |
| White birch [<i>Betula papyrifera</i> Marsh] | 0.50 1.00 1.50 | 0.48 0.99 1.47 | 96 99 98 | |
| White elm [<i>Ulmus americana</i> L.] | 0.50 1.00 1.50 | 0.51 1.05 1.52 | 102 105 101 | |
| Balsam poplar [<i>Populus balsamifera</i> L.] | 0.50 1.00 1.50 | 0.49 0.98 1.49 | 98 98 99 | 98 |

* Each value is the mean of 3 replicates.

Table 2

Recovery of total phosphamidon from fortified forest soil*

| Soil | Phosphamidon Conc. (ppb) | | Recovery (%) | Average Recovery (%) |
|---|--------------------------|-------|--------------|----------------------|
| | Added | Found | | |
| Larose Forest soil (fine sandy loam) | 0.5 | 0.47 | 94 | 98 |
| | 1.0 | 1.02 | 102 | |
| | 1.5 | 1.48 | 97 | |
| | 2.0 | 1.95 | 98 | |

* Each value is the mean of 3 replicates.

RESULTS AND DISCUSSION

The recoveries of phosphamidon insecticide obtained in different spiked foliage and soil samples using the charcoal-Celite column cleanup technique are given in Tables 1 and 2. The average recoveries of the insecticide in conifer foliage ranged from 90 to 92% whereas comparatively low values (average 85%) were obtained from maple foliage samples due to the interferences caused probably by the presence of excess co-extracted polysaccharide impurities. The final cleaned up extracts of maple foliage used in GLC analysis were turbid and milky and all attempts to remove the impurities were

unsuccessful and also resulted in the loss of the insecticide. Mean percentage recoveries of phosphamidon in birch, elm and poplar foliage samples varied from 98 to 102 and the final concentrates were relatively free from co-extractive impurities including lipid materials that were found in conifers.

The use of acetonitrile as an extractant of phosphamidon from forest foliage samples was tried. The solvent is more polar than ethyl acetate (μ 3.37 D* *vs* 1.81 D, see Weissberger *et al* 1967) and extracts more readily the pigments, waxes, terpenes and other hydrophilic polar plant constituents in addition to the insecticide. 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 foliage samples of forest origin.

The new column cleanup technique for phosphamidon was extended to forest soil substrates and the results are summarized in Table 2.

* Dipole moments of molecules are expressed in Debye (D) units.

The average recovery values obtained were high and quantitative ranging from 94 to 102% giving a mean value of 98%. The consistent high values obtained for the soil samples demonstrated that the new column cleanup technique is not only applicable for highly pigmented extracts but also for forest soils. The charcoal-Celite column removed effectively nearly all the co-extractive impurities present in foliage and soil samples that responded to the phosphorus detector thereby giving minimum background interference except probably in maple extracts.

Using similar carbon column chromatography cleanup techniques Brewerton (1963), Watts *et al* (1969) and Beckman and Garber (1969) have reported satisfactory recoveries for various organophosphorus pesticide residues present in agricultural products. Morley (1966) has reviewed the use of carbon column cleanups in 1960's for residue analysis. The new cleanup technique described here for phosphamidon residues in forest environmental samples is rapid, efficient, economical and sensitive. Because of these advantages, the cleanup method is recommended as a standard analytical step in the determination of phosphamidon residues present in forest foliage and soil samples especially while analysing large numbers of them received during the spray season.

SUMMARY AND CONCLUSIONS

A new adsorbent column cleanup chromatographic technique has been developed for the extraction of phosphamidon residues from the foliage of conifers and deciduous trees and forest soil samples. The technique involves the use of Norit® (SG Extra) decolorizing carbon-Celite mixture (3:2 w/w) as the adsorbent and a solution of benzene in

ethyl acetate (1:3 v/v) as the eluting solvent. The mean percent recoveries of phosphamidon from foliage extracts fortified at levels of 0.50 - 1.50 ppm varied from 90 to 103 while the maple extracts gave slightly lower values (85%). Extension of the procedure to soil samples gave quantitative recoveries. Because of its ease of operation, economy and sensitivity, the technique is recommended for quantitative determination of phosphamidon present in different forest environmental samples which give highly pigmented extracts.

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