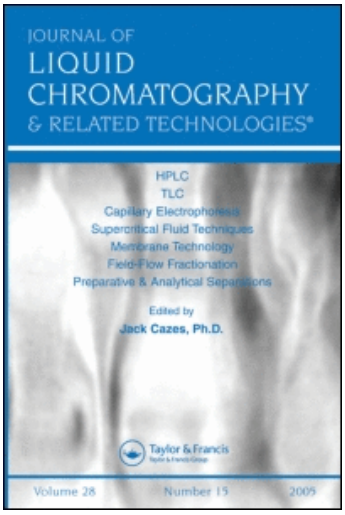

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DETERMINATION OF ORGANOPHOSPHORUS PESTICIDE RESIDUES IN CARROT USING GEL PERMEATION CHROMATOGRAPHY

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ABSTRACT

To investigate the application of gel permeation chromatography to the determination of organophosphorus pesticides in carrot, phorate, dimethoate, pirimiphos-methyl, chlorfenvinphos, quinalphos and triazophos were chosen. Samples were extracted with ethyl acetate and the extracts clean-up by gel permeation chromatography using Bio-Beads SX-3 gel. The high pigment content of samples caused band broadening on the column.

INTRODUCTION

The extraction and partition steps of standard multi residue methods for pesticides provide a degree of clean-up in food matrix. However, food samples contain a wide variety of compounds that are extractable and may give rise to interferences or other problems. The most universally applicable clean-up procedure is gel permeation chromatography or size exclusion chromatography. It uses a macroreticular resin matrix that progressively retards the elution of

compounds based on the inverse of molecular size. Large lipids, and polymeric co-extractives, elute earlier than most pesticides. The technique was first applied by Stalling et al.[1] to the cleanup of organochlorine and polychlorinated biphenyl residues in fatty foods.

Specht and Tillkes [2] developed a GPC system (column: 25mm ID x 40cm long) using polystyrene resin Biobeads SX-3 with cyclohexane-ethyl acetate (1:1) as mobile phase, which was suitable for clean-up of a wide range of pesticides in various foods.

The wide applicability of Bio-Beads SX-3 is indicated by its use in the determination of organophosphorus pesticides residues in fruit and vegetables,[3] in cereals, cereal products and animal feed[4] and in quantitative detection of the pesticides in plant and animal tissues.[5]

The main disadvantages are; -lower molecular weight co-extractives often elute in the pesticide fraction and the separation of the large pesticides is incomplete. For example, interferences from the food matrix can be reduced by increasing the dump fraction, but large pesticides, which elute early, are also progressively removed.[6]

The aim of this work was to investigate the application of gel permeation chromatography to the determination of organophosphorus pesticides in carrots, six pesticides were chosen. Compounds representative of the pesticides which have been used on carrot were; phorate, dimethoate, pirimiphos-methyl, chlorfenvinphos, quinalphos and triazophos.

MATERIALS AND METHODS

The pesticides used as test substances were obtained from Dr. Ehrenstorfer, Augsburg (Germany). Bio-Beads SX-3 (200-400 mesh) was purchased from Bio-Rad Laboratories (UK). Samples of the organically-grown carrots were provided by a health food shop in local market.

Gel Permeation Chromatography (GPC)

Column: Anachem glass preparative chromatography column (45cm x 1cm ID) fitted with a polytetrafluoroethylene (PTFE) bed support and adjustable plunger.

Sample introduction: Rheodyne PTFE rotary valve fitted with a 1mL sample loop.

Solvent delivery system: Kontron Model 420 HPLC pump, flow rate 1mL/min.

Elution solvent: Cyclohexane-Ethyl acetate (1:1).

Column preparation: An appropriate amount (30g) of Bio-Beads SX-3 was placed in a 500mL conical flask and an adequate volume of elution solvent (200mL) was added. The gel was left to stand for 24h and fully swollen gel was packed into the column. After the gel had settled, the plunger was depressed until a bed height of 35cm was obtained. Elution solvent was pumped through the column at a flow rate of 1mL/min for 2h prior to use.

Gas Chromatography (GC)

A Carlo Erba 4200 gas chromatograph equipped with a flame ionization detector (FID) and 25m x 0.32mm ID fused silica column containing a 0.5 micron film thickness of BP-1 non-polar bonded phase was operated under the following conditions; Helium carrier gas flow rate was 2mL/min, injector and detector temperatures were 250°C and 260°C respectively, the oven temperature programmes were; 1) initial temperature isothermal at 150°C for 2 min, and from 150°C to 230°C at 15°C/min, then 20 min at 230°C, 2) initial temperature isothermal at 100°C for 5 min, and from 100°C to 260°C at 5°C/min, then 20 min at 260°C. The samples were injected in the split mode with a split ratio of 30:1. Results were collected using a Spectra Physics 4270 integrator.

Preparation of Samples

Extraction

Chopped carrot (50g) were placed in the spark-proof blender and ethyl acetate (50mL) and sodium sulphate (30g) added and then homogenized at high speed for 3min. The homegenate was filtered and re-extracted with further ethyl acetate (20mL). This step was repeated and the extracts were combined. The combined extracts were concentrated to approximately 20mL in vacuo using a rotary evaporator.

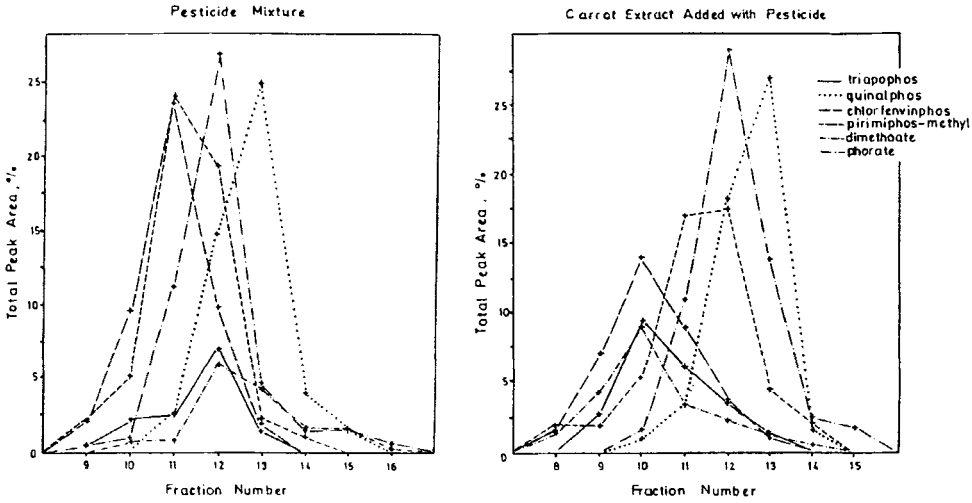

Figure 1. Elution Profiles of the Pesticides from the Bio-Beads SX-3 column.

Fortification

Carrot extract (20 mL), which contained no pesticide, was fortified by adding a known volume of the standard mixture solution of the pesticides. The concentration of the pesticides was 0.2 mg/mL.

Pesticide Elution Profile

A standard mixture solution (0.2mg/mL) of pesticides prepared in cyclohexane:ethyl acetate (1:1). A 1 mL aliquot of the standard mixture solution was injected onto the column and twenty 2mL fractions of the column eluate were collected. The GPC column was washed for a further 10 min before the next sample was introduced. The total run time was 50 min.

Each fraction was reduced to 0.1 mL using a stream of N_2 and then analysed using GC to determine the amount of pesticide pesent. The same procedure was carried out on the fortified carrot extract.

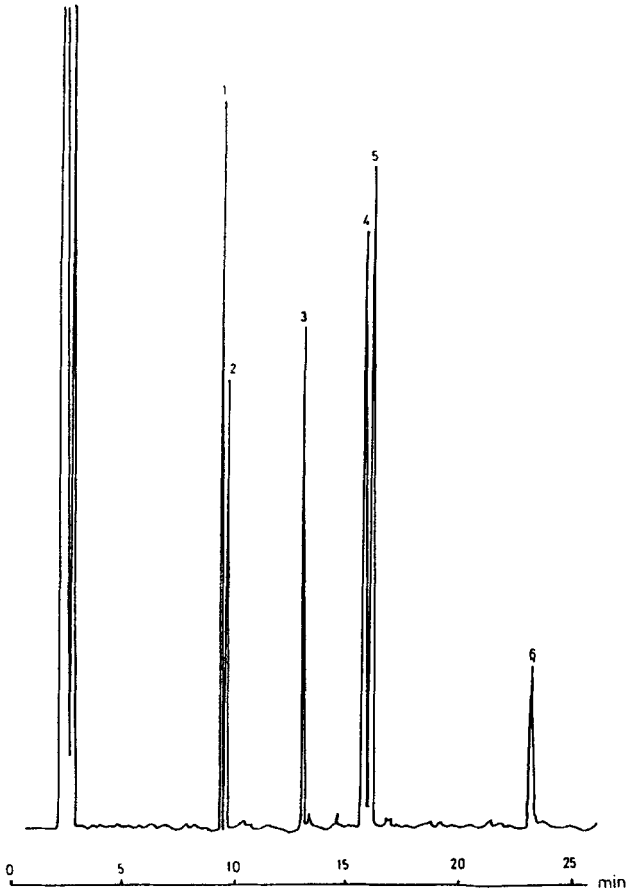

Figure 2. Separation of six OP pesticides on a BP-1 fused silica capillary column. Temp. prog: 150°C for 2 min., 15°C min^{-1} to 230°C, Peaks: 1. Phorate, 2. Dimethoate, 3. Pirimiphos-methyl, 4. Chlorfenvinphos, 5. Quinalphos, 6. Triazophos.

RESULTS AND DISCUSSION

The fractions from the GPC column containing pesticides are shown in Figure 1. The peak areas of the pesticides, which were obtained from the GC integrator, were plotted against the fraction number. The GC chromatogram of the pesticide mixture obtained with a 25m BP-1 non-polar column is given in Fig. 2.

Table 1

Elution Profile of the OP Pesticides from a Bio-Beads SX-3 GPC Column

Pesticide	Elution Volume for Pesticides mL	Volume Fraction No	Elution Volume for the Fortified Carrot Extract, mL	Volume Fraction No
Phorate	15 - 33	8 - 17	17 - 31	9 - 16
Dimethoate	17 - 33	9 - 17	13 - 29	7 - 15
Pirimiphos-methyl	15 - 29	8 - 15	13 - 29	7 - 15
Chlorofenvinphos	15 - 27	8 - 14	13 - 27	7 - 14
Quinalphos	19 - 31	10 - 16	17 - 29	9 - 15
Thiazophos	15 - 27	8 - 14	145 - 29	8 - 15

The elution volume required from the Bio-Beads SX-3 column for each of the pesticides in the standard mixture solution and in fortified carrot extract are given in Table 1. For each pesticide, there was a volume range in which the compound eluted from the GPC column. The separation between the pesticides was incomplete since their molecular weights were close to each other. Chamberlain (1990) has reported a similar elution profile for some organophosphorus pesticides for cereals using a Bio-Beads SX-3 GPC column.

The 'dump volume' was determined as 15 mL and the 'collect volume' was determined as 20 mL from the graph in which is shown the elution profile of pesticides from carrot extracts. The elution volumes of pesticides from the GPC column demonstrated a small difference between the pesticide mixture alone and the carrot extract which is fortified by pesticides. Separation between the co-extractives and the pesticides was incomplete and the co-extractives from the carrot caused band broadening on the column.

This effect arose from relatively high pigment content of carrot, particularly β-carotene which is the dominant pigment in carrot. To reduce the pigment effect, a less concentrated carrot extract, equivalent to 1 g/mL (50g. of carrot was homogenised with 50 mL ethyl acetate and the homogenate was washed twice with 20 mL of ethyl acetate, then the extract was concentrated to ca. 40 mL instead of 20 mL), was injected onto the column, but the broadening was not completely prevented.

Interferences from the carrot could be reduced by increasing the dump fraction but large pesticides, such as chlorfenvinphos, which elute early, would also be progressively removed. Separation mechanisms other than size exclusion, i.e. adsorption and partition, may also be involved. The prevalence of one type of the mechanism over the other is largely determined by the mobile phase and packing pore size. With Bio-Beads SX-3 gel (2000 molecular weight exclusion limit), both size exclusion and adsorption occurred in the presence of the poorly solvating mobile phase. The technique provided adequate clean-up and further clean-up was not required before applying onto the gas chromatography column.

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