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GEL PERMEATION CHROMATOGRAPHIC CLEAN-UP OF ORGANOPHOSPHORUS PESTICIDE IN OIL MATRIX

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Liquid-liquid partition chromatography is widely used as a clean-up procedure for organophosphorus pesticides from various matrices. However, gel permeation is now increasingly used for multi-pesticides determination. This investigation evaluated the gel permeation chromatography (GPC) for clean-up of monocrotophos in an oil matrix.

The gel used in this study was Bio-Beads SX-3 and the GPC solvent system cyclohexane:ethyl acetate (1:1 v/v). The analyte was determined in a gas chromatograph fitted with a flame photometric detector in the phosphorus mode. Recovery of monocrotophos was 74.3%. 101.6% with a standard deviation of 3.409-13.453. The method is used for monitoring monocrotophos in edible oil.

INTRODUCTION

GPC is widely used in analysis of biochemical samples. Stalling *et al. (1972)* reported a GPC clean-up technique using the polystyrene gel, Bio-Beads SX-2, and cyclohexane solvent. They separated lipids from fish extract with good recovery of pesticides. Following this, they developed, in 1974, another system, Bio-Beads SX-3 and toluene-ethyl acetate, for quantitative recoveries of non-ionic chlorinated pesticides and polychlorinated biphenyl compounds. Guinivan *et al.* (1981) used preparative GPC to clean-up extracts of Southern Pea vines containing chlorpyrifos and its major metabolites. The extract was chromatographed on a Bio-Beads SX-3 column and the separated fractions analysed by gas chromatography with electron capture detection. Recoveries of chlorpyrifos and 3,5,6-trichloro-2-pyridinol were 100% for levels of 0.2 ppm and above, and 62% for levels between 0.01 and 0.2 ppm. A rapid method developed by Hopper (1981) used OPRVA-2000

with methylene chloride-acetone system to determine standard pesticides such as organochlorines, PCBs, etc., in fortified bacon fat. Recoveries for nine of the standard pesticides ranged from 93%.103%. Another system, based on Bio-Beads SX-3 with methylene chloride-n-hexane by Hopper (1982), was used to determine industrial chemicals and organophosphates and chlorinated pesticides recovered from fats. With this system, the recoveries of industrial chemicals, organophosphates and chlorinated pesticides from butterfat ranged from 96%-106% with less than 1% fat in the pesticide fraction. Bottomley and Baker (1984) developed a multi-residue method based on partition chromatography using dichloromethane and an acidic aluminium oxide column. They reported recoveries of organophosphorus pesticides from fortified grain between 72%-120% for barley and 54%-129% for wheat. An automated GPC method by Daft et al. (1990) involved loading the sample onto the system by means of a small volume displacement pump, which is an improvement over manual syringe loading. Chamberlain (1990) used Bio-Beads SX-3 with a dichloromethane-hexane system to determine organophosphorus, organochlorine, synthetic pyrethroid pesticides and insect growth hormone regulators in cereal products. The mean recovery for organophosphorus was 99%, organochlorine 94%, synthetic pyrethroid 99% and insect growth regulators 99%.

The effiiciency of Bio-Beads SX-3 with an organic solvent to separate multi-pesticide residues, especially organophosphorus, from their co-extractives is obvious from the work cited above. This prompted us to investigate the use of Bio-Beads SX-3 for determining organophosphorus pesticides in this case monocrotophos residues in palm oil. Monocrotophos is a fast acting insecticide with both systemic and contact action. It is sold as colourless hygroscopic crystals with a melting point between 54°C-55°C/0.005 mm Hg. The density is 1.33 g ml^{-1} and it has a solubility in water of 1 kg kg^{-1} at 20°C. The dosage rates against lepidopterous larvae range from 5-1000 and it persists for 7-14 days. The acceptable daily intake (ADI) for man is 0.006 mg kg^{-1}. In the oil palm plantation, monocrotophos is used against insects such as caterpillars and bagworms of Lepidoptera. Coleoptera, Hymenoptera, Orthoptera and Hemiptera also damage oil palm in the nursery (Hartley, 1977). An outbreak of insect attack can result in severe crop losses and therefore insecticides need to be applied.

The objective of this work is to evaluate gel permeation chromatographic clean-up technique for the determination ofmonocrotophos residues in an oil matrix. The system used in this study was based on the Chamberlain (1990) system of Bio-Beads SX-3 with the elution system replaced with cyclohexane:ethyl acetate (1:1 v/v).

EXPERIMENTAL

Apparatus

The GPC column used was an advanced glass purification column model AP-1 glass, 45 x 1 cm ID with adjustable plunger (Waters, Milford, USA).

GPC Elution Solvent System

Cyclohexane:ethyl acetate (Merck, Germany) (1:1 v/v).

Stock Solution

A 0.001 g monocrotophos (Riedel-De-Haen, Germany) was dissolved in 50 ml GPC elution solvent to make a stock solution of 20.0 µg ml^{-1}.

Working Standard Solution

A 1.0 ml of the stock solution was diluted with cyclohexane:ethyl acetate 1:1 (v/v) to obtain a monocrotophos concentration of 1.0 µg ml-i. For calibration, appropriate dilutions of the working solution of 1.0 µg ml^{-1} were made to give the following monocrotophos concentrations: 0.04, 0.06, 0.08, 0.40, 0.60 and 0.80 µg ml^{-1}. Concentration of 1.2 µg ml-' was obtained by diluting the stock solution with the GPC elution solvent.

Gas Chromatographic Analysis

A 1.0 µl of monocrotophos standard solution

was injected into the gas chromatograph (Hewlet Packard, Palo Alto, CA) fitted with DB-1 megabore column of length 30 m x i.d. 0.53 mm (Folsom, California) and a flame photometric detector in the phosphorus mode. The gas chromatograph operating conditions were as follows: injector and detector temperature 250°C; oven initial temperature 90°C; carrier gas helium at a flow rate of 20 psi and total flow ± 30 ml min^{-1}; detector gas pressures of 20 psi for hydrogen and 50 psi for air. The temperature profile was a programmed rise from 90°C (initial holding time of 6 min) to 200°C (and held for 6 min) at 6°C min^{-1}. The equilibration time was 0.5 min.

Working Solution

A 10 ml of 20.0 µg ml^{-1} stock solution was diluted with the GPC elution solvent to obtain a monocrotophos solution of 10.0 µg ml-'.

Spiked Oil for Recovery Studies

A 2.5 g of RBD palm olein was weighed into a 25 ml volumetric flask and 3.0 ml of the *10* µg ml^{-1} working solution added. The mixture was diluted with the elution solvent to make up to 25 ml. The concentration of monocrotophos in the spiked oil sample was 1.20 µg ml-'. A 2.0, 1.0, and 0.2 ml of the working solution were diluted with the elution solvent to obtain solutions with monocrotophos concentrations of 0.80, 0.40, and 0.08 µg ml^{-1}, respectively.

GPC Column Preparation

A 50 g of the packing material Bio-Beads SX-3 (Bio-Rads, Richmond, USA) -were suspended in 100 ml elution solvent for 24 hr. The gel was then transferred into a vacuum flask and degassed for 5 min. The slurry was packed into the GPC column and, after the bed had settled, the plunger adjusted (or depressed) to give a bed height of approximately 20-35 cm.

GPC Clean-up

The GPC - a multisolvent delivery system model 600 E (Waters, Milford, USA) -was used to clean-up the monocrotophos residue. The layout is shown in *Figure 1.* The column was

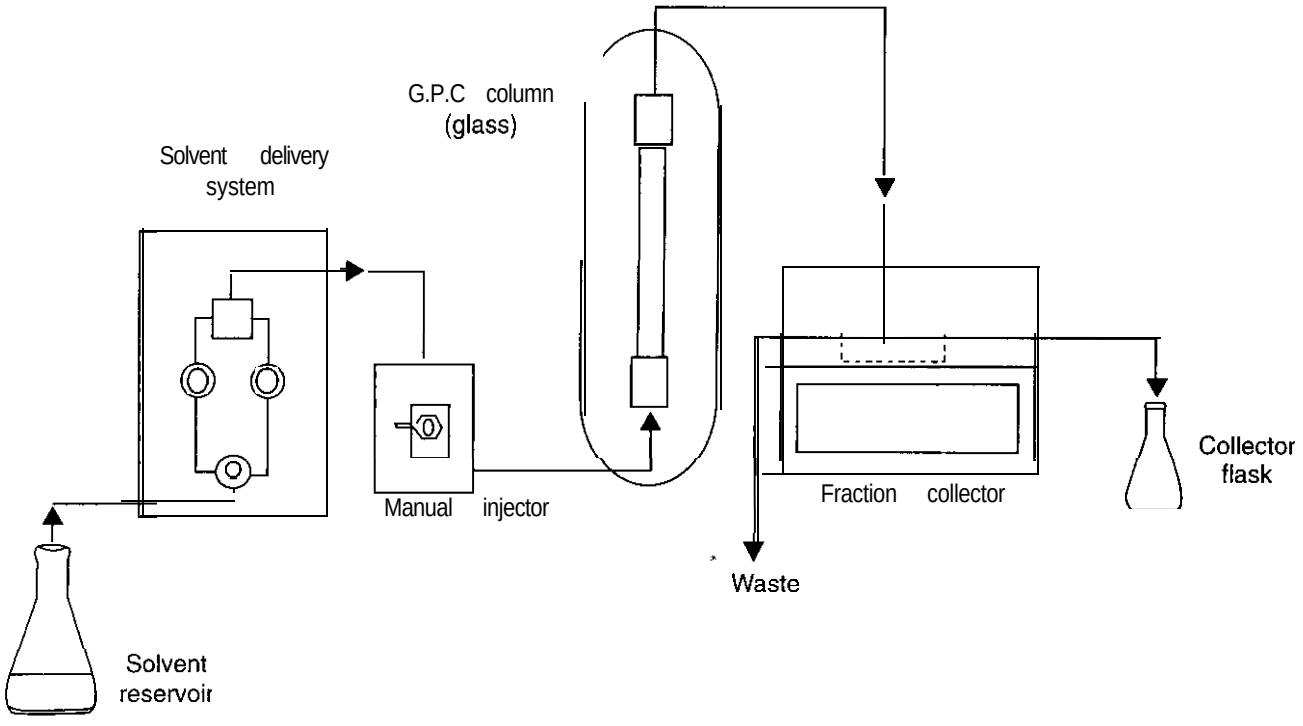

Figure 1. The fluid path of the GPC clean-up system for pesticide residue in oil matrix.

eluted with the GPC elution solvents for 30 min. Following this, 10.0 ml of the 0.08 μg ml^{-1}, monocrotophos- oil mixture was injected into the Rheodyne injector (California, USA) using a syringe fitted with a PTFE filter. A 5.0 ml of the mixture were injected into the column (the remaining 5.0 ml being diverted into a waste flask). The elution solvent was pumped through the column at a constant flow rate of 2.0 ml min^{-1}. A 5.0 ml of the clean-up eluate were collected in a 250 ml round bottomed flask. The solvent was evaporated to 5.0 ml and 1.0 μg of the concentrated eluate was injected into the gas chromatograph for determination. The remaining monocrotophos-oil mixtures were analysed in a similar manner.

RESULTS AND DISCUSSION

Although monocrotophos was completely eluted from the column by the first 20-30 ml of eluate, 50 ml of eluate were nevetheless collected. Gas chromatographic analysis of the eluate gave a chromatogram with a single monocrotophos peak. The retention time for standard monocrotophos was 22.936 min as shown in *Figure* 2. *Figure* 3 shows the chromatogram of recovered monocrotophos at 0.08 μg ml^{-1}. The absence of other peaks in the gas chromatogram indicated that the system Bio-Beads SX-3 with a solvent system of cyclohexane: ethyl acetate was effective in separating monocrotophos from the pure oil matrix. The calibration and recovery data were calculated based on the peak areas at different concentrations.

Calibration Data

Table 1 shows the calibration data based on three triplicate injections of standard monocrotophos. The coefficient of variation was less than 10% for concentrations of 0.08, 0.80 and 1.2 μg ml-', and between 10% and 20% for the other levels. This variation is within experimental error. Even though the R^2 of 0.9573 is slightly low, it is within the 95% confidence limits

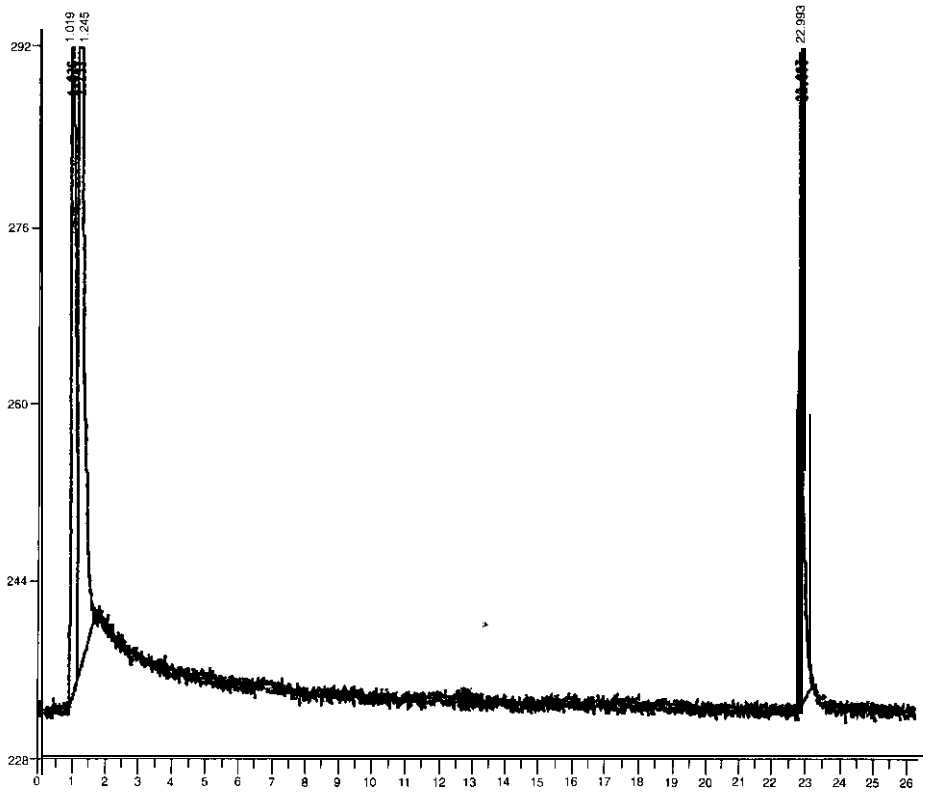

Figure 2. Gas chromatographic peak of standard monocrotophos.

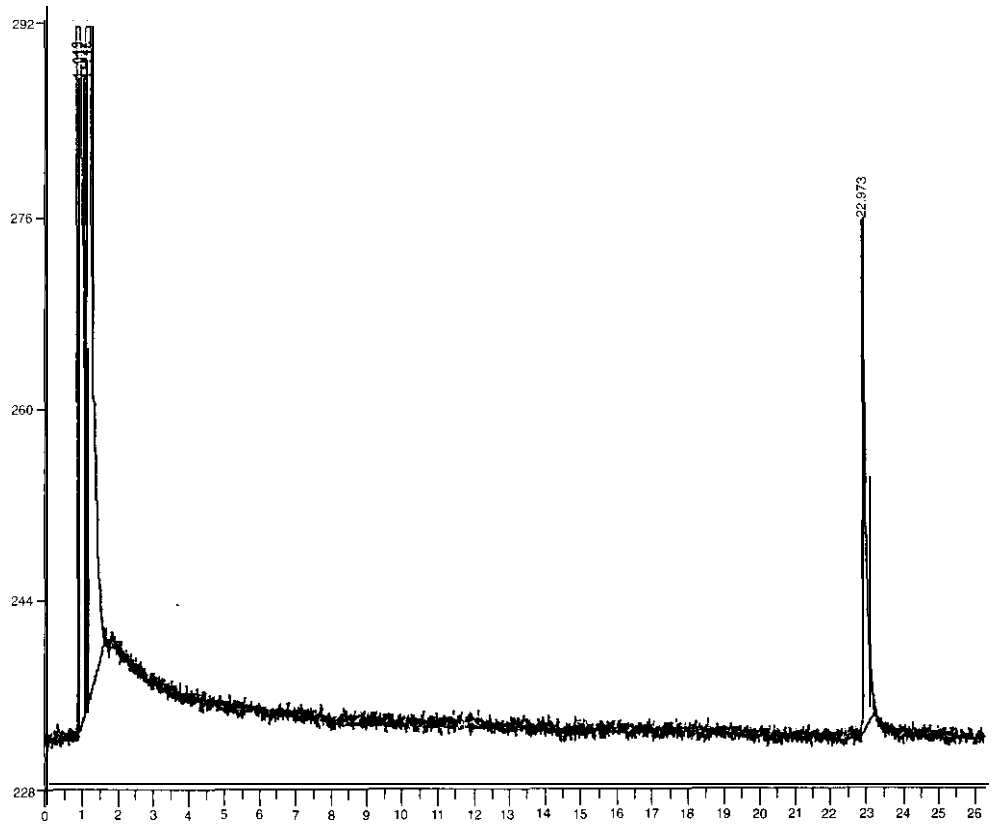

Figure 3. Gas chromatographic peak of 0.08 ug ml $^{-1}$ monocrotophos recovered from spiked oil

TABLE 1. DATA ON CALIBRATION CURVE

Amount injected (µg ml^{-1})	Mean peak area (arbitrary units)	Standard deviation	Coefficient of variation (%)
0.06	*859.04*	158.717	18.5
0.08	3 946.08	215.731	5.5
0.40	4 916.77	758.170	15.4
0.60	13 835.70	1 831.155	13.2
0.80	29 507.89	724.643	2.5
1.20	44 492.78	3 312.059	7.4
R^2	0.9573		
Slope	37 860 (± 3 541)		

Recovery of Monocrotophos from Spiked Oil Matrix

Table 2 shows the recovery data based on chromatographic peak areas of monocrotophos from spiked RBD palm olein. The recoveries ranged from 74.3%-101.6%. The coefficients of variation for lower concentrations were in excess of 10% and those of the higher concentrations less than 10%. The limit of detection was 0.01 µg ml-' (Long and Wineforfner, 1983).

Recoveries of monocrotophos at the lower concentrations were lower than at the higher concentrations. However, the range of recovery was acceptable as shown by data in literature. Guinivan et al. (1981) recovered 100% and 62% organophosphorus pesticides from Southern Pea vines at levels of 0.2 and 0.01-0.2 ppm respec-

TABLE 2. RECOVERY OF MONOCROTOPHOS FROM SPIKED RBD PALM OLEIN

Amount added (μg ml^{-1})	Mean peak area (arbitrary units)	Amount found (μg ml-')	Recovery (%)	Standard deviation	Coefficient of variation (%)
0.08	2 932.42	0.0595	74.3	8.667	11.7
0.40	4 385.76	0.3568	89.2	13.456	15.1
0.80	26 822.67	0.7272	90.9	3.409	3.8
1.20	45 226.92	1.2198	101.65	6.389	6.3

tively. Using another method, Luke and Richards tested the Unitrex system for analysis of organophosphorus pesticide residue and obtained recoveries from 84%-99% with coefficients of variation of 3% to 5%. Recoveries of pesticide residue from spiked milk at levels of 0.1 and 1.0 g ml-' were **82.1%.93.8%** and 79.7%-96.6% respectively (Toyoda et al., 1990). Recoveries of 23 organophosphorus pesticides from oils and fatty materials by Di Muccio et al. (1990) were 82%-111%. Thus, the recovery ranges in this experiment are in line with published results,

CONCLUSION

GPC without an extra Florisil or Alumina column was found suitable for clean-up of monocrotophos residue in oil matrix. The range of recoveries was from 74.3%-101.5%. Therefore, the procedure can be used as a multi-residue clean-up technique for all organophosphorus pesticides in oil matrix.

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