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DETERMINATION OF CHLORPYRIFOS IN REFINED PALM OLEIN BY GC-FPD AND GC-ECD

Keywords: chlorpyrifos, liquid-liquid extraction, silicic acid, gas chromatography, electron capture detector, flame photometric detector.

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Chlorpyrifos (a pesticide containing phosphorous and chlorine) from oil matrix was determined using gas chromatography (GC) with a flame photometric (FPD) and electron capture (ECD) detectors. In the first experiment, the detector (FPD) used gave recoveries (from palm olein samples spiked with chlorpyrifos at levels of 0.04-0.10 μg ml^{-1}) ranging from 89% to 100% with coefficients of variation from 2.9% to 10.8%. In the second experiment, ECD showed recoveries (from palm olein spiked with chlorpyrifos at levels 0.02-0.10 μg ml^{-1}) of greater than 97% with coefficients of variations ranging from 0.5% to 2%. The results showed that using GC, the ECD and FPD are suitable detectors for quantification of chlorpyrifos in oil matrix.

INTRODUCTION

Pesticides are used in agriculture and are essential in the management of pests for the production of sufficient supply of quality food (Mohamad, 1996). Organophosphorus compounds (a group of P-containing pesticides) are widely used for controlling insects and pests. Chlorpyrifos, which is part of this group, is sold under various trade names, such as Lorsban™ and Dursban™. Typically, chlorpyrifos is used to eliminate/control the population of flies, household pests, mosquitoes and various crop pests in soil and on foliage and ectoparasites on cattle and sheep. It has a broad range of insecticidal activity and is effective by contact, ingestion and vapour action but is not systemic (Worthing, 1987).

Analysing for pesticide residues in oil matrix and fat is difficult. The analysis of fats and oils for pesticide residues by GC requires the preliminary extraction of the compound of interest from the bulk of lipids and contaminants. This

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is to prevent damage to the injector and column by non-volatiles and late eluting materials (Erney et al., 1993).

The standard method of analysis involves the use of liquid-liquid extraction in the isolation and fractionation of the organophosphorous pesticide from its matrix (Williams, 1984; 1990; Pesticide Analytical Manual, 1987). However, Gillespie et al. (1995), reported a method using diatomaceous earth columns and normal solid phase extraction (SPE) cartridges instead. With this method, separation of the pesticides from lipids was 98% while the average recoveries from fortified samples were greater than 89%. Furthermore, the method provided several advantages over the official AOAC (Williams, 1984) method in several respects, including clean-up, efficiency, speed and volume of solvent required.

Ana et al. (1993) investigated a GC method for determination of 25 organophosphorus pesticides applied to horticultural crops. They used GC with FPD for quantification of these compounds which were confirmed by gas chromatography-mass spectrometry (GCMS). The recoveries were in the range of 68%-100% with relative standard deviations of less than 35%.

Robert et al. (1987) optimized the sweep codistillation apparatus to quantitatively analyse coumaphos and organophosphorus pesticide residues in animal fat. The recovery of caumaphos was 91% with a coefficients of variations of 6%. Other organophosphorus pesticides (diazinon, chlorpyrifos, ethion and bromophos-ethyl) had recoveries ranging from 90% to 96% and coefficients of variation between 4%-6%.

Sawyer (1985) carried out a collaborative study using the method developed by Luke et al. (1981) for determining multi-residues of pesticides in fruits and vegetables. Chlorpyrifos was quantified by GC equipped with both a Hall 700 A electrolytic conductivity and a FPD detectors. The mean recoveries ranged from 82.6% for acephate fortified at 0.5 ppm in strawberries to 118.1% for 0.0636 ppm fortified chlorpyrifos in lettuce.

James et al. (1984) modified the Dow Chemical Co. method of analysis (Dursban Intecticides Technical Information), for the determination of chlorpyrifos residues in greenhouse vegetables. Recoveries from fortified vegetables were 86% ± 16% and the sensitivity was 0.01 ppm for chlorpyrifos.

In most of the earlier residue studies, quantitation of the pesticide and its oxygen analogs were by phosphorus FPD. Bowman *et al.* (1968) used GC with ECD to determine chlorpyrifos and its major metabolite, 3,5,6-trichloro-2-pyridinol. The results showed that the recoveries from pea vine samples, prior to gel permeation chromatography clean-up, were 100% at a level of 0.2 ppm and 62% at levels ranging from 0.01 to 0.2 ppm.

The objective of this investigation is to study the suitability of the GC method for determination of chlorpyrifos in oil samples using both ECD and FPD. This method was adopted from Cloborn *et al.* (1968) for determination of chlorpyrifos in milk and body tissues of cattle.

MATERIALS AND METHODS

Reagents

The acetonitrile, n-hexane and dichloromethane used were of analytical grade from Merck. Anhydrous sodium sulphate of analytical grade was from R&M chemicals. Silicic acid of analytical grade was from R&M Chemicals and Sigma Company. The chlorpyrifos standard was obtained from Labor Dr. Ehrenstorfer GmbH.

Apparatus. Glass chromatographic column (270 mm x 18 mm) – with sintered glass disc and fitted with a Teflon stopcock.

Experiment 1

The instrument used was GC – Hewlett Packard Model 5890 Series II equipped with ECD. The column use was a HP5 MS as non-polar stationary phase column containing 95% dimethylpolysiloxane and 5% diphenyl, 30 m x 0.25 mm i.d. and 0.25 μm film thickness. The following operating conditions were used: column flow (nitrogen) 2.7 ml min^{-1}; auxillary gas flow (nitrogen) 27 ml min^{-1}; split vent flow 100 ml min^{-1}, ECD anode purge 5 ml min^{-1}, injector temperature 280°C; detector temperature 250°C; injection volume 3 μl (Hewlett-Packard Auto-

injector 5890 series); the oven temperature was programmed to rise from 190°C to 220°C at 5°C min^{-1} and held for 4 min.

Experiment 2

GC – the instrument was a Hewlett Packard Model 5890 linked to an HP 9153 Work Station Model 9453 CO20 and equipped with FPD. The column used was DB-1 capillary column 30 m x 0.53 mm i.d. with a film thickness of 0.25 µm. Operating conditions: detector and injector temperatures at 280°C and 250°C respectively; injection volume 1 µl; oven temperature programmed to rise at 20°C min^{-1} from 40°C to 150°C and held for 1 min, then at 4°C min^{-1} from 150°C to 210°C and held for 5 min.

Preparation of standard curve. A standard solution ofchlorpyrifos was prepared by dissolving 5 mg of chlorpyrifos in 50 ml n-hexane in a 50 ml volumetric flask. Working standard solutions of 0.10, 0.08, 0.06, 0.04 and 0.02 µg ml^{-1} were prepared by diluting the standard solution with appropriate volumes of n-hexane.

Extraction method. Refined palm olein sample (25 g each) were spiked with 0.02-0.1 µg ml^{-1} chlorpyrifos. Each of the spiked samples was dissolved in 100 ml hexane and extracted four times using 50 ml portions of acetonitrile. All the four portions were drained into a separating funnel containing 100 ml n-hexane and the mixture was shaken gently. The acetonitrile (lower phase) was drained into a round bottom flask and evaporated to a volume of 25 ml using a rotary evaporator. This concentrated solution was transferred into a 250 ml separating funnel and 80 ml of 5% aqueous Na_2SO_4 added. This mixture was then extracted with four portions of 50 ml hexane. The upper phases were combined and filtered through anhydrous Na_2SO_4 into a 300 ml round bottom flask. The solution was then concentrated to about 5-10 ml for chromatographic clean-up.

Preparation of Silicic Acid Column and Clean-up Procedure

A chromatographic column containing 1.5 cm bottom layer of anhydrous sodium sulphate, 5.0 cm (8.5 g) of silicic acid and 1.5 cm top layer of anhydrous sodium sulphate was prepared for the clean-up process. Packing of the silicic acid column was carried out under vacuum by connecting a Buchner flask to the column and attaching it to a vacuum line. The silicic acid was washed successively by eluting it with 25 ml of 7.5% v/v dichloromethane in hexane at the rate of 3-5 ml min^{-1}.

The extract was transferred to the column and washed with 180 ml of 7.5% v/v dichloromethane in n-hexane and the eluate collected in a round bottom flask. The solution was concentrated to 5 ml using a rotary evaporator and subsequently evaporated to dryness under a stream of nitrogen. The residue was redissolved using an accurately measured volume of 5 ml n-hexane, and samples injected into the gas chromatography system. Experiments using silicic acid from two suppliers (R&M Chemicals and Sigma Company) were evaluated to observe the efficiency of the separation.

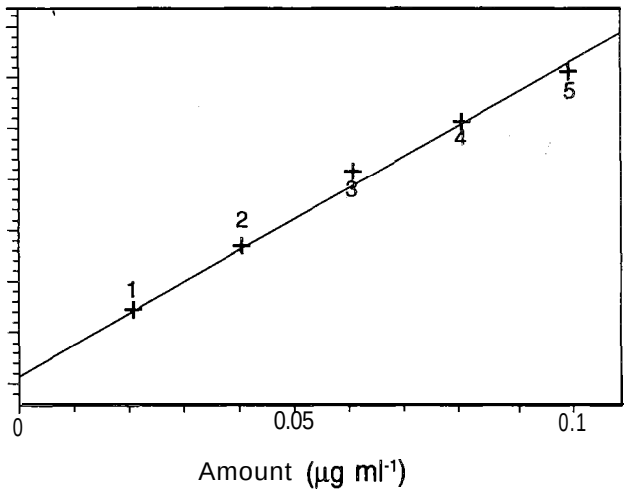
RESULTS AND DISCUSSION

Liquid-liquid partition chromatography is widely used for organsphosphorus pesticides in oil matrix. The methods used in this study involved two key steps, namely, liquid-liquid extraction and the clean-up process. Using an appropriate ratio of n-hexane and acetonitrile, chlorpyrifos was preferentially partitioned into the polar acetonitrile layer while the bulk of the lipids remained solubilized in the non-polar hexane layer (Walters, 1990). In the second step, namely, the clean-up process, residual fat was further removed from the chlorpyrifos by means of column chromatography. The chlorpyrifos residue was then analysed using GC with FPD and ECD.

To determine the reproducibility of the injection technique and linearity of the FPD, repeated injections of 0.02-0.10 µg ml^{-1} of standard chlorpyrifos in n-hexane were made into the capillary gas chromatographic column. *Table 1* shows the calibration data obtained from triplicate analysis, with each solution being injected thrice. The percentage correlation variation from the triplicate injections was 3.3%-

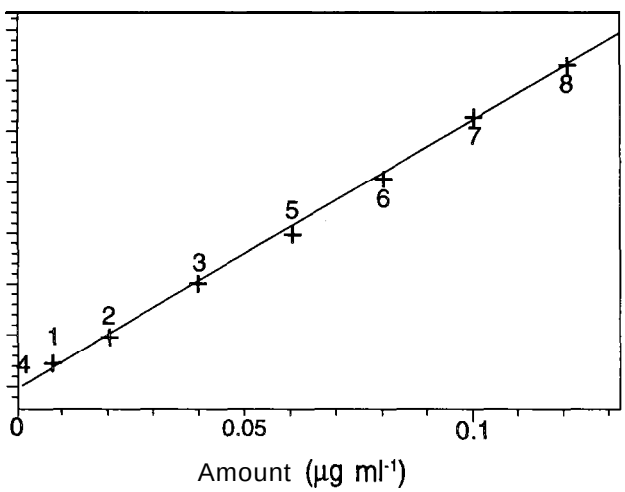
TABLE 1. DATA ON CALIBRATION CURVE (GC-FPD)

Conc. of chlorpyrifos standard (μg ml^{-1})	Mean peak area arbitrary unit	S.D.	C.V. (%)
0.02	133.44	15.69	11.75
0.04	268.67	15.57	5.79
0.06	413.66	18.37	3.58
0.08	513.66	18.37	3.58
0.10	563.70	20.90	3.34



Correlation: 0.99724
Residual std. dev.: 19.46538
Formula: y = mx + b
m : 6256.85714
b : 15.29048
x : amount (μg ml^{-1})
y : arbitrary units

Figure 1. Calibration curve of standard chlorpyrifos against gas chromatographic peak area (GC-FPD).



Correlation: 0.99948
Residual std. dev.: 1922.96125
Formula: y = mx + b
m : 1.23619e6
b : -419.70691
x : amount (μg ml^{-1})
y : arbitrary units

Figure 2. Calibration curve of standard chlorpyrifos against gas chromatographic peak area (GC-ECD).

11.8%. Figure 1 shows the calibration curve of standard chlorpyrifos against the gas chromatographic peak area using GC with FPD. The linear regression (r^2) was found to be 0.9972 and the equation derived from the calibration area data was y = 6251.74x +15.29, where y is the area of chlorpyrifos obtained from the GC analysis and x is the concentration of chlorpyrifos in μg ml^{-1}. The reproducibility of the injection technique and linearity were acceptable.

The efficiency of the method was tested by adding known amounts of chlorpyrifos to an oil which is free from chlorpyrifos residue. *Figure 3b* shows the chromatogram of blank refined palm olein without chlorpyrifos residue.

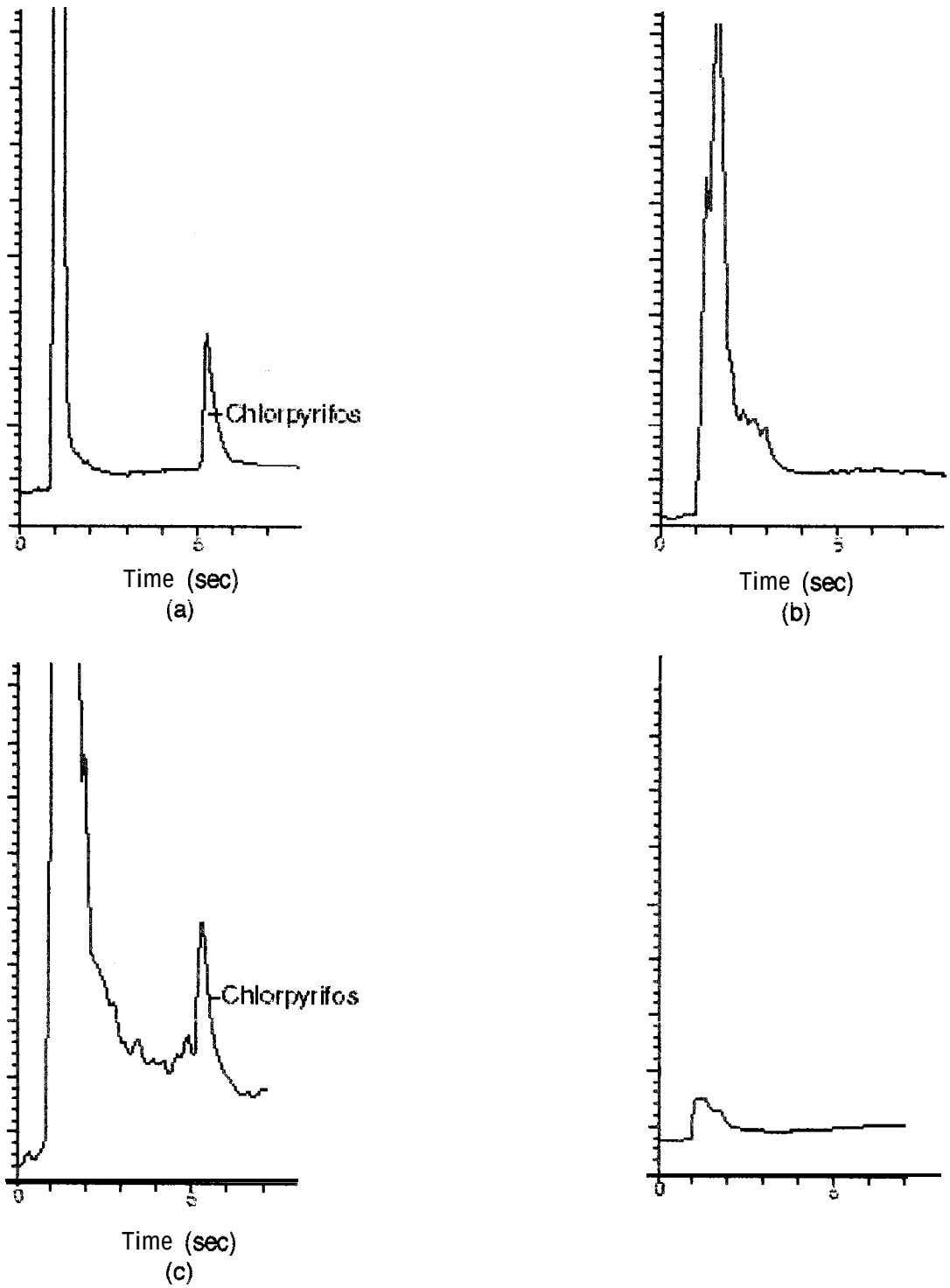


Figure 3. Chromatograms obtained by using GC-ECD for a) *chlorpyrifos standard solution (0.1 µg ml-1), b) blank sample of oil, c) a spiked sample of oil at a fortification level of 0.1 µg ml-', and d) blank hexane.*

TABLE 2. DATA ON RECOVERY OF CHLORPYRIFOS IN REFINED PALM OLEIN (GC-FPD)

Conc. of chlorpyrifos standard (μg/ml^{-1})	Recovery (%)	S.D.	C.V. (%)
0.10	**100.20**	**10.80**	10.80
0.08	96.84	2.18	2.25
0.06	88.72	2.56	2.90
0.04	93.80	8.25	8.80

The recovery ofchlorpyrifos residue by using GC-FPD was determined by analysing refined palm olein samples spiked with chlorpyrifos standard solution at four different levels of 0.1, 0.08, 0.06 and 0.04 μg ml^{-1}. *Table 2* shows the recoveries and coefficients of variation of chlorpyrifos from 0.02-0.1 μg ml^{-1}. They ranged from 88.7% to 100.2% and from 2.9% to 10.8% respectively. This is comparable to the result obtained by Cloborn et al. (1968) where the recovery was 75% to 100%. The recovery results were satisfactory for residue analysis.

An equivalent silicic acid from another company was used in the clean-up process. The same procedure was repeated to determine the recovery of chlorpyrifos from an oil matrix. *Figure* 2 shows the calibration curve of standard chlorpyrifos by GC with ECD and r^2 = 0.9994. The response was linear from 0.008 to 0.12 μg ml^{-1}, with a regression equation of y = 1.23619e6 - (419.71) where y is gas chromatographic peak area of standard chlorpyrifos solution and x the concentration of chlorpyrifos in μg ml^{-1}. The detector response was tested between 0.008 to 0.12 μg ml^{-1} with the response being linear over the range. *Table 3 shows* the data for the calibration curve (GC-ECD) which was obtained from triplicate analysis, with each solution being injected thrice. The coefficients of variation was low (0.02%-2.1%), indicating accurate delivery of samples by the autosampler. *Figure 3a* shows the chromatograms obtained by gas liquid chromatography with the HP-5MS capillary column corresponding to the chlorpyrifos standard solution. Parts b and c of *Figure* 3 show the chromatograms corresponding to a blank sample of palm olein and a spiked sample of palm olein at a fortified level of 0.04 μg ml^{-1} for chlorpyrifos residue. *Figure 3d* shows the chromatogram of hexane obtained by GC with HP-5MS capillary column, by GC with ECD.

The recovery and sensitivity of the method using ECD were determined at five levels of concentration, 0.02, 0.04, 0.06, 0.08 and 0.10 μg ml-" of chlorpyrifos. The results are shown *in Table* 4. For each concentration, duplicate analysis were carried out and each solution was injected thrice. The percentage recovery of chlorpyrifos using GC with ECD ranged from 97% to 105%. The coefficients of variation at levels of 0.02 to 0.1 μg ml^{-1} ranged from 0.5% to 2%. As expected, the trend observed was that at higher levels of concentration the

TABLE 3. DATA FOR CALIBRATION CURVE (GC-ECD)

Conc. of chlorpyrifos standard (μg ml^{-1})	Mean peak area arbitrary unit	C.V. (%)
0.008	12 433	2.0
0.02	22 866	2.1
0.04	47 050	2.0
0.06	71 714	1.5
0.08	97 186	2.0
0.10	125 372	0.02
0.12	148 692	0.84

TABLE 4. RECOVERY OF CHLORPYRIFOS RESIDUE (GC-ECD)

Conc. of chlorpyrifos std. ($\mu g\ ml^{-1}$)	Area arbitary unit	A.V. area arbitrary unit	% Rec.	% A.V. rec.	% A.V. rec. (set 1,2)	SD	% C.V.	A.V. % C.V. (Set 1,2)
0.02 Set 1	27 803		104					
	27 268	27 659	102	103		342	1.2	
	27 905		104					
					101			2
0.02 Set 2	26 267		99					
	26 546	25 985	100	99		,744	2.8	
	25 140		97					
0.04 Set 1	42 343		100					
	42 596	42 199	101	100		486	1.2	
	41 657		99					
					100			1.5
0.04 Set 2	41 132		99					
	41 285	41 605	99	100		691	1.7	
	42 398		101					
0.06 Set 1	69 141		99					
	69 930	69 368	100	99		489	1.2	
	69 304		99					
					100			1
0.06 Set 2	68 907		99					
	69 973	69 541	100	100		561	0.8	
	69 742		100					
0.08 Set 1	85 421		101					
	83 504	84 123	96	98		1 124	1.3	
	83 444		96					
					97			0.7
0.08 Set 2	83 023		96					
	82 927	82 981	96	96		49	0.05	
	82 994		96					
0.1 Set 1	121 912		109					
	122 360	122 033	109	108		286	0.2	
	121 826		106					
					105			0.5
0.1 Set 2	113 615		101					
	115 487	114 436	103	102		956	0.8	
	114 206		102					

Notes:
A.V. = average.
Conc. = concentration.
Std. = standard.
Rec. = recovery.
S.D. = standard deviation.
C.V. = coefficient variation.

percent recovery was better compared to that at a lower levels of concentration.

In this study, the results were more reproducible when compared to the study using FPD since an automated injector was used. One of the advantages of using ECD is its ability to detect halogen atoms, in this case chlorine. This enables detection of both chlorpyrifos [0,0-diethyl-0- (3,5,6-trichloro-2-pyridyl)-phosphorothioate] and its major metabolite (3,5,6-trichloro-2-pyridinol). FPD only allows detection of the phosphorus atom. Therefore FPD cannot detect the metabolite of chlorpyrifos since there is no phosphorus atom in the molecules. With this added sensitivity, GC with ECD method is the preferred method for the detection of chlorpyrifos. Guinivan *et* al. (1981) found that the lower recovery data for 0.0% ppm chlorpyrifos and 3,5,6-trichloro-2 pyridinol spiked in southern pea vines did not sacrifice the accuracy of the residue quantification.

CONCLUSION

From the results obtained, it can be concluded that the GC methods using ECD and FPD described in this paper are sensitive enough for the determination of chlorpyrifos in refined palm olein up to 0.02 $\mu g\ ml^{-1}$ and 0.04 $\mu g\ ml^{-1}$ respectively. It can also be used for monitoring chlorpyrifos in edible oil.

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REFERENCES

ANA, A; MARIANO, C; AMADEO, R and FERNANDEZ, A (1993). Gas chromatographic analysis of organophosphorus pesticides of horticultural concern. *Journal of Chromatography A, 655:*293-300.

BOWMAN, M C; BRAZA, M and LEUCM, D B (1968). *Journal Agric. Food Chem., 16:*796-802.

CLOBORN, H V; MANN, H D and OEHLER, D D (1968). Dursban determination in milk and tissues of cattle. *Journal of the A.O.A.C, 52(6):* 1243-1245.

ERNEY, D R; GILLESPIE, AM and GILVYDIS, D M (1993). Explaination of the matrix-induced chromatographic response enhancement of organophosphorus pesticides during open tubular column gas chromatography with splitless or hot column injection and flame photometric detection. *Journal of Chromatography, 638: 57-*63.

GILLIESPIE, A M; DOLY, S L; GILVYDIS, D M; SCHNEIDER, F and WALTERS, S M (1995). Multicoloumn solid-phase extraction clean-up of organophosphorus and organochlorine pesticide residue in vegetable oils and butterfat. *Journal of A.O.A.C International, 78(2):*431-437.

GUINIVAN, R A; THOPSON, N P and BARDALAYE, P C (1981). Simultaneous electron capture detection of chlorpyrifos and its metabolite, 3, 5, 6-trichloro - 2 - pyridinol, after gel permeation chromatography. *Journal of A.O.A.C. International, 64(5):*1201-1204.

JAMES, J P; JAMES, H S and JOSEPH, H F (1984). Gas chromatographic determination of chlorpyrifos residues in greenhouse vegetables after treatment of potting media wit& dursban for imported fire ant. *Journal of A.O.A.C. International, 67(6):*1091-1094.

LURE, A M; FROBERG, J E; DOOSE, GM and MASUMOTO, H T C (1981). Improved multiresidue gas chromotographic determination of organophosphorus, organonitrogen and organohalogen pesticides in produce; using flame photometric and electrolytic conductivity detectors. *J. Assoc. of Anal. Chem. 64(5):*1187-1195.

MOHAMAD, R (1996). Inaugural lecture of pesticide usage: concern and options. Presented at University Putra Malaysia. 10 February 1996. p. l-18.

PESTICIDE ANALYTICAL MANUAl (1987). *Methods for Individual Residues, Volume II.* U.S. Food and Drug Administration.

ROBERT, LB; CLINTON, N Rand RODERICK, GM (1987). Optimization ofsweep codistillation apparatus for determination of coumophos and other organophosphorus pesticide residues in animal fat. *Journal of A.O.A.C. International, 70(3):*442-445.

SAWYER, L D (1985). The Luke *et al.* method for determining multipesticides residues in fruits and vegetables: collaborative study. *Journal of A.O.A.C. International, 68(1):*64-71.

WORTHING, *C* R *(1987). The Pesticide Manual - A World Compendium.* Eight edition Published by the British Crop Protection Council. p. 179-180.

WALTERS, S M (1990). Clean-up techniques for pesticides in fatty food. *Analytica Chimica Acta, 236:*77-82.

WILLIAMS, *S* (1984). *AOAC. Official Methods of Analysis.* 14th ed; AOAC Arlington, VA.

WILLIAM, *S* (1990). *AOAC. Official Methods of Analysis.* 15th ed; AOAC Arlington, VA.