Malaysian Journal of Chemistry, 2002, Vol. 4, No. 1, 0021 - 0027

Optimization of Extraction and Clean-up Procedures for Chlorpyrifos Residue Determination in Oil Matrix

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Abstract : An improved method for extraction and clean-up techniques for extraction of chlorpyrifos residue from oil matrix was established after a series of trials. The method involved 2 steps, namely, the extraction of analyte from the chlorpyrifos-spiked oil matrix followed by clean up of analyte. Liquid-liquid extraction using petroleum ether saturated acetonitrile was used to exclude the oil matrix as well as to extract the chlorpyrifos. Optimum clean-up and recovery of the analyte was carried out using solid phase extraction (SPE) cartridges packed with silica and eluents of different composition and polarity. Analysis of the eluate was by gas chromatography with electron capture detector. Optimization of all these factors has resulted in greater than 90% recovery of chlorpyrifos from spiked samples. As a result of polarity variation of the eluting solvent, the volume of the eluent was significantly reduced, without sacrificing the extraction efficiency of chlorpyrifos residue. The proposed method is applicable for the analyses of chlorpyrifos residue in vegetable oils.

Abstrak : Satu pembaikan kaedah pengekstrakan dan pembersihan untuk residu *chlorpyrifos* dari minyak telah di bangunkan selepas beberapa siri percubaan dilakukan. Kaedah ini melibat dua langkah, iaitu, pengekstrakan analit dari minyak yang telah di suntik *chlorpyrifos* didalamnya diikuti dengan kaedah pembersihan. Pengekstrakan cecair-cecair menggunakan petroleum eter tertepu asetonitril bertujuan untuk menyah minyak dan mengekstrak *chlorpyrifos*, Pengoptimuman pembersihan dan perolehan semula analit tersebut telah dijalankan dengan menggunakan kaedah pengekstrakan fasa pepejal (SPE) yang telah dipadatkan dengan silika dan eluen dari komposisi yang polaritinya berbeza. Analisa keatas bahan eluen dilakukan menggunakan kromatografi dengan alat pengesan penangkap elektron. Pengoptimuman factor-faktor di atas telah menghasilkan perolehan semula *chlorpyrifos* dari minyak yang disuntik melebihi 90%. Isipadu eluen telah banyak dapat dikurangkan dengan kepelbagaian polariti pelarut eluen tetapi tidak mengurangkan kecekapan pengekstrakan residu *chlorpyrifos*. Kaedah yang dicadangkan ini boleh digunakan untuk analisa *chlorpyrifos* residu dalam minyak sayur.

Received : 11.01.02; accepted : 19.09.02

Introduction

With the phasing out of organochlorine (OC) insecticides, the organophosphorus (OP) compounds are now widely used for insect control. One of the organophosphorus compounds used is commonly known as chlorpyrifos. The chemical name of chlorpyrifos is *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate (IUPAC). Because of its versatility, chlorpyrifos is used globally in a myriad of pest control situations. From corn agriculture in the United States to termite control in Japan, cotton agriculture in Egypt, citrus horticulture in Spain and oil palm plantations in Malaysia, chlorpyrifos has been successfully employed to combat insect and arthropod pests threatening production of food and fiber and maintenance of human health (1-3).

Separation of trace levels of pesticides from edible oil in preparation for their analysis by gas chromatography (GC) requires pre-treatment of oil samples (4). A method had been reported for determination of organophosphorus (OP) in oil and butterfat by using a diatomaceous earth column and C18 bonded silica SPE cartridge to separate the compound of interest from oil. The average recoveries of OP and OC were greater than 89% (5). A study was attempted to analyse OP pesticides in molluscs by using GC-ECD and nitrogen phosphorus detector (NPD) after extraction with acetonitrile-acetone and cleanup with column chromatography using silica gel (6).

Hopper (7) reported a different cleanup technique for pesticides and herbicides in fatty food. He developed a gel permeation chromatography

system for the rapid cleanup of fat from pesticides and herbicides. This system allows a wide range of compounds including the OC, OP, herbicides, carbamates and drugs to elute in the cleanup fraction.

A semi-preparative HPLC employing an ODS-bonded silica column to collect fractions of pesticides from edible oil and butter was also investigated (8). Using acetonitrile as the mobile phase, OC and OP pesticides of a wide range of polarity were eluted in the first 40mL of the eluate. Recoveries of selected pesticides from spiked samples, which were subsequently determined by gas chromatography with electron capture detector, ranged from 80-108%.

A method for clean up and determination of chlorpyrifos (Dursban) in milk and tissue of cattle was reported in 1968 (9). The recovery of Dursban from milk and body tissue of cattle ranged from 75-100%. The method was successfully adopted and modified accordingly for the extraction of chlorpyrifos residue in oil matrix, offering much improved extraction efficiency and significant reduction in solvent usage (10).

Recently, an improved and efficient pesticide residue analysis in fruits and vegetables was developed (11). The method offers a few advantages such as the elimination of methylene chloride harzadous waste by replacing it with acetonitrile during the extraction process. Sample size and solvent consumption were also reduced. In the cleanup process, C18 cartridges were used and these provided cleaner extracts for chromatographic analysis.

Recently, a new monitoring system using solid phase extraction and gas chromatography mass spectrometry was established for determination of ninety pesticides and related compounds in river water (12). The limits of detection were reported to be from 0.01-0.1µg/mL and overall recoveries ranged from 72-118%.

An alternative method for determination of pesticides by using supercritical fluid (SFC) extraction was developed by several researchers (13-18). A study had been reported on collection efficiencies of chlorpyrifos in off-line supercritical fluid extraction (19). Two types of trapping solvent were used, namely, methanol and acetone. Trapping in methanol captured about 70% of chlorpyrifos and trapping in acetone managed to extract 95% of chlorpyrifos. Acetone was a better trapping solvent than methanol.

This paper proposes a method for determination of chlorpyrifos using GC-ECD after pre-treatment of sample in the extraction step by liquid-liquid extraction followed by clean-up procedures using commercial solid phase extraction (SPE). The objective of this investigation was to further increase the extraction, efficiency of trace chlorpyrifos residue in oil matrix, using minimum amount of solvent.

Material and Method

Reagents and apparatus

All solvents used were of analytical grade.

(**a**) Solvents - petroleum ether (60-80°C), acetonitrile, hexane and ethyl acetate.
(**b**) Sample - refined bleached and deodorized palm olein (RPOo) was obtained from Lam Soon Sdn. Bhd.
(**c**) Standard - chlorpyrifos standard 99.0% purity was purchased from Ehrenstorfer Co. Germany.
(d) SPE cartridges (silica,1g) were obtained from Supelco.

Apparatus / Instrument

(**a**) Rotary evaporator (Heidolph VB 2000, German)
(**b**) N-evaporator (Organomation Associates.Inc., USA)
(**c**) Vortex mixture (Thermolyne, USA)
(**d**) Centrifuge (Hettich,USA)
(**e**) Solid phase extraction manifold (IST,United Kingdom).
(**f**) Hewlett-Packard Model 5890 (USA) gas chromatography with an electron capture detector (ECD) was used. The column was a non-polar capillary column with 5% diphenyl (HP 5MS), 30m length, 0.25mm i.d. and 0.25 µm film thickness.

GC parameter: Parameters used for GC-ECD were as follows: column flow (nitrogen) 2.7mL/min, auxillary gas (nitrogen) 27mL/min, anode purge for ECD was 5mL/min. The injector temperature was set at 250°C in splitless mode with split valve off for 0.75 minutes.

Sample preparation

Refined palm olein (RPOo) samples were heated at 60°C and shaken to ensure homogeneity. The sample was then weighed (3g) and spiked with 1 mL of 1µg/mL chlorpyrifos standard solution.

Preparation of chlorpyrifos standard solution

A standard solution of chlorpyrifos was prepared by dissolving 5 mg of chlorpyrifos in 50 mL n-hexane in a 50-mL volumetric flask. Working standard solutions: 0.1, 0.08, 0.06, 0.04 and 0.02 µg/mL were prepared by diluting the standard solution with appropriate amounts of n-hexane.

Extraction

The extraction procedure was a modification of the method proposed by Gillespie and Walters (5) for organophosphorus pesticide residue in oil matrix. This preliminary partitioning step eliminated a major portion of the oil.

An accurately weighed spiked RPOo sample of 3.0g (±0.001g) was transferred into a 20-mL centrifuge tube and 0.5mL of petroleum ether was added. The tube was then stoppered and the content mixed on a vortex mixer for 15 seconds. The stopper was removed and 4 mL petroleum of ether-saturated

acetonitrile was added to the tube and the resulting solution was again mixed for 30 seconds. The tube was then centrifuged at high speed (3,000 rpm) for 2 min. The top acetonitrile layer was transferred into a round-bottomed flask with the aid of a glass pasteur capillary pipette. The above steps (addition of ether-saturated acetonitrile) were repeated 5 times to give six extracts which were kept in separate round bottom flasks and labeled as Espe1, Espe2, Espe3, Espe4, Espe5 and Espe6. All the six extracts were placed in a water bath at 50°C and purged to dryness with nitrogen (N-evaporator). The residue remaining in each of the round bottomed flasks was redissolved with 10mL hexane and 3µl was injected into the GC-ECD.

SPE Clean-up

Experiment 1

The SPE cartridge was attached to the SPE manifold and a 20-mL vial was placed at the tube outlet for collecting the extract. The cartridge was pre-washed once with 3mL hexane, and the washings discarded. The combined extracts (Espe1 – Espe6) that have been evaporated to about 1 mL were transferred onto the SPE cartridge. Chlorpyrifos was eluted as follows and each fraction was collected in separate round bottom flasks:

P1spe 1 : 1st fraction = 1mL hexane
P1spe 2 : 2nd fraction = 1mL hexane
P1spe 3: 3rd fraction = 1mL hexane
P1spe 4: 4th fraction = 2mL 2% EA in hexane
P1spe 5: 5th fraction = 2mL 2% EA in hexane
P1spe 6: 6th fraction = 2mL 2% EA in hexane

The extracts in each round bottom flask were dried using N_2. The residue was then redissolved in 10mL n-hexane and 3 µl injected into the GC-ECD. Quantification of the analyte in each fraction was made by comparison with chlorpyrifos standard solution.

Experiment 2

The procedure for experiment 2 was similar to experiment 1 except that the elution pattern was different. For this experiment, the cartridge was eluted according to the elution pattern described below:

P2spe1: 1st fraction = 1mL hexane
P2spe2: 2nd fraction = 4 mL 2% ethyl acetate in hexane
P2spe3: 3rd fraction = 2mL 2% ethyl acetate in hexane
P2spe4: 4th fraction = 4mL 2% ethyl acetate in hexane

Experiment 3

In this experiment, the procedure was similar to experiment 1, however the elution patterns is as follows:-

P3spe 1: 1st fraction = 1mL hexane
P3spe 2: 2nd fraction = 1mL hexane
P3spe 3: 3rd fraction = 2 mL 5% ethyl acetate in hexane
P3spe 4: 4th fraction = 2 mL 5% ethyl acetate in hexane
P3spe 5: 5th fraction = 4 mL 5% ethyl acetate in hexane

Recovery of chlorpyrifos for the clean-up process using cartridge was then calculated.

Results and Discussion

Calibration curve

The calibration curve was plotted to ensure that all instruments were working properly. To establish the calibration curve, a series of working standard chlorpyrifos 0.005µg/ml to 1.0µg/ml solution were injected into GC-ECD. Table 1 shows the calibration data obtained triplicate analysis, with each solution being injected twice. The linear regression (R^2) was found to be 0.9994 and the equation derived from the calibration area data was $y = 76960.71x + 53.29$, where y is the area of chlorpyrifos obtained from GC analysis and x is the concentration of chlorpyrifos in µg/mL. The reproducibility and linearity of the injection technique was acceptable.

Optimization of Extraction Procedure. To obtain the maximum extraction of analyte in the extraction process, the experiment was carried out as discussed in the extraction procedure. Figure 1 shows the percent recovery of chlorpyrifos for each extract with acetonitrile. Chlorpyrifos was found in all extract Espe1, Espe 2, Espe 3, Espe 4 and Espe 5 except in the last extract (Espe 6). The first extract showed the highest recovery followed by the second, third, fourth and fifth extraction. This figure also shows that the percent extraction and the relative standard deviations are almost similar for triplicate analysis. The consistency was attributed to the time controlled vortex mixing. As shown in figure 1, only five extractions were required for complete extraction of chlorpyrifos from the oil matrix. This is probably because chlorpyrifos is very much less polar and hence it presents a greater resistance to acetonitrile partitioning (5).

The elution system for clean up with silica gel was also optimized. Figure 2 shows that chlorpyrifos was found in all P1spe 2, P1spe 3, P1spe 4 and P1spe 5 fractions, except the first (P1spe 1) and last fraction (P1spe 6). It was found that recovery of chlorpyrifos increased with increasing volume of the eluting solvent (2% ethyl acetate in hexane). The highest (about 75%) was in the fifth fraction, (P1spe 5) while the lowest was in the second fraction (P1spe 2). Before the pesticide was eluted, a washing step with hexane was performed to eliminate as many interfering compounds as possible. Based on the results in Figure 2, chlorpyrifos was only found in the

second (P1spe 2) and third fractions (P1spe 3) after the SPE column was eluted with 1mL hexane. Therefore, the method was modified by washing the silica cartridge with only 1 mL of hexane as this volume of solvent did not elute the chlorpyrifos from the cartridge. This was followed by washing of the silica cartridge with 2% ethyl acetate in hexane. The adsorptions of chlorpyrifos onto the stationary phase (silinol) was strong and it was retained by the stationary phase for a period of time. Chlorpyrifos was eluted when the chlorpyrifos molecules partitioned between 2% ethyl acetate in hexane and the silinol stationary phase. Since chlorpyrifos is less polar it was eluted by 2% ethyl acetate in hexane.

From the results, a new elution pattern was developed. This is to obtain better and faster results by reduction of eluting solvent. Results of the elution pattern of chlorpyrifos eluted from the column are shown in Table 2. Chlorpyrifos was found in all fractions except fraction 1 (P2spe 1). The second fraction (P2spe 2) gave the highest recovery as compared to third fraction as (P2spe 3). It was also observed that no chlorpyrifos was eluted in the fourth fraction (P2spe 4). The total chlorpyrifos recovery found in P2spe 2 and P2spe3 was 99%. Most of the chlorpyrifos eluted completely with 6 mL of 2% ethyl acetate in hexane.

From the above elution pattern, a new elution system was optimized for the clean up of the silica cartridge using a more polar eluent. Results of the eluted pattern of chlorpyrifos from the column with 2% and 5% ethyl acetate in hexane are shown in Table 2. The use of a more polar solvent was to increase the polarity of the mobile phase so that chlorpyrifos could be eluted faster from the cartridge. The maximum recovery (92%) was found in second fraction (P3spe 2) when the silica cartridge was eluted with 2 mL of 5% ethyl acetate in hexane. The remaining chlorpyrifos residue (8%) was collected in the third fraction (P3spe 3) when it was eluted with 2 mL 5% ethyl acetate in hexane. Based on the results obtained 1 mL hexane is needed to wash the silica cartridge to ensure that a clean cartridge is used for the chlorpyrifos clean-up steps. From the above experiment, it was also found that 4 mL of 5% ethyl acetate in hexane was sufficient to elute chlorpyrifos from the silica column. The current method showed a successful adoption of liquid-liquid extraction and clean-up SPE method (5,6) in the analysis of chlorpyrifos residue in oil matrix. The method offers significant improvement to our earlier work (10) in terms of extraction efficiency, ease of operation and smaller amount of solvent used.

The detection limit of the method, determined according to International Union of Pure & Applied Chemistry, IUPAC (20), was found to be 11 pg/mL.

Conclusion

From our investigation, we proposed that extraction of chlorpyrifos from oil matrix could be carried out using 20mL of petroleum ether saturated acetonitrile. This was then followed with a clean-up step through a silica SPE cartridge. The cartridge was cleaned with 1 mL of n-hexane prior to use. The isolated chlorpyrifos from the liquid-liquid extraction process was then eluted through the cartridge using 6mL of 2% ethyl acetate in n-hexane. Alternatively, 4mL of 5% ethyl acetate in n-hexane could be used as the eluting solvent.

Acknowledgement

The authors wish to thank the Director General of MPOB for permission to carry out this project and for publishing this paper. Thanks are also extended to Puan Rosliza and Puan Rosilawati for her invaluable technical assistance.

Table1. Calibration curve data

Concentration of Chlorpyrifos (µg/mL)	[a]Mean peak area, Arbitrary units
0.005	460
0.008	704
0.01	952
0.02	1808
0.04	3354
0.06	4909
0.08	6734
0.1	8631
0.2	1.4×10^4
0.4	2.9×10^4
0.6	4.6×10^4
0.8	6.3×10^4
1.0	7.7×10^4
N	26
R^2	0.9994
Slope	76960.71x + 53.29

[a] For 52 injections

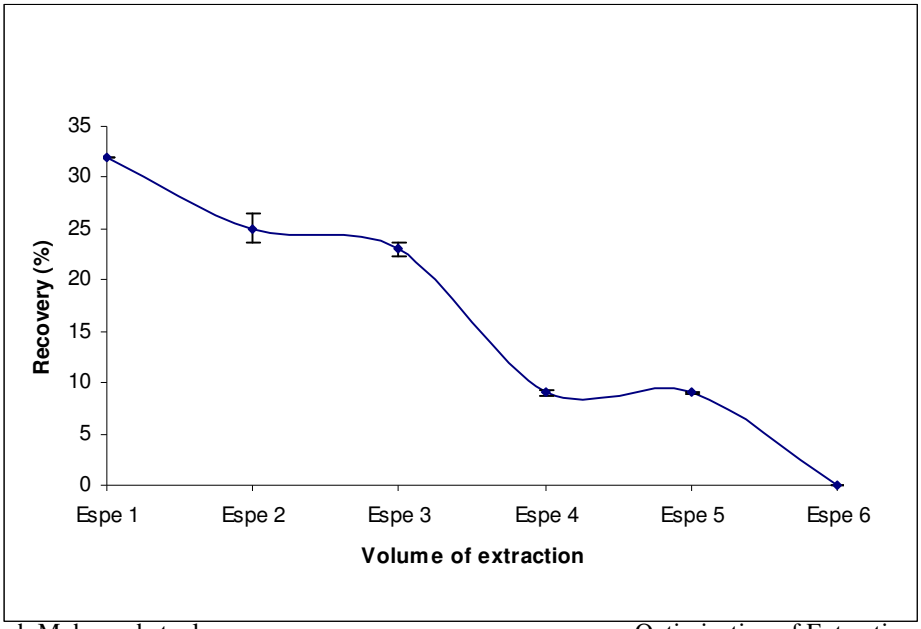

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Figure 1. GC-ECD results showing the percent recovery of chlorpyrifos for each extract in the extraction process.

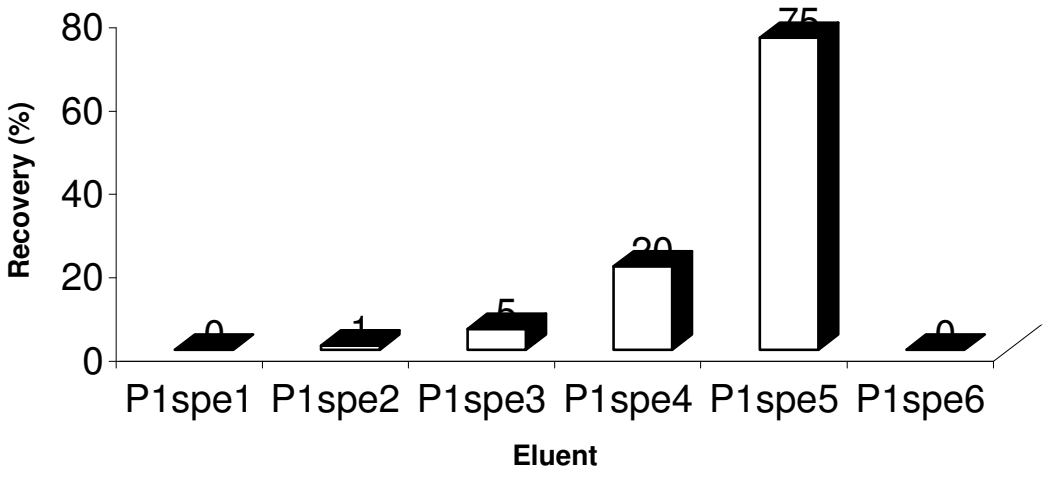

Figure 2. Elution pattern of chlorpyrifos from silica cartridge using 2% ethyl acetate (EA) in hexane

EA = Ethyl acetate
P1spe1 = First elution with 1 mL hexane
P1spe 2 = Second elution with 1 mL hexane
P1spe 3 = Third elution with 1 mL h9exane
P1spe 4 = Fourth elution with 2 mL 2% EA in hexane
P1spe 5 = Fifth elution with 2 mL 2% EA in hexane
P1spe 6 = Sixth elution with 2 mL 2% EA in hexane

Table 2. Recovery of chlorpyrifos from silica cartridge using 5% ethyl acetate (EA) in hexane

Fractions of each eluate	% Recovery	
	2% EA in hexane	5% EA in hexane
1 mL hexane	0	0
4mL	91	0
2mL	8	92
2 mL	0	8
Total	99	100

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