The Malaysian Journal of Analytical Sciences Vol. 12 No. 1 (2008)

COMPARATIVE STUDY ON CLEANUP PROCEDURES FOR THE DETERMINATION OF ORGANOPHOSPHORUS PESTICIDES IN VEGETABLES

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Key words: Solid-phase extraction, organophosphorus pesticides, gas chromatography

Abstract

A study was carried out to compare the cleanup procedures for the determination of organophosphorus pesticides in vegetables. Eleven organophosphorus pesticides were extracted with acetone and methylene chloride. Extracts were cleanup by solid-phase extraction (SPE) mixed-mode column using quaternary amine and aminopropyl (SAX/NH_2) or octadecyl (C_{18}) sorbents. The pesticides were determined by gas chromatography with flame photometric detector. The recovery results obtained from the SPE SAX/NH_2 and C_{18} cleanups in carrot, cucumber and green mustard samples were in the range of 71.0 % to 115 %. Lower recoveries were obtained for polar pesticides, methamidophos and dimethoate. These results were compared to the method currently used in the laboratory which does not include any cleanup.

Abstrak

Satu kajian telah dijalankan untuk membandingkan kaedah-kaedah pembersihan untuk menentukan racun perosak organopfosforus di dalam sayur-sayuran. Sebelas racun perosak organofosforus diekstrak dengan aseton dan dwiklorometana. Ekstrak dibersihkan dengan pengekstrakan fasa pepejal (SPE) menggunakan amina quaterina dan aminopropil (SAX/NH_2) atau turus oktadesil, $C_{18.}$ Racun perosak ditentukan dengan kromatografi gas yang dilengkapkan dengan pengesan fotometrik nyala. Pengembalian racun perosak dalam tiga jenis sayur-sayuran iaitu lobak merah, timun dan sawi hijau adalah di antara 71.0 % dan 115 %. Pengembalian yang rendah diperolehi untuk racun perosak yang berkutub iaitu methamidophos dan dimethoate. Keputusan ini dibandingkan dengan kaedah tanpa pembersihan yang digunakan di makmal pada masa ini.

Introduction

Organophosphorus (OP) pesticides have replaced the organochlorine pesticides due to concern regarding the persistence and polluting effect of these compounds to the environment. However, due to its low persistency, a greater number of applications to a crop may be necessary during the course of the growing season. Numerous methods have been developed for the analysis of OP pesticides in fruits and vegetables. Some of these methods advocate the use of solid-phase extraction (SPE) cartridges. A method using acetonitrile for extraction of pesticide residues in fruits and vegetables was reported [1]. The pesticides were detected on gas chromatograph (GC) equipped with flame photometric detector (FPD). A simple and efficient cleanup method for GC determination of twenty-three OP pesticides in crops was reported [2]. The sample was extracted with acetone and benzene. Cleanup was performed on silica catridges. It was reported that water-soluble pesticides such as dichlorvos and dimethoate gave poor recoveries in all crops. A method for the determination of twenty-eight OP pesticides in fatty and non-fatty foods was reported [3]. Extraction was carried out using acetone and mixture of acetone-water. Carbon-celite was used as cleanup. A multi-residue method for determination of forty-three OP insecticides in plant and animal tissues was reported [4]. The OP insecticides were extracted with methanol-dichloromethane (1 : 9) and cleaned up using gel permeation chromatograph and silica gel mini columns. Determination of OP pesticides in fruits and vegetables using octadecyl, carbon and aminopropyl cartridges was reported [5]. The pesticides were determined by GC equipped with mass selective detector. Gravity-fed C_{18} SPE as cleanup for detection of pesticides in spinach, oranges, tomatoes and peaches was also reported [6]. The method was used to analyze forty-eight OP pesticides. A method using SPE cleanup of chlorpyrifos, methidathion and methyl parathion in oranges was reported [7]. Samples were extracted with anhydrous sodium acetate with ethyl acetate. The ethyl acetate extract was concentrated and cleaned up by passing through tandem

SAX and PSA SPE columns. In this study, the cleanup procedures for the determination of OP pesticides in vegetables using the SPE mixed-mode column; quaternary amine and aminopropyl (SAX/NH_2) and octadecyl (C_{18}) sorbents were employed.

Experimental

Eleven OP pesticides; namely methamidophos, dimethoate, diazinon, tolcofos-methyl, fenitrothion, chlorpyrifos, phenthoate, prothiofos, triazofos, cyanofenfos and azinphos-ethyl were selected for this study. These pesticides were fortified in carrot, cucumber, and green mustard at 0.5 and 0.1 ppm levels. Three replicate fortifications for each matrix type were prepared. Extraction was carried out based on procedures described by Steinwandter [8]. 10 g of sample was homogenised in a blender containing 100 mL acetone, 75 mL dichloromethane and 15 g sodium chloride for three minutes. The organic phase was transferred to a beaker and 3 g of sodium sulphate was added to remove the remaining water. For SPE SAX/NH_2 cleanup, the tube was conditioned with 10 mL of acetone : petroleum ether (1 : 2). 2 mL of extract was transferred to the SPE tube, followed by eluting with 10 mL of conditioning solvent at the flow rate of 1 mL/min. For SPE C_{18}, the tube was conditioned with 10 mL hexane : petroleum ether (1 : 1 v/v). 2 mL of extract was transferred to the tube, followed by eluting with 10 mL conditioning solvent. The eluates collected were analysed by GC-FPD on a HP 5, 15 m x 0.53 mm x 1.5 μm column. A Hewlett-Packard GC 5890 Series II equipped with FPD was used for the determination of the OP pesticides. GC conditions were: injector temperature, 260 °C; detector temperature 250 °C; carrier flow (nitrogen) 4 mL/min; oven temperature, 120 °C (1.0 min), rate 30 °C/min to 150 °C, rate 5 °C/min to 270 °C (10 min); air flow : 80 mL/min; hydrogen flow : 67 mL/min.

Results and Discussion

Recoveries of the OP pesticides at 0.5 ppm and 0.1 ppm levels from carrot samples with SAX/NH_2, C_{18} cleanup and without cleanup are shown in Table 1. At 0.5 ppm fortification level, the recoveries obtained for eleven OP pesticides using the method without cleanup were within the acceptable range of 70 -120 % [9]. The recoveries for these pesticides ranged from 76 % to 108.3 % with CV of 5.0 - 11.0 %. Comparable results were obtained for the SPE SAX/NH_2 cleanup for nine OP pesticides. Their recoveries were in the range of 92.0 % to 115.0 % with CV of 4.0 - 7.2 %. A low recovery of 30.3% was obtained for dimethoate, while methamidophos was totally absorbed in the SPE during the cleanup. Both dimethoate and methamidophos were more polar as compared to the other OP pesticides. The solubility of methamidophos and dimethoate in water are 200,000 mg/L and 25,000 mg/L respectively as compared to 0.05 mg/L for prothiofos [10]. The low recoveries of these polar pesticides were because of their strong retention by polar anion exchange sorbents, SAX/NH_2. For the C_{18} cleanup, the recoveries obtained were within the acceptable range except for methamidophos. Their recoveries were in the range of 80.0 % to 107.7 % with CV of 1.5% to 5.9 %. The recovery obtained for methamidophos was 6.7 %.

Table 1. Recovery of OP pesticides from carrot samples using different cleanup methods

Pesticide	0.5 ppm			0.1ppm		
	SAX/NH_2	C_{18}	No cleanup	SAX/NH_2	C_{18}	No cleanup
	% Rec.[(a)] ± CV	% Rec.[(a)] ± CV	% Rec.[(a)] ± CV	% Rec.[(a)] ± CV	% Rec.[(a)] ± CV	% Rec.[(a)] ± CV
Methamidophos	0	6.7 ± 2.5	76.0 ± 5.0	0	40.7 ± 5.9	108.3 ± 10.5
Dimethoate	30.3 ± 17	107.7 ± 2.3	108.3 ± 6.0	71.0 ± 6.6	99.7 ± 8.7	118.0 ± 3.5
Diazinon	92.0 ± 6.1	92.7 ± 3.2	89.3 ± 9.3	95.7 ± 3.5	93.7 ± 3.1	97.3 ± 4.2
Tolcofos-methyl	113.3 ± 5.8	92.0 ± 1.7	98.7 ± 10.7	94.7 ± 4.0	93.3 ± 6.7	97.0 ± 2.7
Fenitrothion	108.7 ± 5.7	91.7 ± 1.5	102.0 ± 6.1	92.3 ± 4.7	97.0 ± 4.6	110.1 ± 1.2
Chlorpyrifos	112.3 ± 5.8	84.7 ± 5.9	99.3 ± 11	92.7 ± 1.2	97.0 ± 2.0	96.0 ± 2.0
Phenthoate	109.7 ± 5.7	92.3 ± 2.1	97.0 ± 8.9	99.3 ± 2.5	91.0 ± 2.7	94.0 ± 1.7
Prothiofos	115.0 ± 7.2	80.0 ± 1.0	103.7 ± 10	93.0 ± 4.4	85.3 ± 2.5	94.0 ± 1.0
Triazofos	105.3 ± 4.0	102.0 ± 2.0	95.7 ± 8.1	98.0 ± 6.1	92.7 ± 5.7	124.0 ± 1.0
Cyanofenfos	112.7 ± 4.0	88.0 ± 1.7	99.7 ± 7.2	90.3 ± 5.5	96.0 ± 4.4	99.7 ± 0.6
Azinphos-ethyl	102.3 ± 6.8	102.3 ± 5.0	97.3 ± 9.2	97.0 ± 4.4	92.0 ± 6.6	112.0 ± 1.0
AV	107.9	91.7	98.1	92.4	93.8	104.2
SD	7.2	7.2	4.1	8.0	4.0	10.9

AV = average mean CV = coefficient of variation SD = standard deviation (a) n = 3

At 0.1 ppm fortification level, all the OP pesticides except methamidofos showed good recoveries of 71 % to 99.3 % using the SAX/NH_2 cleanup with CV of 1.2 % to 6.6 %. None of the methamidofos was recovered in this cleanup. The higher recovery obtained for dimethoate as compared to 0.5 ppm level showed that the amount of semi polar pesticide being retained by the SAX/NH_2 varied depending on the concentration of the pesticides. Better recoveries were obtained for method without cleanup. The recoveries for the OP pesticides without cleanup ranged from 94.0 % to 124.0 % with CV of 0.6 % to 10.5 %. For C_{18} cleanup, the recoveries obtained for 10 OP pesticides were from 85.3 % to 99.7 % with CV of 2.0 % to 8.7 %. Methamidophos had lower recovery of 40.7 %.

Recoveries of the OP pesticides at 0.5 ppm and 0.1 ppm levels from cucumber samples with SAX/NH_2, C_{18} cleanup and without cleanup are shown in Table 2. At 0.5 ppm fortification level, the recoveries for 9 OP pesticides using the SAX/NH_2 cleanup were within the acceptable range of 80.3 % to 95.3 % with CV of 2.5 % to 7.1 %. Low recovery of 51.7 % was obtained for dimethoate. Methamidophos was totally absorbed during the cleanup. Good recoveries were obtained for method without cleanup. Their recoveries ranged from 69.3 % to 111.0 % with CV of 1.2 % to 6.2 %. For the C_{18} cleanup, the recoveries were from 76.3 % to 104.0 % with CV of 1.5 % to 7.9 %. Low recovery of 8 % was obtained for methamidophos.

Table 2. Recovery of OP pesticides from cucumber samples using different cleanup methods

Pesticide	0.5 ppm			0.1ppm		
	SAX/NH_2	C_{18}	No cleanup	SAX/NH_2	C_{18}	No cleanup
	% Rec.[(a)] ± CV	% Rec.[(a)] ± CV	% Rec.[(a)] ± CV	% Rec.[(a)] ± CV	% Rec.[(a)] ± CV	% Rec.[(a)] ± CV
Methamidophos	0	8.0 ± 4.4	69.3 ± 1.5	0	30.7 ± 1.5	92.0 ± 2.7
Dimethoate	51.7 ± 6.0	104.0 ± 6.2	111.0 ± 6.2	43.3 ± 8.5	111.0 ± 9.5	100.3 ± 10.7
Diazinon	86.7 ± 4.6	82.7 ± 3.2	96.3 ± 4.0	80.7 ± 1.5	86.7 ± 7.1	98.7 ± 1.5
Tolcofos-methyl	87.0 ± 5.3	76.3 ± 1.5	94.3 ± 3.1	71.0 ± 1.0	94.3 ± 8.1	94.0 ± 2.7
Fenitrothion	89.0 ± 6.1	79.7 ± 2.1	98.3 ± 1.5	77.3 ± 3.5	95.7 ± 8.7	101.0 ± 2.7
Chlorpyrifos	82.0 ± 7.0	87.0 ± 7.2	91.7 ± 4.2	92.3 ± 3.1	88.7 ± 7.0	94.3 ± 1.5
Phenthoate	95.3 ± 5.9	92.3 ± 7.0	97.3 ± 1.2	85.3 ± 3.8	103.0 ± 6.0	87.0 ± 7.9
Prothiofos	80.3 ± 7.1	92.3 ± 3.2	92.3 ± 4.0	95.3 ± 2.5	82.7 ± 1.2	93.7 ± 7.1
Triazofos	94.0 ± 3.5	98.3 ± 7.9	97.0 ± 2.0	79.7 ± 1.5	104.3 ± 1.2	99.3 ± 7.1
Cyanofenfos	91.3 ± 6.1	86.7 ± 7.4	89.3 ± 1.5	80.3 ± 4.0	95.7 ± 7.5	91.0 ± 1.7
Azinphos-ethyl	91.3 ± 2.5	96.3 ± 6.7	101.0 ± 4.0	88.3 ± 6.4	89.0 ± 7.9	111.7 ± 2.1
AV	88.5	88.0	95.3	79.3	93.3	98.6
SD	5.1	7.5	3.7	14.6	7.3	11.0

AV = average mean CV = coefficient of variation SD = standard deviation (a) n = 3

At 0.1 ppm fortification level, the recoveries obtained for the nine OP pesticides using SAX/NH_2 cleanup ranged from 71 % to 95.3 % with CV of 1.0 % to 6.4 %. A low recovery of 43.3 % was obtained for dimethoate, while methamidophos was totally absorbed in the sorbent. Good recoveries were obtained for method without cleanup. Their recoveries ranged from 87.0 % to 111.7 %, with CV of 1.5 % to 10.7 %. For C_{18} cleanup, the recoveries obtained for 10 OP pesticides ranged from 86.7 % to 111 % with CV of 1.2 % to 9.5 %. Low recovery of 30.7 % was obtained for methamidophos.

The recovery results obtained from green mustard samples with SAX/NH_2, C_{18} cleanup and without cleanup are shown in Table 3. At 0.5 ppm fortification level, the recoveries obtained for 10 OP pesticides using SAX/NH_2 cleanup were within the acceptable range. They were in the range of 82.0 % to 102.0 % with CV of 2.7 % to 9.9 %. The only pesticide with low recovery was methamidophos. As compared to carrot and cucumber, higher recoveries were obtained for dimethoate. For semi polar pesticides such as dimethoate, the amount of absorption into the SAX/NH_2 also varies depending on the samples types. Therefore, there is a wide variation of recoveries obtained among the three types of vegetables tested. Good recoveries were obtained from the method without cleanup. Their recoveries were in the range of 74.3 % to 118.3 % with CV of 0.6 % to 6.1 %. For C_{18} cleanup, the recoveries obtained were in the range of 71.0 % to 97.3 % with CV of 2.0 to 8.3 %. Lower recovery of 38.0 % was obtained for methamidophos.

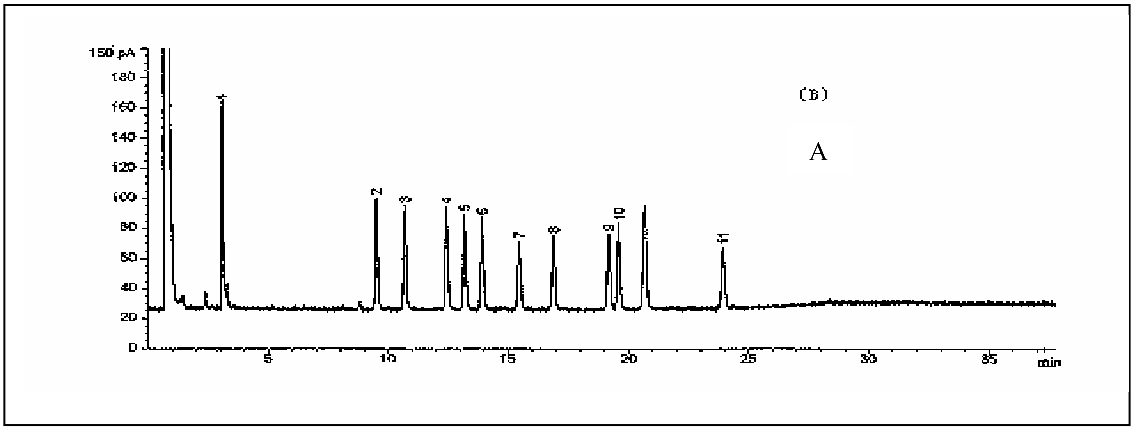
At 0.1 ppm fortification level, the recoveries obtained for nine OP pesticides using SAX/NH_2 cleanup ranged from 77.0 % to 88.0 % with CV of 0.7 % to 5.7 %. As encountered in the cucumber and carrot samples, the recovery for dimethoate was low; 34.5 %, while, methamidophos was totally absorbed by the SPE sorbent. Good recoveries were obtained for method without cleanup. Their recoveries ranged from 86.3 % to 114.7 % with CV of 2.5 % to 10.4 %. For the C_{18} cleanup, the recoveries obtained were in the range of 83.7 % to 106.3 % with CV of 1.7% to 8.7 %. The recovery obtained for methamidophos was 37.0 %.

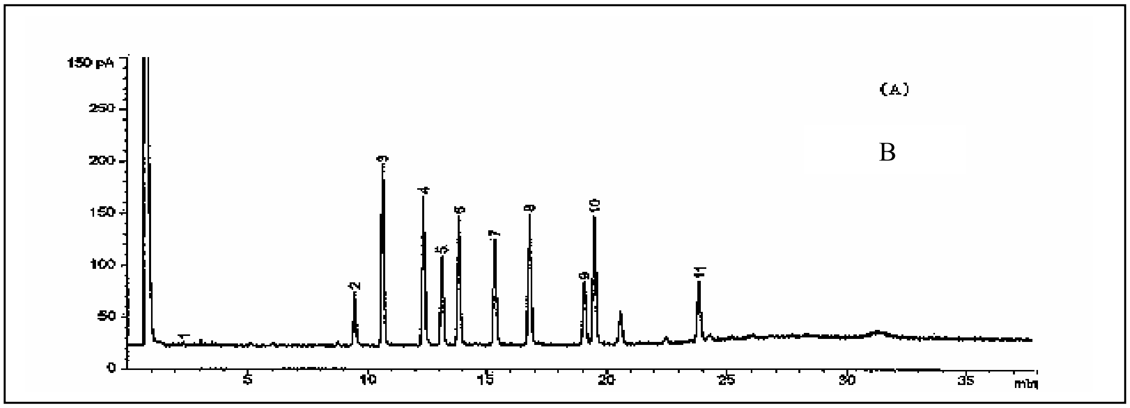
The chromatograms for SPE cleanups and without cleanup are shown in Figure 1. All the chromatograms showed no interference peaks which co-eluted with the 11 OP pesticides. The FPD in phosphorus mode is selective and specific responding only to phosphorus compounds. It was noted that a large peak at 21 min has substantially reduced after the SAX/NH_2 and C_{18} cleanups.

Table 3 Recovery of OP pesticides from green mustard samples using different cleanup methods

Pesticide	0.5 ppm			0.1ppm		
	SAX/NH_2	C_{18}	No cleanup	SAX/NH_2	C_{18}	No cleanup
	% Rec.[(a)] ± CV	% Rec.[(a)] ± CV	% Rec.[(a)] ± CV	% Rec.[(a)] ± CV	% Rec.[(a)] ± CV	% Rec.[(a)] ± CV
Methamidophos	15.3 ± 4.5	38.0 ± 27.8	74.3 ± 1.5	0	37.0 ± 10.2	93.3 ± 10.4
Dimethoate	102.0 ± 9.9	97.3 ± 8.3	118.3 ± 6.1	34.5 ± 9.2	96.7 ± 8.7	99.0 ± 7.6
Diazinon	88.3 ± 6.5	84.0 ± 2.0	92.3 ± 1.5	84.0 ± 5.7	94.7 ± 3.8	91.3 ± 2.5
Tolcofos-methyl	92.0 ± 4.6	80.0 ± 2.0	89.7 ± 1.5	80.5 ± 0.7	104.3 ± 2.3	89.0 ± 4.0
Fenitrothion	91.3 ± 4.7	80.0 ± 7.6	93.3 ± 5.9	77.0 ± 5.7	96.3 ± 3.8	100.3 ± 6.8
Chlorpyrifos	94.0 ± 5.2	71.0 ± 7.9	96.7 ± 2.1	77.5 ± 2.1	83.7 ± 3.1	90.3 ± 3.2
Phenthoate	89.7 ± 4.5	85.7 ± 7.6	93.3 ± 3.2	88.0 ± 1.4	99.3 ± 6.0	86.3 ± 4.9
Prothiofos	90.7 ± 7.4	71.0 ± 3.6	97.3 ± 0.6	78.0 ± 2.8	84.0 ± 5.0	91.3 ± 6.7
Triazofos	86.0 ± 2.7	77.5 ± 7.9	89.7 ± 4.0	80.5 ± 7.8	105.7 ± 4.0	96.3 ± 6.7
Cyanofenfos	88.0 ± 5.3	80.7 ± 5.5	91.3 ± 3.2	84.5 ± 2.1	88.0 ± 1.7	88.3 ± 4.0
Azinphos-ethyl	82.0 ± 4.6	83.0 ± 3.6	92.3 ± 6.1	87.5 ± 3.5	106.3 ± 6.7	114.7 ± 8.1
AV	90.4	81.0	95.5	77.2	95.8	94.7
SD	5.3	7.6	8.4	15.5	8.9	8.4

AV = average mean CV = coefficient of variation SD = standard deviation (a) n = 3





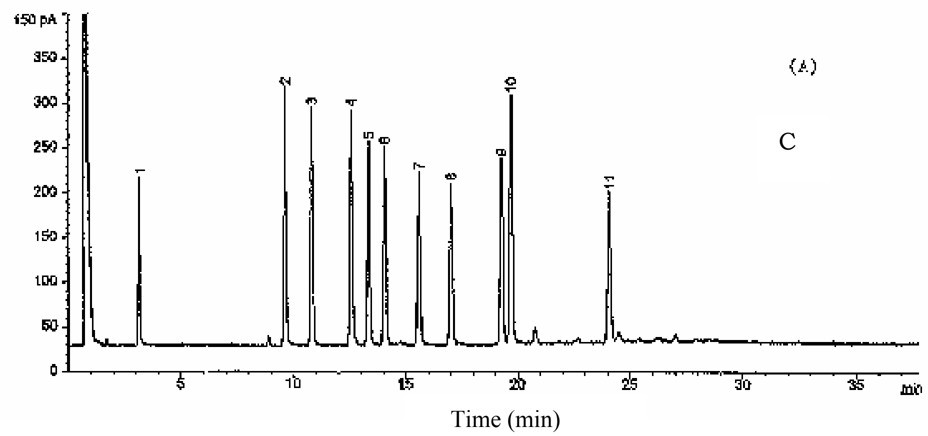


Figure 1. Typical GC chromatogram of vegetable sample without cleanup (A), after SAX/NH_2 cleanup (B) and C_{18} cleanup (C). Peaks : 1, methamidophos; 2, dimethoate; 3, diazinon; 4, tolcofos-methyl; 5, fenitrothion; 6, chlorpyrifos; 7, phenthoate; 8, prothiofos; 9, triazofos; 10, cyanofenfos; 11, azinphos-ethyl

Conclusion

The studies on the SPE cleanup methods showed that SAX/NH_2 and C_{18} have the potentials to be used as cleanup method for OP pesticides in the laboratory. The advantage of the SPE method over the current method without cleanup is that the former removed a substantial amount of coloured compounds and matrix interferences from the samples. This can reduce the cost of GC maintenance especially for the GC inlet and column. Except for more polar pesticides such as methamidofos and dimethoate, comparable results were obtained for the other OP pesticides using SPE cleanup methods. No interfering peaks were encountered in the chromatograms. In spite of the limited range of this study, it can be predicted that the SPE cleanup can be successfully extrapolated to other pesticides and vegetables. The benefits of the SPE method compared to the other cleanup methods were the reduction of organic solvents use, possibility of concentration the samples, less sorbent is used, no cross-contamination and shorter analysis time.

Acknowledgement

The authors wish to thank the Universiti Malaysia Sarawak and Director of Agriculture Sarawak and Senior Assistant Director (Research) for their support of this project and permission to publish this paper. The technical assistance rendered by staff of the Pesticide Residue Laboratory especially Mr. Phillip Gudom is greatly acknowledged.

References

1. Lee, S.M., Papathakis, M.L., Feng, H.M., Hunter, G.F. & Carr. J.E. (1991) Multipesticide residue method for fruits and vegetables. *Fresenius J. Anal. Chem.*, **339**, 376 – 383.
2. Sasaki, K., Takashhi, S. & Saito, Y. (1987) Simplified cleanup method and gas chromatographic determination of organophosphorus pesticides in crops. *J. Assoc. Off. Anal. Chem.*, **70**, 460 – 464.
3. Leoni, V., Caricchia, A.M. & Chiavarini, S. (1992). Multiresidue method for quantitation of organophosphorus pesticides in vegetable and animal foods. *J. AOAC Int.* **75**, 511 - 518.
4. Holstege, D.M., Scharberg, D.L., Richardson, E.R. & Moller, G. (1991). Multiresidue screen for organophosphorus insecticides using gel permeation chromatography-silica gel cleanup. *J. Assoc.Off. Anal. Chem.* **72,** 394 – 399.
5. Fillion, J., Saure, F. & Selwyn, J. (2000) Multiresidue methods for the determination of residues of 251 pesticides in fruits and vegetables by gas chromatography with florescence detection. *J.AOAC Int.*, **83**, 698 – 713.
6. Cook, J., Beckett, M.P., Reliford, B., Hammock, W. & Engel, M. (1999) Multiresidue analysis of pesticides in fresh fruits and vegetables using procedures developed by the Florida Department of Agriculture and Consumer Services *J. AOAC Int.*, **82**, 1419 - 1435.
7. Yamazaki, Y. & Ninomiya, T. (1999) Determination of benomyl, diphenyl, o-phenylphenol, thiabendazole, chlopyrifos, methidation and methyl parathion in oranges by solid-phase extraction, liquid chromatography and gas chromatography. *J. AOAC Int*, **82**, 1474 – 1478.
8. Steinwandter, H. (1985) Universal 5-min on-line method for extracting and isolating pesticide residues and industrial chemicals. *Fresenius Z. Anal. Chem.*, **322,** 752 – 754.

9. Parker, G.A. (1991). Validation of methods used in the Florida Department of Agriculture and Consumer Services Chemical Residue Laboratory. *J. Assoc. Off. Anal.Chem*. **74,** 868 - 871.
10. Worthing, C.R., & Hance, R.J. (1991). *The Pesticide Manual*, 9th Ed. Surrey : Unwin Brothers Limited.