

Determination of Pyrethroid Pesticides in Vegetables by Solid-Phase Extraction Cleanup and Gas Chromatography

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ABSTRAK

Satu kaedah yang ringkas dan cepat telah dihasilkan untuk menentukan lima jenis racun perosak pirethroid pada sayur-sayuran. Sisa baki racun perosak diekstrak dengan aseton dan metilena klorida. Ekstrak dibersihkan dengan turus ekstraksi fasa pepejal amina kuaterner dan aminopropil. Racun perosak ditentukan dengan kromatografi gas yang dilengkapi dengan pengesan tangkapan elektron. Pemulihan daripada tiga jenis sayur-sayuran iaitu lobak merah, timun dan sawi hijau pada aras 0.25 - 0.5 mg/kg adalah di antara 72.0 dan 117.0%. Keputusan ini adalah setanding dengan yang diperolehi daripada kaedah pembersihan melalui gel silika.

ABSTRACT

A simple and rapid method has been developed for the simultaneous determination of five pyrethroid pesticides in vegetables. Residues were extracted with acetone and methylene chloride. Extracts were cleaned up by solid-phase extraction mixed-mode column using quaternary amine and aminopropyl sorbents. The pesticides were determined by gas chromatography with electron capture detector. The recoveries from three vegetables: carrot, cucumber and green mustard, spiked at 0.25-0.5 mg/kg were 72.0 to 117.0%. The results were comparable to those obtained by the silica gel cleanup method.

Keywords: Solid-phase extraction, pyrethroid pesticides, gas chromatography

INTRODUCTION

The use of pyrethroid insecticides in agriculture has increased rapidly since the development of the first pyrethroid, permethrin, in the 1970s. This is because of their desirable environmental properties of short persistence and non-toxicity to mammals. These features, combined with their broad spectrum of insecticidal activity and the comparatively low application rates required for insect control, have made the pyrethroid an environmentally safe and acceptable alternative to organochlorine pesticides. In Malaysia, they are widely used for vegetable cultivation throughout the growing season. As these insecticides have a shorter preharvest interval of between 3 - 5 days, they are the preferred choice of insecticides near harvest, as they do not pose a residue problem. Based on the residue survey carried out by the Department of Agriculture, Sarawak from 1987 - 2000, these insecticides constitute only a small portion of the total violation even though they are the most widely used pesticides by vegetable farmers in Sarawak (Chai 2000).

Pyrethroid pesticides are usually more difficult to determine than the organophosphates because of their appreciably lower levels of application. These compounds consist of a mixture of stereoisomers. Therefore, separation is more easily carried out on capillary column. Several methods for the determination of pyrethroids have been reported. Baker *et al.* (1982) developed a multi-residue method for the determination of pyrethroids in fruit and vegetables. Residues were first extracted with hexane and acetone. The extracts were then cleaned up on silica gel column and determined by gas chromatography (GC) with electron capture detector (ECD) and liquid chromatography (LC). Procedures based on GC have also been reported by Bolgyo *et al.* (1983) for the determination of six pyrethroid pesticides in fruits, vegetables and leaves by using acetone and methylene chloride extraction and carbon-magnesia-diatomaceous earth and alumina cleanup. Bottomley *et al.* (1984) have determined pyrethroid pesticides in grain using LC. Samples were extracted with acetone and methanol and cleaned up on aluminium oxide column.

Pang *et al.* (1994) described a simple and rapid packed column GC method for the simultaneous determination of ten pyrethroid insecticides in grains, fruits and vegetables using acetone and petroleum ether extraction. The extracts were cleaned up on a florisil column or a column consisting of a mixture of florisil, charcoal and alumina. A GC method for the determination of nine pyrethroid insecticides in products of animal origin was reported (Pang *et al.* 1994). Pang *et al.* (1995) determined nine pyrethroid insecticides in fruits and vegetables using methanol and toluene as extractants followed by florisil and charcoal column chromatography cleanup. Braun *et al.* (1982) reported a multi-residue method for the determination of pyrethroid pesticides in celery and animal products.

Numerous methods for the determination of the residues of pyrethroid pesticides in different matrices using solid phase extraction (SPE) cleanup have been developed. Bennett *et al.* (1997) developed a method for the determination of permethrin and other pesticides in liquid whole milk using octadecyl (C18) and aminopropyl (NH₂) silica cartridges. Jasson *et al.* (2000) reported good recoveries for twenty six pesticides including pyrethroid pesticides, lambda-cyhalothrin, cyfluthrin, esfenvalerate and deltamethrin in honey using Isolute ENV+. Mogadati *et al.* (1999) described a method using C18 and LC-amino for the cleanup of soil extracts prior to gas chromatography/mass spectrometry (GC/MS) determination. Sheridan *et al.* (1999) applied GC/MS for the determination of pyrethroid pesticides in fruits, vegetables and milk using quaternary amine (SAX) and ethylenediamine-N-propyl (PSA) as sorbents. Cook *et al.* (1999) reported C18, florisil and aminopropyl SPE cleanup for the determination of cypermethrin, fenvalerate, permethrin and other pesticides in fruits and vegetables. Fillion *et al.* (2000) described the removal of co-extractives in fruits and vegetables using C18 and NH₂ SPE cleanup, followed by GC/MS and LC fluorescence detection.

This paper reports the extraction of pyrethroid pesticides in vegetables using acetone and methylene chloride followed by clean up on SPE SAX/NH₂ and determination on GC-ECD. The results obtained were compared to the current method using silica gel column chromatography cleanup.

METHOD

Chemicals and Reagents

All the chemicals and reagents were of analytical grade. Pesticide standards were obtained from Dr. Ehrenstorfer™, Germany. SPE tubes, Isolute SAX/NH₂ (1 g sorbent in 6 mL tube) were purchased from International Sorbent Technology.

Instrumentation

A Hewlett-Packard 6890 gas chromatograph equipped with electron capture detector was used for the determination of pyrethroid pesticides. GC conditions were: injector temperature, 260°C; detector, 280°C; carrier flow (nitrogen) 1.2 mL/min; oven temperature, 120°C (0.5 min), rate 10°C/min to 180°C, rate 6°C/min to 240°C, rate 10°C/min to 280°C (12 min). Pyrethroid pesticides were analysed on HP Ultra 1, 25 m x 0.32 mm x 0.5 mm column.

Procedures

The method was validated using five pyrethroid pesticides, namely lambda-cyhalothrin, permethrin, cypermethrin, fenvalerate and deltamethrin fortified in carrot (*Daucus carota*), cucumber (*Cucumis sativus*) and green mustard (*Brassica chinensis*). Each sample was fortified with 0.5 ppm of each pesticide except lambda-cyhalothrin with 0.25 ppm. Three replicate fortifications for each matrix type were prepared.

Extraction was carried out using procedures described by Steinwandter (1985). A weight of 50 g sample was homogenised in a blender containing 100 mL acetone, 75 mL dichloromethane and 15 g sodium chloride for 3 min. The homogenised mixture was allowed to separate into organic and aqueous layers. The organic layer was transferred to a beaker and 3 g of sodium sulphate was added to remove any remaining water.

For the current method, 2 mL of extract was transferred to a chromatographic column filled with 10 g of silica gel. The column was eluted with 80 mL of methylene chloride-hexane mixture (1:1 v/v). The eluant was analysed for pyrethroid pesticides on GC-ECD.

For the SPE method, SAX/NH₂ sorbent was used for pyrethroid cleanup. The SAX/NH₂ was conditioned with 10 mL of acetone : petroleum ether (1:2). 2 mL of the extract was transferred to the SPE tube. The SPE tube was eluted with 10 mL of conditioning solvent at a flow rate of 1 mL/min. The eluant was analysed for pyrethroid pesticides using GC-ECD.

Statistical Analysis

The t-test was used to determine the significance of the difference between the current method and SPE method in terms of recoveries. They were considered significantly different when values of t (calculated) $>$ t (critical) at 95% confidence level.

RESULTS AND DISCUSSION

Optimisation of SPE Conditions

The SPE conditions were optimised to obtain good recoveries for pyrethroid pesticides. Among the parameters studied were flow rate and solvent polarity. The studies concluded that a flow rate of 1 mL/min to 1.5 mL/min was sufficient to recover the pyrethroid pesticides above 67% when the combination of acetone/hexane or acetone/petroleum ether was used. The flow rate can be increased to 3 - 4 mL/min without affecting the efficiency of recovery. Flow rates of less than 1 mL/min resulted in low recoveries of less than 35% for all the pesticides studied.

Development of an optimum solvent system for pyrethroid pesticides was based on several criteria. Solvents, which were hazardous or expensive to be disposed of, were not evaluated. A volatile solvent system must be used, as rapid evaporation of a large volume of solvent would be required in sample preparation without causing the loss of volatile pesticides. The solvent system must be sufficiently polar to extract most polar pesticides. The final sample extract should have only minimal co-extractives interference.

Solvent mixtures consisting of any two of the following solvents, namely acetone, hexane and petroleum ether in various ratios (v/v) were used in this study. Low polarity solvent mixture such as acetone : hexane (5:95) resulted in excellent recoveries for pyrethroid pesticides. Increasing the solvents' polarity did not result in losses of pesticides. An attempt was made to use more polar eluting solvents such as acetone : petroleum ether (4:1). This resulted in good recoveries for all the pyrethroid pesticides studied. The critical factor to be observed was that the SPE tube should not be left dry after the samples were eluted. A volume of 10 mL of eluting solvents was sufficient to condition the SPE tube and to elute the analytes. Increase of the volume of solvent for elution has not improved the recoveries.

The study has found the optimal conditions for the extraction of pyrethroid pesticides to be as follows : (a) solvent mixture : acetone : petroleum ether (1:2); (b) volume of eluting solvent : 10 mL; (c) flow rate of elution : 1 mL/min. The study was conducted at room temperature (25°C).

Recovery Studies

Five pyrethroid pesticides, namely lamda-cyhalothrin, permethrin, cypermethrin, fenvalerate and deltamethrin, were selected for this study. A concentration of 0.5 ppm of pesticides was leached through SPE, SAX/NH₂ using the optimum conditions. The results are given in Table 1. The recoveries for pyrethroid

pesticides ranged from 67.3 to 114.7% with the coefficient of variation (CV) ranging from 3.1 to 11.5%.

Method Validation

Three types of vegetables, namely carrot, cucumber and green mustard, which represent root, cucurbits and brassica family, were selected for this study. As each group contains different chemical compounds, they may co-elute with the pyrethroid pesticides. This may interfere with the GC analysis of sample extracts.

a. Carrot

Recoveries of pyrethroid pesticides from carrot samples using SAX/NH₂ and silica gel column chromatography cleanup are shown in Table 2. For silica gel cleanup (the reference method), the recoveries ranged from 91.0 to 116.7% with CV of 1.2 to 6.7%. Comparable results were obtained from SPE SAX/NH₂ cleanup method with recoveries ranging from 92.3 to 117% with CV of 3.5 to 11.4%. When comparing these two methods, the t-value obtained was 0.65 with 4 degrees of freedom. As this value was lower than the calculated t-value, there was no significant difference between these two methods.

Chromatograms for carrot samples using SAX/NH₂ and silica gel cleanup are shown in Figs. 1 and 2, respectively. Both chromatograms showed no interference to peaks identified for pyrethroid pesticides. However, more unknown peaks were observed in the GC chromatogram of carrot extract after SPE cleanup than that after silica gel cleanup. This may be due to smaller amounts of sorbent used in SPE cleanup (1 g) than those in silica gel cleanup (10 g). The colour of both extracts was yellow. It is proposed that additional cleanup using SPE solvent such as C18 is required to remove the excessive co-extractives present in the sample. Several researchers have used more than two sorbents for the removal of co-extractives (Sheridan and Meola 1999; Cook *et al.* 1999; Fillion *et al.* 2000). Alternatively, a smaller sample size of 10 - 20 g is suggested instead of 50 g. This can reduce the matrix interferences substantially. In the SPE cleanup method, only 10 mL of solvent was used as compared to

TABLE 1
Recovery of pyrethroid pesticides

Pesticide	Recovery ^(a) %	CV %
Lamda-cyhalothrin	85.3	9.5
Permethrin	103.0	11.5
Cypermethrin	114.7	3.1
Fenvalerate	110.3	4.9
Deltamethrin	67.3	3.2

(a) : n = 3

CV : coefficient of variation

80 mL in the conventional silica gel cleanup. The SPE cleanup allows automation and thus shortens the time needed for the analysis. These advantages in the SPE method will be most welcome in routine analysis that requires high output. Contamination can be eliminated in SPE as the tube is discarded after use. No hazardous solvent such as methylene chloride was used in the SPE method.

b. Cucumber

The results of pyrethroid pesticides recovery from spiked cucumber samples using the SAX/NH₂ and silica gel cleanup methods are given in Table 3. For the SAX/NH₂ method, the recoveries were 85 to 97.7% with CV of 3.5 to 9.0%.

TABLE 2
Recovery of pyrethroid pesticides from spiked carrot samples using SAX/NH₂ cleanup and silica gel cleanup

Pesticide	SAX/NH ₂		Silica gel (reference method)	
	Recovery ^(a) %	CV %	Recovery ^(a) %	CV %
Lamda-cyhalothrin	105.7	11.4	100.3	1.2
Permethrin	96.0	3.6	106.3	2.1
Cypermethrin	106.0	3.5	111.7	6.7
Fenvalerate	117.0	3.5	116.7	5.7
Deltamethrin	92.3	4.2	91.0	3.6
AV	103.4		105.2	
SD	9.7		10.0	

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

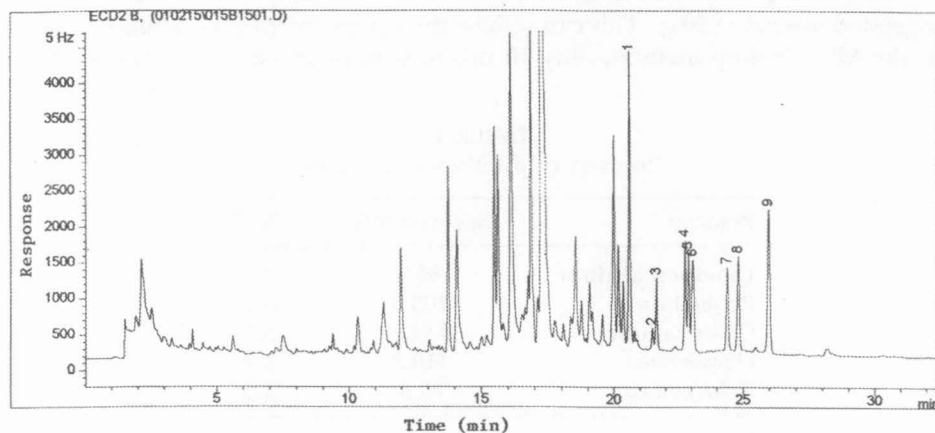


Fig. 1: GC chromatogram of carrot extract after SPE cleanup. Peaks: 1, lamda-cyhalothrin; 2, 3, permethrin; 4, 5, 6, cypermethrin; 7, 8, fenvalerate; 9, deltamethrin

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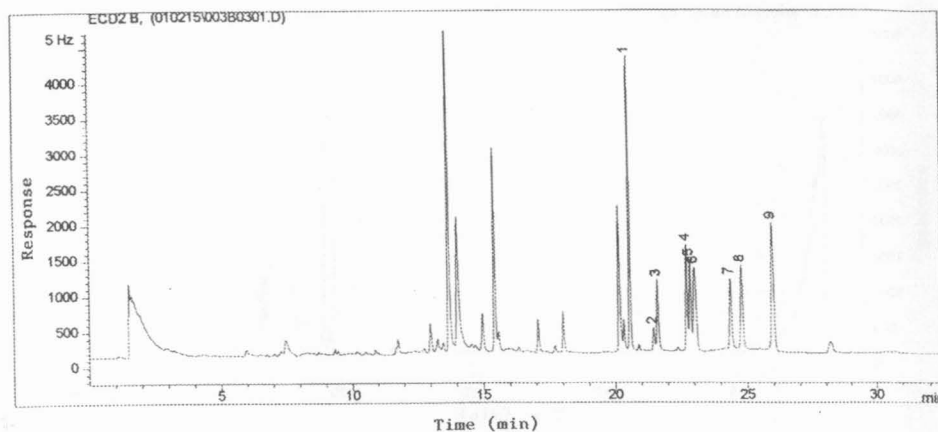


Fig. 2: GC chromatogram of carrot extract after silica gel cleanup. Peaks: 1, lamda-cyhalothrin; 2, 3, permethrin; 4, 5, 6, cypermethrin; 7, 8, fenvalerate; 9, deltamethrin

Corresponding results of 76.3 to 100.0% were obtained for the silica gel method. The CV was between 2.7 to 7.6%. Statistical analysis showed that there was no significant difference between these two methods as the t-value obtained was lower than the critical value. The chromatograms for the SPE and silica gel cleanup methods are shown in Figs. 3 and 4, respectively. The extract of cucumber was colorless after the SPE or silica gel cleanup. The chromatograms showed no interfering peaks after cleanup. This is because cucumber contains less interfering compounds, which may respond more to ECD, compared to the carrot samples. It was concluded that single SPE SAX/NH₂ was sufficient for the cleanup of cucumber samples.

TABLE 3
Recovery of pyrethroid pesticides from spiked cucumber samples using SAX/NH₂ and silica gel cleanup

Pesticide	SAX/NH ₂		Silica gel (reference method)	
	Recovery ^(a) %	CV %	Recovery ^(a) %	CV %
Lamda-cyhalothrin	85.0	3.5	76.3	3.5
Permethrin	93.0	3.5	85.0	2.7
Cypermethrin	93.0	5.6	91.7	5.0
Fenvalerate	97.7	9.0	100.0	7.0
Deltamethrin	96.3	8.7	90.0	7.6
AV	93.0		88.0	
SD	4.9		8.7	

AV : average mean

SD : standard deviation

CV : coefficient of variation

(a) : n = 3

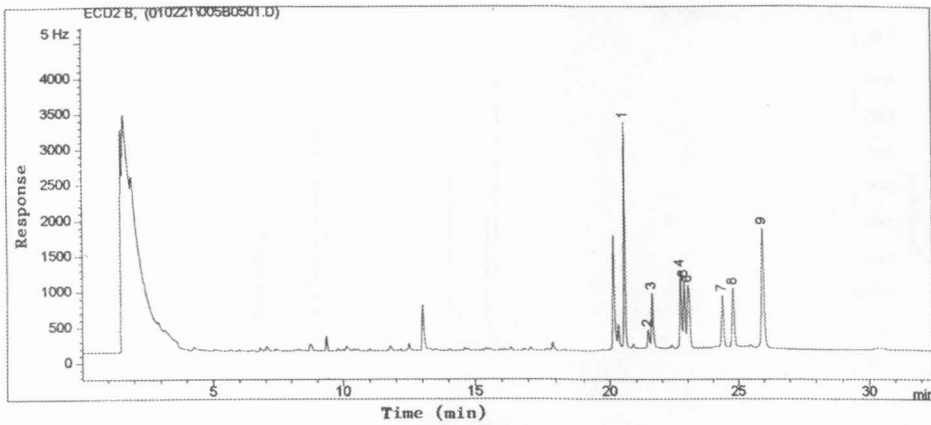


Fig. 3: GC chromatogram of cucumber extract after SPE cleanup. Peaks: 1, lamda-cyhalothrin; 2, 3, permethrin; 4, 5, 6, cypermethrin; 7, 8, fenvalerate; 9, deltamethrin

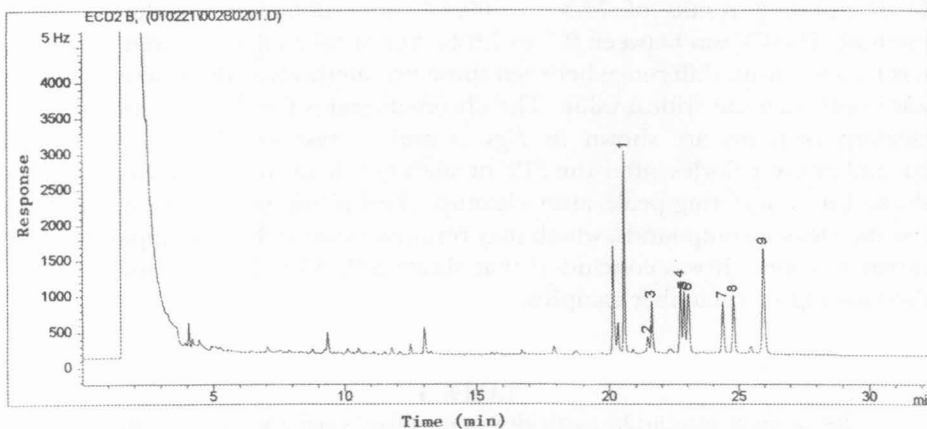


Fig. 4: GC chromatogram of cucumber extract after silica gel cleanup. Peaks: 1, lamda-cyhalothrin; 2, 3, permethrin; 4, 5, 6, cypermethrin; 7, 8, fenvalerate; 9, deltamethrin

c. Green Mustard

The results of pesticide recovery from spiked green mustard samples using SAX/ NH_2 and silica gel cleanup are given in Table 4. The recoveries obtained from the SPE cleanup were slightly lower than those obtained from the silica gel cleanup. However, the recoveries were still within the acceptable range. The recoveries for the SAX/ NH_2 cleanup were 72 to 81.7% with CV of 4.6 to 11.9%. Higher recoveries of 92 to 96% were obtained for the silica gel cleanup with CV of 3.0 to 11.2%. Statistical analysis showed that there was a significant difference between these two methods. Modification in terms of solvent mixture may improve the recoveries. Further optimisation work is required for these types of samples.

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TABLE 4
Recovery of pyrethroid pesticides from spiked green mustard samples using SAX/NH₂ and silica gel cleanup

Pesticide	SAX/NH ₂		Silica gel (reference method)	
	Recovery ^(a) %	CV %	Recovery ^(a) %	CV %
Lamda-cyhalothrin	75.0	6.0	95.7	11.2
Permethrin	75.3	4.6	92.0	3.0
Cypermethrin	73.7	11.9	94.7	8.7
Fenvalerate	81.7	9.6	96.0	8.7
Deltamethrin	72.0	7.6	93.0	6.2
AV	75.5		94.3	
SD	3.7		1.7	

AV : average mean

SD : standard deviation

CV : coefficient of variation

(a) : n = 3

The chromatograms for the SPE and silica gel cleanup are shown in Figs. 5 and 6 respectively. The extract obtained after the SPE cleanup was light green as compared to yellow in the silica gel cleanup. Despite this, no interfering peak was observed in the SPE cleanup method and the chromatogram was comparable to that obtained from the silica gel cleanup. Apparently, both methods had successfully removed the interfering co-extractives prior to GC determination. It was concluded that only one single SPE sorbent was sufficient to perform the sample cleanup. Nonetheless, the relatively lower percentage of recovery from the SPE cleanup method needs further investigation.

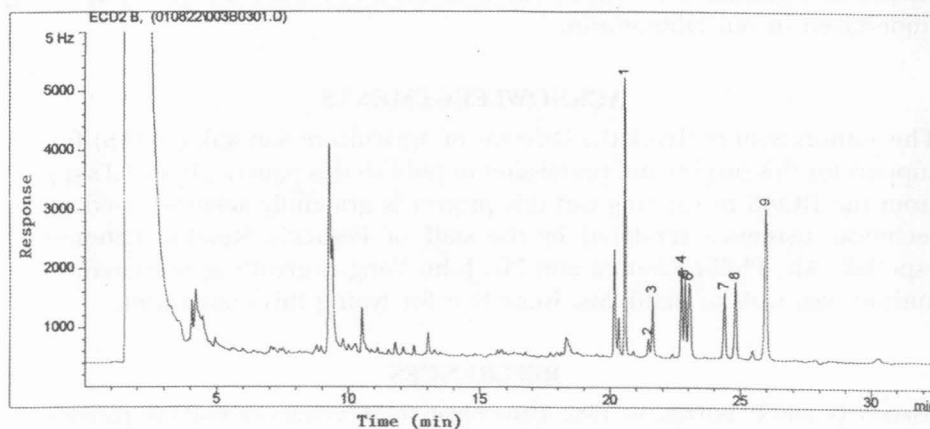


Fig. 5: GC chromatogram of green mustard extract after SPE cleanup. Peaks: 1, lamda-cyhalothrin; 2, 3, permethrin; 4, 5, 6, cypermethrin; 7, 8, fenvalerate; 9, deltamethrin

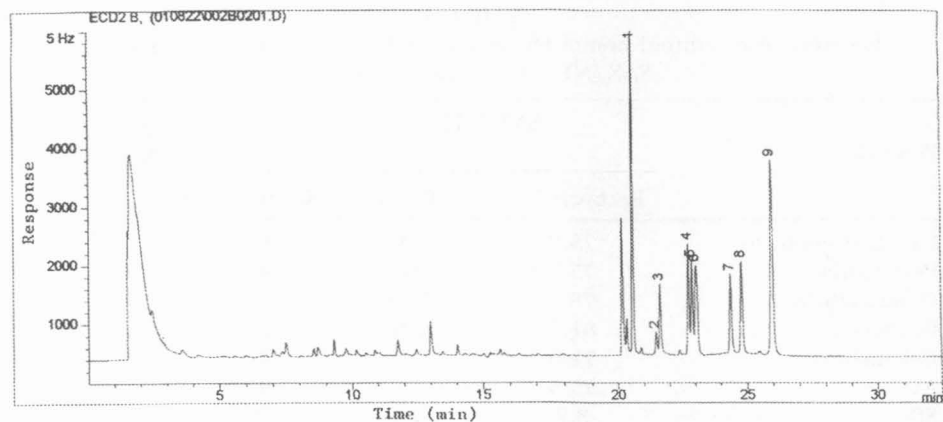


Fig. 6: GC chromatogram of green mustard extract after silica gel cleanup. Peaks: 1, lambda-cyhalothrin; 2, 3, permethrin; 4, 5, 6, cypermethrin; 7, 8, fenvalerate; 9, deltamethrin

CONCLUSION

The results from this study showed that SAX/ NH_2 is suitable for the cleanup of vegetable extracts for pyrethroid pesticides. Recoveries obtained were comparable to those obtained from the established silica gel cleanup method. The SPE sorbent was found to have better efficiency than silica gel, particularly on the cleanup of cucumber and green mustard extracts. This study has shown that the SPE method has great potential as an alternative to the conventional silica gel cleanup method and it is possible to extend the application for the analysis of pesticides in a wider range of vegetables and fruit crops. The benefits of the SPE method are : less solvent consumption, no cross-contamination, shorter analysis time and the process can be automated. Further studies to optimise the sample size and detection limit are currently being undertaken in our laboratories.

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REFERENCES

- BAKER, P.G. and P. BOTTOMLEY. 1982. Determination of residues of synthetic pyrethroids in fruit and vegetables by gas-liquid and high-performance liquid chromatography. *Analyst* **107**: 201 - 212.

Determination of Pyrethroid Pesticides in Vegetables

- BENNETT, D.A., A.C. CHUNG and S.M. LEE. 1997. Multiresidue method for analysis of pesticides in liquid whole milk. *J. of AOAC International* **80**: 1065 - 1077.
- BOLYGO, E. and F. ZAKAR. 1983. Gas-liquid chromatographic screening method for six synthetic pyrethroid insecticides. *J. Assoc. Off. Anal. Chem.* **66**: 1013 - 1017.
- BOTTOMLEY, P. and P.G. BAKER. 1984. Multi-residue determination of organochlorine, organophosphorus and synthetic pyrethroid pesticides in grain by gas-liquid and high-performance liquid chromatography. *Analyst* **109**: 85 - 109.
- BRAUN, H.E. and J. STANEK. 1982. Application of the AOAC multi-residue method to determination of synthetic pyrethroid residues in celery and animal products. *J. Assoc. Off. Anal. Chem.* **65**: 685 - 689.
- CHAI, L.K.A. 2000. Pesticide residues in local vegetables (unpublished).
- COOK, J., M.P. BECKETT, B. RELIFORD, W. HAMMOCK and M. ENGEL. 1999. Multiresidue analysis of pesticides in fresh fruits and vegetables using procedures developed by the Florida Department of Agriculture and Consumer Services. *J. of AOAC International* **82**: 1419 - 1435.
- FILLION, J., F. SAURE and J. SELWYN. 2000. Multiresidue method for the determination of residues of 251 pesticides in fruits and vegetables by gas chromatography with fluorescence detection. *J. of AOAC International* **83**: 698 - 713.
- JANSSON, C. 2000. Multiresidue method for the gas chromatographic determination of pesticides in honey after solid-phase extraction cleanup. *J. of AOAC International* **83**: 714 - 727.
- MOGADATI, P., J.B. LOUIS and J.D. ROSEN. 1999. Multiresidue determination of pesticides in high organic content soils by solid-phase extraction and gas chromatography/mass spectrometry. *J. of AOAC International* **82**: 705 - 715.
- PANG, G.F., Y.Z. CHAO, X.S. LIU and C. FAN. 1995. Multiresidue liquid chromatographic method for simultaneous determination of pyrethroid insecticides in fruits and vegetables. *J. of AOAC International* **78**: 1474 - 1480.
- PANG, G.F., C.L. FAN and Y.Z. CHAO. 1994. Packed-column gas chromatographic method for the simultaneous determination of 10 pyrethroid insecticide residues in fruits, vegetables and grains. *J. of AOAC International* **77**: 738 - 747.
- PANG, G.F., T.S. ZHAO, Y.Z. CHAO and C.L. FAN. 1994. Cleanup with two florasil columns for gas chromatographic determination of multiple pyrethroid insecticides in products of animal origin. *J. of AOAC International* **77**: 1634 - 1638.
- SHERIDAN, R.S. and J.R. MEOLA. 1999. Analysis of pesticide residues in fruits, vegetables, and milk by gas chromatography/tandem mass spectrometry. *J. of AOAC International* **82**: 982 - 990.
- 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.