

Solid Phase Extraction Cleanup for the Determination of Organochlorine Pesticides in Vegetables

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Abstract: A simple solid-phase extraction (SPE) cleanup method has been developed for the determination of organochlorine (OC) pesticides in vegetables. Pesticide residues are extracted with acetone and dichloromethane. Extracts are further cleaned up with an octadecyl, C₁₈ SPE column. The pesticides retained in the column were eluted out with hexane and petroleum ether and quantitatively determined by gas chromatography (GC) using micro electron capture detector (μ -ECD). The recovery obtained for nine OC pesticides in three vegetables; carrot, cucumber and green mustard fortified at 0.1 mg/kg and 0.5 mg/kg were in the range of 62.0 % to 105.0 %. The results obtained were compared with the silica gel chromatography column cleanup currently used in the laboratory.

Abstrak : Satu kaedah yang ringkas telah dihasilkan untuk menentukan sembilan jenis racun perosak organoklorin pada sayur-sayuran. Sisabaki racun perosak diekstrak dengan aseton and metilena klorida. Ekstrak dilarutkan melalui turus ekstraksi pepejal oktadesil, C₁₈. Racun perosak yang terserap dalam turus kemudiannya dielusikan dengan heksana dan petroleum eter dan ditentukan dengan kromatografi gas yang dilengkapi dengan pengesan tangkapan electron mikro. Pengembalian untuk sembilan jenis racun perosak organoklorin pada sayur-sayuran iaitu lobak merah, timun dan sawi hijau pada aras 0.1 mg/kg dan 0.5 mg/kg adalah di antara 62.0 % dan 105.0 %. Keputusan ini di bandingkan dengan kaedah pembersihan turus kromatografi gel silika yang digunakan di makmal pada masa kini.

Key words: solid-phase extraction, silica gel cleanup, organochlorine pesticides

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Introduction

Organochlorine (OC) pesticides have been used extensively worldwide in the early 1950s. However, due to their persistency in the environment, most of these pesticides are no longer allowed to be used in many countries. But some developing countries still allow their use in agriculture and public health. Because of its highly lipophilic properties, these pesticides have led to contamination of the environment and the food chain. The occurrence of the OC pesticides in the food has been reported [1,2].

Several methods have been reported for the analysis of OC pesticides using the SPE cleanup. A method for the determination of OC pesticides in honey has been reported [3]. The sample was extracted with methanol, followed by a cleanup step through a C₁₈ cartridge and final elution with hexane. The recoveries were found to be efficient in most of the OC pesticides studied. A method for the determination of OC pesticides in fatty food using SPE florisil cartridges cleanup has also been reported [4]. Petroleum ether-ethyl ether was used as eluent for the SPE. Hsu *et al.* [5] reported the C₁₈ and florisil SPE cleanups for halogenated organic pesticides in broccoli, carrot, celery and orange. The C₁₈ was eluted with n-hexane, followed by 5 %

acetone/hexane and 10 % of acetone/hexane was used to elute the florisil. Lee *et al.* [6] reported a method for 7 chlorinated hydrocarbons using C₁₈ SPE cleanup for potato, tomato, orange, carrot, broccoli and melon. The sample was extracted with acetonitrile and the extract was leached through the SPE without using any solvent for elution. Holstege *et al.* [7] reported a multi-residue method capable of detecting 17 OC pesticides in plant and animal tissue samples. Samples with high lipid content were cleaned up by automated gel permeation chromatography and silica gel mini-columns. Highly pigmented samples were cleaned up with florisil and aminopropyl SPE columns. Fillion *et al.* [8] reported a method for the determination of pesticides in fruit and vegetable samples. Co-extractives are removed by passing the acetonitrile extract through octadecyl (C₁₈), carbon and aminopropyl SPE columns. Bordet *et al.* [9] reported a method for the determination of OC and other pesticides in milk, fish, eggs and beef fat. Extracts were cleaned up on SPE C₁₈ and florisil. The SPE C₁₈ was conditioned with petroleum ether, acetone, and methanol and acetonitrile was used eluent. Yague *et al.* [10] reported a multi-residue method for the determination of OC pesticides in yogurt. C₁₈ and alumina columns were used as

cleanup. The C₁₈ column was conditioned with light petroleum, acetone and methanol and the pesticides were eluted with n-hexane.

This study looks into the possibility of using the SPE C₁₈ as an alternative cleanup method to the silica gel chromatography cleanup currently used in the Agriculture Research Centre laboratory for the analysis of pesticide residues in vegetables.

Materials and Methods

Chemicals and Reagents

All the chemicals and reagents used were of analytical grade. Pesticide standards were obtained from Ehrenstorfer Company. SPE tubes, Isolute C₁₈ (1 g sorbent in 6 ml tube) were purchased from International Sorbent Technology.

Instrumentation

An Agilent 6890 GC equipped with micro Electron Capture Detector (μ ECD) was used for the determination of the OC pesticides. The GC conditions were: Injector temperature, 260°C; detector 300°C; carrier flow (nitrogen) 1.2 ml/min; oven temperature, 100°C (0.2 min), rate 20°C/min to 180°C, rate 2°C/min to 250°C, rate 50°C/min to 300°C (5 min). The OC pesticides were analysed on an Ultra 1, 25 m x 0.32 mm x 0.5 μ m column.

Procedures

Nine OC pesticides, namely gamma-benzenehexachloride (γ -HCH), heptachlor, alpha-endosulphan, dieldrin, beta-endosulphan, p,p'-DDT (dichlorodiphenyltrichloroethane), o,p'-DDT, endosulfan sulphate and p,p'-DDD (1,1-dichloro-2,2-bis(p-chlorophenyl)ethane) 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 [11]. 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 homogenised mixture was allowed to separate into its organic and aqueous layers. The organic phase was transferred to a beaker and 3 g of sodium sulphate was added to remove the remaining water. For the current method, 2 ml of extract was transferred to a chromatographic column containing 10 g of silica gel. The OC pesticides were eluted with 60 ml of hexane-methylene chloride mixture (4 : 1 v/v). For the SPE method, the C₁₈ tube was used for the cleanup of the extract. The tube was conditioned with 10 ml hexane : petroleum ether (1 : 1 v/v). Then, 2 ml of extract was transferred to the tube, followed by 10 ml hexane : petroleum ether (1 : 1 v/v) for elution. Both eluates were analysed on GC- μ ECD.

Results and Discussion

Development of SPE Conditions

The selection of the solvent system for the SPE was based on several criteria. Solvents that are hazardous or expensive to dispose of were not evaluated. A volatile solvent system must be used, as rapid evaporation of a large volume would be required in the sample preparation without causing loss of volatile pesticides. The solvent system must be sufficiently polar to extract most polar pesticides. The final extract should have minimum matrix co-extractives. Halogenated solvents, such as methylene chloride and acetonitrile, were eliminated from consideration because of hazard and higher disposal cost. Toluene, propanol, higher alcohols, isooctane and cyclohexane were eliminated because of insufficient volatility. In order to obtain good recoveries for the OC pesticides, parameter affecting the SPE conditions such as solvent polarity was optimised.

Solvent mixtures or single solvent system consisting of any of three solvents, namely acetone, hexane, methanol and petroleum ether were used in this study. The solubility of the OC pesticides used in this study ranges from totally not soluble such as DDT to 7 mg/l for gamma-HCH [12]. It was found that the non-polar solvent system such as hexane : petroleum ether (1 : 1 v/v) resulted in better recoveries for all the pesticides studied. Increase the solvent polarity by using hexane : acetone (2 : 1 v/v and 1 : 1 v/v), petroleum ether : acetone (1 : 1 v/v), acetone : petroleum ether (1 : 1 v/v) did not result in good recoveries for the OC pesticides. This is due to non-polar nature of all the OC pesticides used in this study. As C₁₈ is the most non-polar sorbent available, extremely non-polar compound is often difficult to elute from the C₁₈. Using 100 % hexane also did not result in good recoveries for the OC pesticides tested. The critical factor to be observed was that the SPE tube should not be left dry after eluting the samples since low recoveries may result. 10 ml eluting solvent was sufficient to condition the SPE tube and to elute the analytes. A flow rate of 1 ml/min was sufficient to elute the analytes. The optimal conditions for the OC pesticides were found to be as follows: (a) solvent mixture: hexane : petroleum ether (1 : 1), (b) volume of eluting solvent : 10 ml, (c) flow rate for elution : 1 ml/min.

Recovery Studies And Method Validation

Of the nine OC pesticides used in this study, only endosulfan is commonly used for vegetables cultivation, while others are present in the vegetables as a result of contamination from the environment. For this study, the OC pesticide standards were spiked at 0.5 ppm and the results are given in Table 1. The recoveries obtained for 7 OC pesticides, namely alpha-endosulfan, dieldrin, beta-endosulfan,

pp'-DDT, op'-DDT, endosulfan sulphate, and pp'-DDT were in the range of 66.3 % to 76.3 % with CV of 0.6 % to 4.4 %. Low recoveries of 51.3 % and 31.7 % were obtained for gamma-HCH and heptachlor respectively. Three types of vegetables, namely carrot, cucumber and green mustard represent the root, cucurbit and brassica families were selected for this study to illustrate the efficiency of the cleanup method for different sample matrix. As different matrix compounds are present in these vegetables, they may co-elute with the OC pesticides. These vegetables were previously analysed to ensure they do not contain any pesticides, thereby provide a true blank for spiking and recovery.

(a) Carrot

Recoveries for the OC pesticides fortified in carrot samples at 0.5 ppm and 0.1 ppm using the C₁₈ and the silica gel cleanup are given in Table 2. At 0.5 ppm fortification level, the recoveries for the C₁₈ cleanup ranged from 65.7 % to 82.0 % with CV of 2.3 % to 7.2 %. The recoveries obtained for the gamma-HCH and heptachlor were higher compared to those using the pesticide standards only. This is due to the matrices enhancement effect of the carrot samples enabling more pesticides to elute out from the SPE tube. High recoveries of 73.0 % to 106.0 % were obtained for the silica gel cleanup. The CV was in the range of 1.0 % to 5.8 %. At 0.1 ppm fortification level, the recoveries obtained using the C₁₈ cleanup were in the range of 67.0 % to 105.0 % with CV of 1.5 % to 7.9 %. Better recoveries were obtained from the silica gel cleanup. Their recoveries

ranged from 63.0 % to 113.0 % with CV of 1.2 to 8.6 %.

The chromatograms for the carrot samples using the C₁₈ and the silica gel cleanup are shown in Figure 1. Both chromatograms showed no interfering peaks between 8 min and 21 min where the OC pesticides peaks were eluted. However, the chromatogram obtained from the silica gel cleanup contained more co-extractives peaks than the C₁₈ cleanup. The colour of the carrot extracts before the C₁₈ cleanup was yellow, while after cleanup it was light yellow. Similar observation was noted in the silica gel cleanup. Therefore, both methods removed a substantial amount of colour compounds from the carrot extracts. These compounds significantly reduce the GC performance and the lifetime of GC chromatographic columns. Besides, they also clog the GC inlet. In a study by Hsu *et. al.* [5] on halogenated pesticides, it was found that there was no difference between the ECD chromatograms of the matrix blanks and the ones with C₁₈ cleanup. The final carrot solutions after the C₁₈ SPE cleanup showed only slight reduction in the color intensity. In their study, different solvent systems were used. The water extract was loaded onto a C₁₈ cartridge, which was conditioned with 2 ml of methanol and followed by 5 ml water. The tube was rinsed with 30 % acetonitrile/water, water and finally eluted with 2 ml of n-hexane or 5 % acetone/hexane depending on the type of the pesticides. This study concluded that a single C₁₈ tube is sufficient for the cleanup of the carrot extracts. The limit of detection (LOD) for the OC pesticides using the SPE C₁₈ and the silica gel cleanup was 0.01 mg/kg.

Table 1 : Recovery of OC pesticides (n = 3)

Pesticide	% Recovery ^(a) ± CV
γ-HCH	51.3 ± 4.0
Heptachlor	31.7 ± 2.1
α-endosulphan	66.3 ± 1.5
Dieldrin	71.3 ± 0.6
β-endosulphan	72.0 ± 4.0
p,p'-DDD	72.0 ± 4.4
o,p'-DDT	71.7 ± 5.1
Endosulfan sulphate	74.0 ± 2.7
p,p'-DDT	76.3 ± 3.1

CV = coefficient of variation

Table 2 : Recovery of OC pesticides from carrot samples using C₁₈ and silica gel cleanup (n = 3)

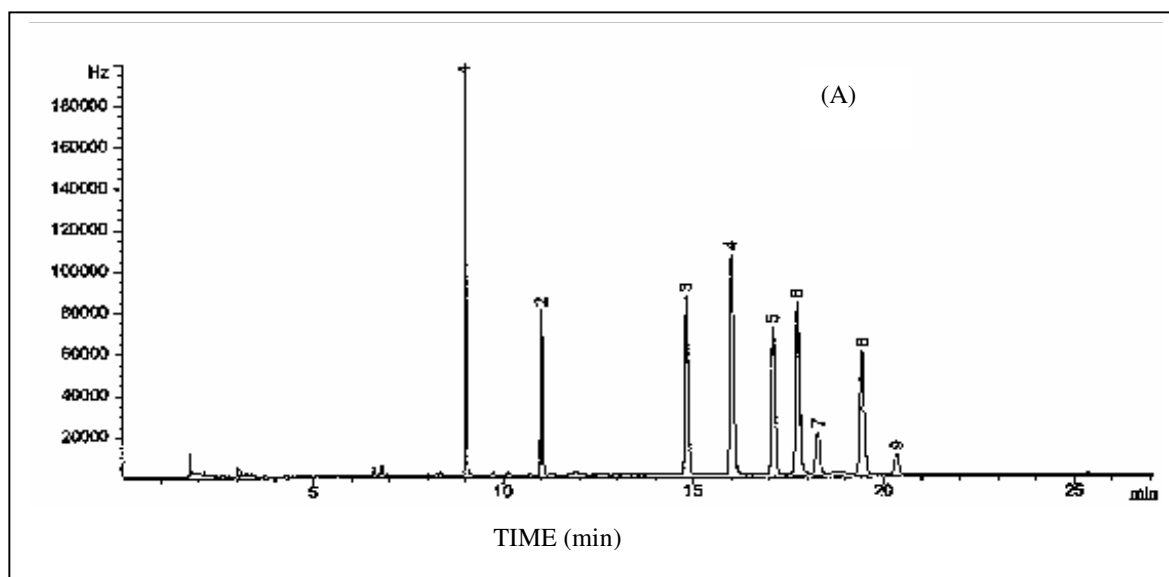
Pesticide	0.5 ppm		0.1 ppm	
	C ₁₈	Silica gel	C ₁₈	Silica gel
	% Rec ± CV	% Rec ± CV	% Rec ± CV	% Rec ± CV
γ-HCH	65.7 ± 2.3	81.7 ± 5.8	75.7 ± 6.7	63.0 ± 5.3
Heptachlor	66.3 ± 5.7	73.0 ± 2.7	67.0 ± 7.9	67.7 ± 8.6
α-endosulphan	75.3 ± 5.1	102.0 ± 3.6	81.7 ± 1.5	71.7 ± 4.7
Dieldrin	82.0 ± 4.6	103.3 ± 4.0	92.3 ± 4.7	82.0 ± 3.0
β-endosulphan	79.7 ± 4.5	104.0 ± 3.0	93.7 ± 2.1	89.0 ± 6.1
p,p'-DDD	77.7 ± 3.2	106.0 ± 1.7	102.7 ± 3.1	112.7 ± 2.9
o,p'-DDT	65.7 ± 7.2	106.0 ± 1.0	98.3 ± 3.5	113.0 ± 4.4
Endosulphan sulphate	80.3 ± 3.5	105.7 ± 4.2	105.0 ± 2.0	94.7 ± 7.4
p,p'-DDT	71.7 ± 5.0	103.3 ± 1.5	72.7 ± 2.1	73.3 ± 1.2
AV	73.8	98.3	87.7	85.2
SD	6.6	12.1	13.8	18.6

AV = Average mean

SD = Standard deviation

CV = Coefficient of variation

Rec = recovery



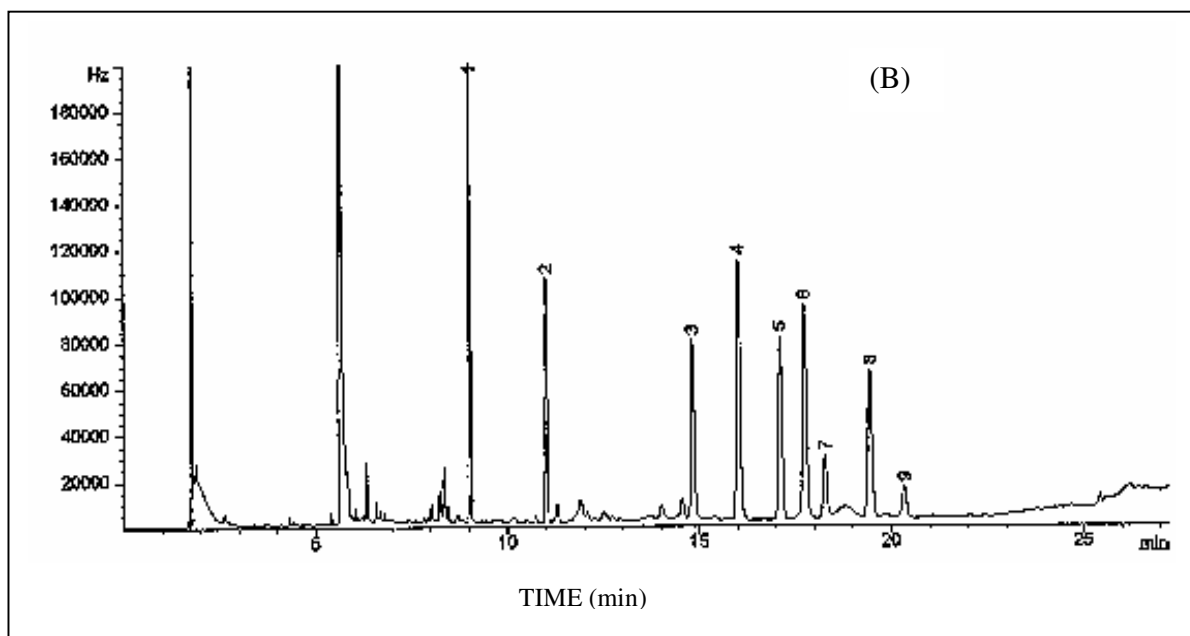


Figure 1 : GC chromatogram of carrot extract after SPE C_{18} cleanup (A) and silica gel cleanup (B). Peaks : 1, gamma-HCH; 2, heptachlor; 3, alpha-endosulfan; 4, dieldrin; 5, beta-endosulfan; 6, p,p'-DDD; 7, o,p'-DDT; 8, endosulfan-sulphate; 9, p,p'-DDT

b) Cucumber

Recoveries for the OC pesticides fortified in cucumber samples at 0.5 ppm and 0.1 ppm with the C_{18} and the silica gel cleanup are given in Table 3. At 0.5 ppm fortification level, the recoveries for all the OC pesticides tested using the C_{18} cleanup were within the acceptable range of 70–120 % [13]. The recoveries obtained were in the range of 74.0 % to 80.7 % with CV of 1.2 to 7.0 %. For the silica gel cleanup, the recoveries for all the OC pesticides tested were also within the acceptable range. Their recoveries ranged from 71.0 % to 109.0 % with the CV of 1.5 to 7.0 %. At 0.1 ppm fortification level, the recoveries obtained using the C_{18} cleanup were in the range of 65.7 % to 100.3 % with CV of 1.5 to 8.7 %. While, recoveries of 61.7 % to 110.0 % were obtained for the silica gel cleanup. The CVs were in the range of 3.2 to 10 %.

The chromatograms for the cucumber samples are given in Figure 2. As less co-extractive was found in the cucumber samples, no interfering peaks were found in the chromatograms between 8 to 21 min regions where the OC pesticides were eluted. The chromatograms were also cleaner as compared to the carrot samples. In general, cleaner chromatograms were obtained using the C_{18} cleanup. The colour of the cucumber extracts was light green before the C_{18} cleanup, while after it was colorless. Similar colour intensity was observed for the silica gel cleanup. Therefore, both cleanups removed a substantial amount of coloured compounds from the cucumber samples. This study concluded that a single SPE tube is sufficient for the cleanup of the cucumber samples. The LOD obtained for the OC pesticides using the SPE C_{18} and the silica gel cleanup was 0.01 mg/kg.

Table 3 : Recovery of OC pesticides from cucumber samples with C₁₈ and silica gel cleanup (n = 3)

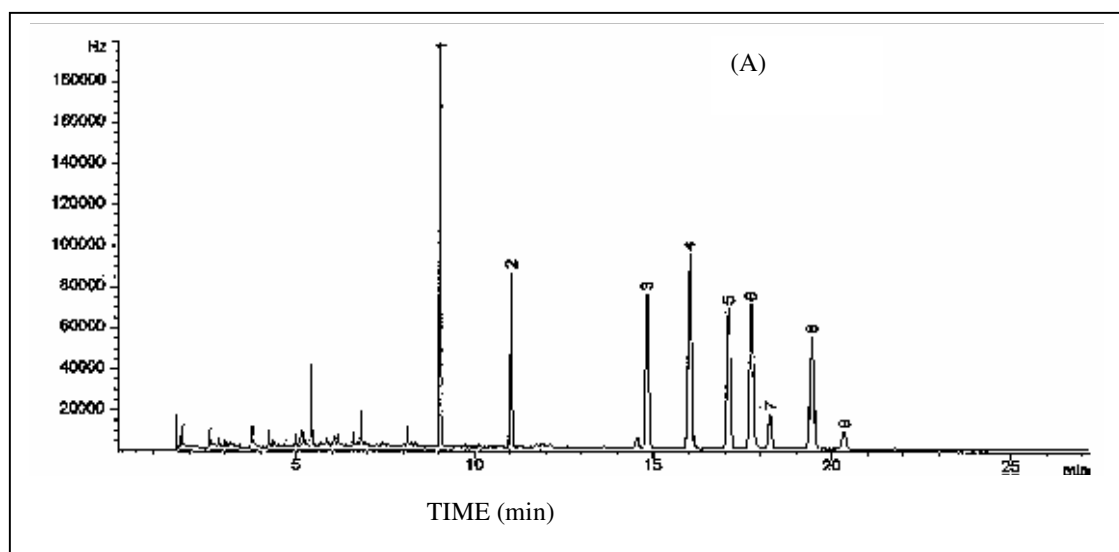
Pesticide	0.5 ppm		0.1 ppm	
	C ₁₈ % Rec ± CV	Silica gel % Rec ± CV	C ₁₈ % Rec ± CV	Silica gel % Rec ± CV
γ-HCH	78.3 ± 0.6	71.0 ± 7.0	74.0 ± 8.7	61.7 ± 5.0
Heptachlor	71.0 ± 3.6	92.7 ± 9.2	65.7 ± 5.1	63.0 ± 5.0
α-endosulphan	80.7 ± 2.3	88.7 ± 2.5	96.3 ± 4.0	69.7 ± 10.0
Dieldrin	82.3 ± 1.2	95.0 ± 3.0	79.7 ± 6.8	76.0 ± 5.0
β-endosulphan	82.7 ± 2.1	101.3 ± 5.1	91.7 ± 4.0	79.3 ± 8.1
p,p'-DDD	74.0 ± 2.7	109.0 ± 2.7	100.3 ± 8.5	105.7 ± 3.2
o,p'-DDT	77.3 ± 5.1	104.7 ± 3.8	73.0 ± 4.0	110.0 ± 4.6
Endosulphan sulphate	81.0 ± 2.0	107.3 ± 6.0	95.7 ± 1.5	87.3 ± 4.2
p,p'-DDT	77.0 ± 2.0	106.3 ± 1.5	65.7 ± 2.3	75.3 ± 5.0
AV	78.3	97.3	82.5	80.9
SD	3.9	12.1	13.7	17.2

AV = average mean

CV = coefficient of variation

SD = standard deviation

Rec = recovery



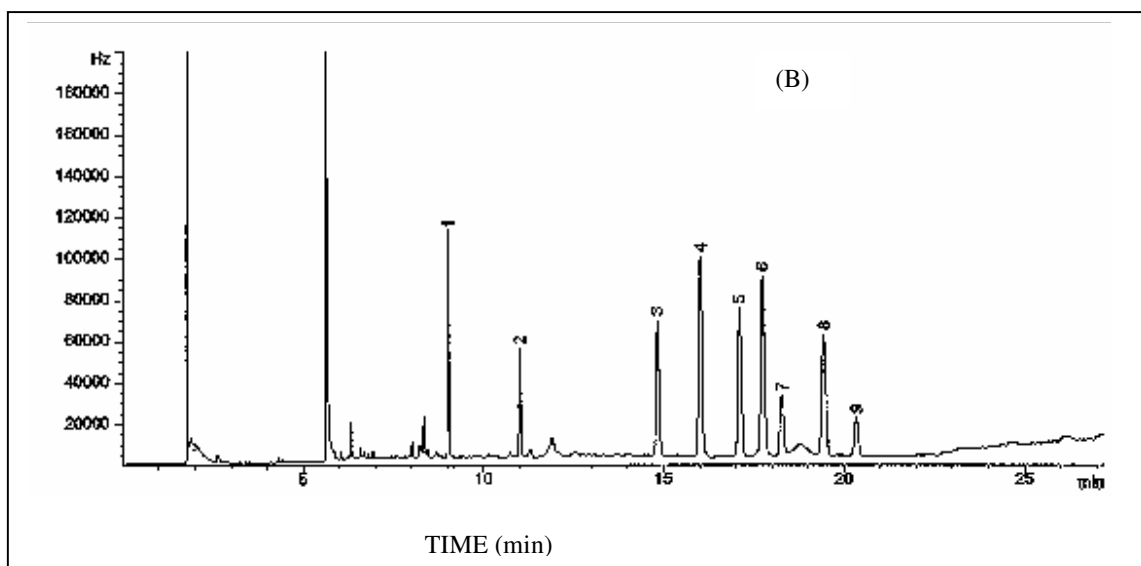


Figure 2 : GC chromatogram of cucumber extract after SPE C_{18} cleanup (A) and silica gel cleanup (B). Peaks : gamma-HCH; 2, heptachlor; 3, alpha-endosulfan; 4, dieldrin; 5, beta-endosulfan; 6, p,p'-DDD; 7, o,p'-DDT; 8, endosulfan-sulphate; 9, p,p'-DDT

(c) Green mustard

Recoveries of the OC pesticides fortified in green mustard samples at 0.5 ppm and 0.1 ppm with the C_{18} and the silica gel cleanups are given in Table 4. At 0.5 ppm fortification level, the recoveries obtained using the C_{18} cleanup for the OC pesticides ranged from 62.7 % to 85.0 % with CV of 1.5 % to 8.1 %. High recoveries of 72.0 % to 103.3 % were obtained for the silica gel cleanup. The CV ranged from 1.0 to 8.4 %. At 0.1 ppm fortification level, the recoveries obtained using the C_{18} cleanup were in the range of 62.0 % to 86.7 % with CV of 1.5 to 8.1 %. Higher recoveries of 71.7 % to 100.3 % were obtained for the silica gel cleanup. The CV ranged from 1.0 to 8.4 %.

The chromatograms for green mustard using the C_{18} and the silica gel cleanup are shown in Figure 3. The chromatograms showed no interfering peaks between 8 min to 21 min where the OC pesticides were eluted. In general, cleaner chromatograms were obtained using the C_{18} cleanup than the silica gel cleanup. The colour of the extract was dark green, while after cleanup it was light green. Similar observation was noted for the method using the silica gel cleanup. Both cleanups removed a substantial amount of coloured compounds from the green mustard samples. This study concluded that a single SPE cleanup is sufficient for the cleanup of the green mustard samples. The LOD obtained for the OC pesticides using the SPE C_{18} and the silica gel cleanup was 0.01 mg/kg.

Conclusion

The crops selected for this study represented

divergent chemical problems with regard to the cleanup. While some crops may be high in sugars, others high in chlorophyll or fats or waxes. The results from this study showed that the C_{18} is suitable and has the potential to be used as alternative cleanup to the conventional column chromatography for the determination of OC pesticide residues in vegetables. Most of the recoveries obtained in three crops tested, namely carrot, cucumber and green mustard were within the acceptable range. However, the overall recoveries obtained from the C_{18} cleanup were lower than those obtained from silica gel cleanup method. It was found that both C_{18} and silica gel cleanups removed a substantial amount of matrix co-extractives from the vegetables tested as the colour intensity of the extracts were lighter after the cleanup. This showed that C_{18} has better efficiency than the silica gel as only 1 g of the sorbent was used in the SPE as compared to 10 g in the later method. No interfering peaks were observed in the chromatograms obtained using both cleanup methods. In spite of the limited range of this study, it can be predicted that the C_{18} cleanup can be successfully extrapolated to other pesticides, fruits and vegetables. The benefits of the SPE method compared to the silica gel method were the use of organic solvents is reduced, possibility of concentration the samples, less sorbent used, no cross-contamination, shorter analysis time and the technique is easy to automate. However, due to smaller size used in the SPE, samples must be well mixed to obtain representative ones before a sub-sample is taken for the analysis.

Table 4 : Recovery of OC pesticides from green mustard samples with C₁₈ and silica gel cleanup (n = 3)

Pesticide	0.5 ppm		0.1 ppm	
	C ₁₈	Silica gel	C ₁₈	Silica gel
	% Rec ± CV	% Rec ± CV	% Rec ± CV	% Rec ± CV
γ-HCH	77.3 ± 6.7	72.3 ± 5.0	70.7 ± 5.1	71.7 ± 3.2
Heptachlor	66.7 ± 4.6	72.0 ± 5.6	62.0 ± 4.0	73.7 ± 4.2
α-endosulphan	78.7 ± 5.5	100.0 ± 6.1	76.3 ± 6.7	94.7 ± 5.0
Dieldrin	85.0 ± 2.7	103.3 ± 4.7	86.7 ± 4.0	98.7 ± 3.1
β-endosulphan	73.7 ± 1.5	95.0 ± 1.7	86.0 ± 5.3	100.3 ± 6.1
p,p'-DDD	76.0 ± 4.6	101.0 ± 4.6	82.0 ± 1.0	97.0 ± 4.4
o,p'-DDT	62.7 ± 8.0	100.0 ± 1.0	82.0 ± 2.0	81.3 ± 3.1
Endosulphan-sulphate	73.3 ± 2.5	97.7 ± 4.7	86.3 ± 4.9	98.0 ± 7.2
p,p'-DDT	70.3 ± 8.1	102.7 ± 8.4	72.3 ± 7.6	72.0 ± 7.0
AV	73.7	93.8	78.3	87.5
SD	6.6	12.5	8.5	12.6

AV = average mean

CV = coefficient of variation

SD = standard deviation

Rec = recovery

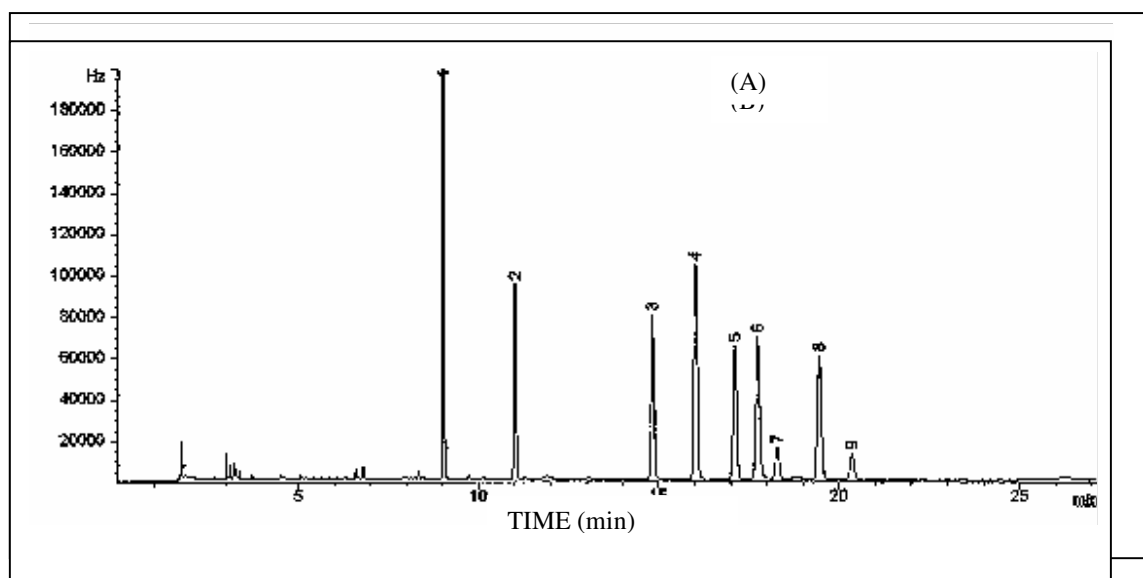


Figure 3 : GC chromatogram of green mustard extract after SPE C₁₈ cleanup (A) and silica gel cleanup (B).
Peaks : gamma-HCH; 2, heptachlor; 3, alpha-endosulfan; 4, dieldrin; 5, beta-endosulfan; 6, p,p'-DDD; 7, o,p'-DDT; 8, endosulfan-sulphate; 9, p,p'-DDT

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