

Full Length Research Paper

Supercritical carbon dioxide extraction of cypermethrin in different fresh vegetables using anhydrous sodium sulfate for sample preparation and extraction

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Supercritical fluid extraction (SFE) is a promising method of extraction for pesticide residues in fruits and vegetables. A rapid and high percentage of recoveries extraction of cypermethrin insecticides from fresh yardlong bean, carrot and eggplant vegetables matrixs using drying agent and SC-CO₂ was developed in this study. In vegetable samples, which typically contain 80 - 95% water, but samples dry with Na₂SO₄ showed 96 - 105% recoveries in SFE. Moreover, the performance of drying agents (Na₂SO₄) and their combination such as Na₂SO₄ -H₂O (6.7 + 1), Na₂SO₄ - H₂O (2 + 1) and Na₂SO₄ - H₂O (1 + 1) were evaluated. Na₂SO₄-H₂O (1 + 1) was shown moisture conveyanced by SFE/mg highest (93) performance. Cypermethrin recovered from yardlong bean and carrot with critical pressure, P_C at 20 lbs and critical temperature, T_C 40 °C. On the other hand, the recovery rate of similar insecticides from egg plant was 96% with P_C at 31.5 lbs and 45 °C in T_C. The recovery rate in yardlong bean matrices was 100%; 105% was in carrot matrix. The retention time (t_R) was 49.6 min of cypermethrin standard and similar t_R was found in formulated. The lowest detectable limit (LDL) of cypermethrin insecticides was 0.02 µg/mL with gas chromatography-electron capture detection (GC-ECD). GC-ECD temperature was of injection port and detector 280 and 300 °C, respectively, with split less. However, the method is useful to recover non-polar insecticides from the fresh vegetable; thus, further research could be continued with co-solvents and different drying agents to recover the polar residues from different types of fresh vegetables.

Key words: Lowest detectable limit, retention time, drying agents, recovery rate.

INTRODUCTION

The increase in the use of pesticides throughout the 20th century coincided with the development of industrial methods of cultivation and increased consumer demand. Consumers' aesthetic preferences have bolstered the use of pesticides, since most consumers avoid vegetables that

are less than perfect in color or shape, even if they are of equivalent nutritional value. Most vegetables are exposed to chemical pesticides. Part of these pesticides remain on the vegetables in the form of residues, a certain quantity of which penetrates the vegetable itself (systemic contamination), while others remain on the surface (topical contamination) but its residue not only pose problems for international trade but also damage the public health. For example, China's exports have suffered due to vegetable safety issues, as food safety standards in developed

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countries such as Europe, Japan and the US are more strictly enforced (Calvin et al., 2006; Jin et al., 2008). It has been reported that the substantive application of pesticides may cause pesticide residues in food crops, which is especially true for vegetables which among other crops receive the highest application of pesticides (Ngowi et al., 2007). Cypermethrin, (RS)-cyano (3-phenoxyphenyl) methyl (1RS)- cis-trans-3-(2,2-dichloroethenyl)-2,2-dimethyl-cyclopropane carboxylate was first synthesized in 1974 as a highly active synthetic pyrethroid insecticide effective against a wide range of pests in agriculture, public health and animal husbandry (Environment Agency, 2009). It is related chemically to natural insecticides called pyrethrins produced by certain sorts of plant, notably the pyrethrum extract (which comes from the chrysanthemum plant) but highly stable to light at temperatures below 220°C. In agriculture, its main use is against foliage pests and certain surface soil pests and residues do not accumulate in the environment when applied to crops, residues may occur in surface waters and soils. (Environment-agency, 2009).

The most frequently applied supercritical fluid is carbon dioxide, a non-polar solvent. If polar compounds as pesticides have to be extracted, the addition of a polar solvent to the supercritical fluid, called cosolvent, may improve the extraction because of the increase in the polarity of the supercritical fluid and thus the recovery of pesticides (Abaroudi et al., 2002; King et al., 2006). Even though supercritical CO₂ is a good solvent only for the extraction of non-polar to moderately polar compounds, the extraction of polar compounds can be improved by the addition of small quantities of polar organic solvents employed as modifiers (Rial-Otero et al., 2007). Limitations encountered in classical sample handling techniques such as SFE have been overcome by employing modern techniques that are faster and environmentally friendly (Rodil et al., 2007). The pressure and temperature of the supercritical fluid can be varied so as to effect selectivity during extraction (Fidalgo-Used et al., 2007). SFE is unapplicable to water samples directly (plant materials/tissues: fruit and vegetable typically consist of 80 - 95% water) and extreme moisture levels (high or low) can affect extraction of both the most polar and non-polar pesticide analytes. SFE faces two major limitations. Firstly, polar compounds require the addition of a modifier to either the fluid or the matrix, thereby decreasing the selectivity of the extraction; also, the range of pesticides that can be quantitatively extracted under the same conditions is limited. Secondly, results are strongly dependent on the matrix and typically small sample size, which leads to new optimizations each time a new matrix is considered. The physical structure of the matrix is of prime importance, as the extraction efficiency is strongly related to the ability of the supercritical fluid to diffuse within the matrix. For that reason, the extraction conditions of the same group of pesticides may differ from one matrix to another (Camel, 1998). Different chromatographic

methods for the determination of chlorpyrifos residues and its main metabolites in food and environmental samples have been developed (Carabias-Martínez et al., 2000; López-Roldán et al., 2004). Wilson et al. (2001) stated that gas chromatography (GC) coupled to electron capture detector (ECD) have been applied for the determination of cypermethrin. This work is aimed at i) Solving the moisture content problems with a quite simple preparation, ii) To establish a rapid and high percentages of recovery and iii) Find out an extraction protocol using SFE system of cypermethrin from yard long bean, carrot and eggplant matrixes..

MATERIALS AND METHODS

The present study has been undertaken to a rapid and high percentages of recoveries extraction protocol of cypermethrin from yardlong bean, carrot and eggplant matrixs using SFE system. It has been carried out at the Toxicology Laboratory of Department of Plant Protection, Faculty of Agriculture and Department of Food Science, Faculty of Food Science and Technology, University Putra Malaysia (UPM), Selangor, Malaysia.

Instruments

Supercritical fluid extraction (SFE), consists of an extraction vessel placed in a column oven (Model CO 1560, Jasco, Japan), back pressure regulator (Model BP1580-81, Jasco, Japan). The carbon dioxide was chilled to -5°C before a HPLC pump (Model PU-150, Jasco, Japan) and the liquefied CO₂ poured into the extractor at a constant flow of 4.0 mL min⁻¹.

Gas chromatography-electron capture detection (GC-ECD): Model G1540N, Agilent Technologies, USA.

Matrixes, pesticides and pesticide standards

The studied vegetables matrixes such as yardlong bean, carrot and eggplant were purchased from local market called Pasamalam. The cypermethrin insecticides standard is purchased from Sigma-Aldrich Labochemikalien GmbH, Germany supplied by local representative.

Fluid, solvent and drying agent

A special siphoned 99.995% pure carbon dioxide (CO₂) from Malaysian Oxygen Berhad (Selangor, Malaysia) was used in SFE. n-Hexane of a special grade (analytical grade) for pesticide residue analysis and drying agent were purchased from Mallinkrodt, Merck's local representative. Experiments were carried out to compare drying agent properties of Na₂SO₄ and HMX before performing SFE of produce samples. To determine the amount of material transported by the supercritical CO₂, the masses of the loaded vessels were measured before and after SFE. To determine the water retention capabilities of the drying agents, the vessel contents after SFE were transferred to a watch glass and were baked at 250°C for 24 h. The amount of water extracted during SFE was determined by subtracting the post-SFE water content in the sample from the original water content.

Preparation of cypermethrin standard solutions

A standard stock solution of cypermethrin was prepared by dissolving

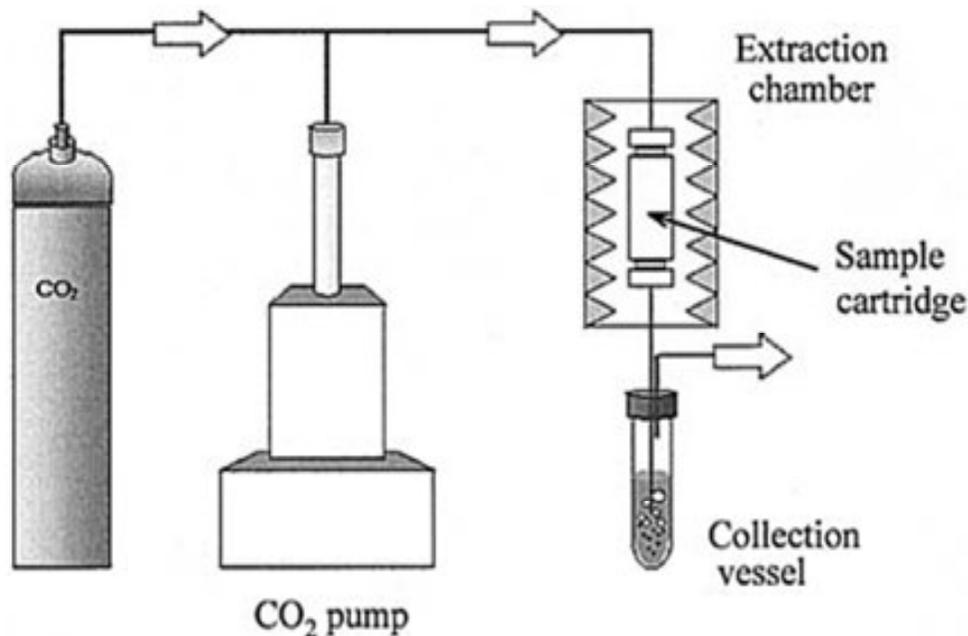


Figure 1. Schematic view of SFE instrument. Sample cartridge volume is approximately 10 ml (Yukiko et al., 2006).

100 mg of cypermethrin in 100 ml hexane in a 100 ml volumetric flask. Working standard solutions of 0.001, 0.005, 0.05, 0.1 and 0.5 $\mu\text{g/ml}$ were prepared by diluting the standard solution with appropriate amount of hexane.

Samples preparation with drying agents

The vegetable samples were purchased randomly, transported to the laboratory and stored for a short time at 4°C until analyzed. Before extraction procedure, the samples were checked for cypermethrin by GC-MS. Samples were washed by placing in a plastic strainer and sluice under normal tap water for 30 s, with gentle rotation by hand and chopped into small pieces as described by Walter et al. (2000). 20 g of chopped samples were taken and 20 g of $\text{Na}_2\text{SO}_4 \cdot \text{H}_2\text{O}$ in a chopper added well in that, a glass rod was used for removing moisture. Kabir et al. (2008) reported that sodium sulphate (Na_2SO_4) was also added (as required) with sample until water was removed from the sample. Similarly, Valverde-García et al. (1996) demonstrated that methamidophos, chlorpyrifos, endosulfans, methiocarb and chlorothalonil were extracted from vegetables when using anhydrous Na_2SO_4 as the drying agent. Filter paper, Whatman # 3 (Maidstone, Kent, UK) was cut into disks which were placed at both ends to keep particles from affecting sealing of the vessels. The vessels were kept cold before extraction in order to reduce losses of degradative pesticides (Lehotay et al., 1995). The homogenized mixtures, 6 g (2.4 g of sample), were loaded into the 7 ml vessels and extracted by SFE and mixing 12 g of sample (after fortification) with 12 g of $\text{Na}_2\text{SO}_4 \cdot \text{H}_2\text{O}$. The solvents were selected on the basis of the criteria described by Lehotay et al. (1995). Thus known and similar concentrated solutions of each of the standard and formulated cypermethrin were prepared. After this, cypermethrin-free samples were fortified with 10 ml of different levels (0-as blank, 0.001, 0.005, 0.05, 0.1 and 0.5 $\mu\text{g/ml}$) cypermethrin formulated solution in yardlong bean, carrot and eggplant after being kept at room temperature for 1 h.

Extraction through SFE

The spike sample was placed in the stainless steel extraction vessel (5.6 cm x 1.6-mm i.d.) and extraction vessel put in column/oven chamber. According to samples versus pesticide nature, put critical temperature (T_c) and critical pressure (P_c) in column-oven and back pressure regulator chamber, respectively. At last, software was switched on and waits for extraction. A piece of fused silica capillary tube (30 cm x 100 μ i.d.) was attached to the outlet of the extractor and the pesticides were directly collected in an aminopropyl cartridge at 10°C. The SFE collection screw capped test tubes were placed on an orbital shaker at 250 rpm for 1 h to desorb. Figure 1 shows the schematic flow diagram of SFE system. The color substance (if any) was removed by passing it through GPC (gel-permeation chromatography).

Fortification and GC calibration

Fortification was done by applying 100 μL of three fortification concentrations solutions (0.001, 0.005, 0.05, 0.1 and 0.5 $\mu\text{g/ml}$) over the surface of sampling media. Then the spiked samples were kept at 4°C inside a freeze drawer overnight. Next day the spiked sample were extracted and analyzed to gas chromatography-electron capture detection (GC-ECD). Three replications were considered for fortification.

For calibrating the GC, yardlong bean, carrot and eggplant samples were spiked with formulated insecticides solutions: 0.001, 0.005, 0.05, 0.1 and 0.5 $\mu\text{g/ml}$ drop-by-drop and stored at 4°C. Sample solutions (1 ml) of each were transferred into 2 ml screw capped GC vials and labeled. All vials placed in GC-auto sampler tray and analyzed with GC-ECD. GC-ECD was calibrated using standard solutions. The instrument parameters were temperature of injection port and detector 280 and 300°C, respectively. Carrier Gas: helium 1.3 ml/min; carrier and make up gas (nitrogen) pressure was 70 and 100 kPa, respectively; split less; mode current: 0.5 μV ; Injector: auto and injection volume 1 μL .

Table 1. Water content of three popular fresh vegetables^a.

S/N	Name of Vegetables	Food Weight (g)	Water Weight	Percent Water
01	Carrot	72	63	87
02	Yard long bean	72	57	79
03	Eggplant	41	38	92

^aJean A. T. (1998), Book: Bowes and Church's food values.

Table 2a. Performance of samples dry with Na₂SO₄ through supercritical fluid extraction.

S/N	Vegetable matrix	Oven Temp. (°C)	Drying time (min)	Recovery* (%)
01	Yard long Bean	48	5	100
02	Carrot		7	105
03	Eggplant		8.5	96

*Samples dried with Na₂SO₄.

Table 2b. Performance of samples dry without Na₂SO₄ through supercritical fluid extraction.

S/N	Vegetable Matrix	Oven Temp. (°C)	Drying Time (min)	Recovery* (%)
01	Yard long Bean	48	5	79
02	Carrot		7	86
03	Eggplant		8.5	71

*Samples dried without Na₂SO₄.

Table 3. Performance of drying agents through supercritical fluid extraction.

Sample matrix	size/g	Moisture in vessel before SFE/mg	Moisture in vessel after SFE/mg	Moisture conveyed by SFE/mg
Na ₂ SO ₄ , anhydrous	5	0	0	0
Na ₂ SO ₄ - H ₂ O (6.7 + 1)	5	664	643	21
Na ₂ SO ₄ - H ₂ O (2 + 1)	5	1044	988	56
Na ₂ SO ₄ - H ₂ O (1 + 1)	5	1410	1317	93

RESULTS AND DISCUSSION

Drying Agents

Vegetables typically consist of 80-95% water and extreme moisture levels (high or low) can affect extraction of both the most polar and non-polar pesticide analytes. Water content of three popular fresh vegetables is summarized in Table 1. Yardlong bean, carrot and eggplant were showed 96-105% recoveries oven dry with Na₂SO₄ through SFE is summarized in Table 2a. Similarly, 71 - 86% recoveries were found in oven dry without Na₂SO₄ through SFE is summarized in Table 2b. The solubility of H₂O in supercritical CO₂ is ≈ 0.3% (Kuk and Montagna, 1983), which corresponds to ≈110 mg of H₂O for the 38 g of CO₂ used in extractions and ≈70 mg of water were

required to dissolve methamidophos in CO₂ under the SFE conditions. The performance of drying agents and their combination were evaluated and the combination Na₂SO₄-H₂O (1 + 1) were shown in Table 3 that moisture conveyed by SFE/mg highest (93) performance. From Table 3, water were lost up to 93 mg from drying agents during SFE. After a series of extractions of samples containing Na₂SO₄, a build-up of fine particles appeared at the frit between the vessel and restrictor. When using Na₂SO₄ in routine applications, this frit should be changed frequently and a filter paper with pores < 2 mm should be used to contain the sample in the SFE vessel. Plugging of the automated variable restrictor is a potential problem with Na₂SO₄, especially if there is no filter placed before the restrictor, but with linear restrictors, plugging due to Na₂SO₄ is not a problem (Valverde-García et al., 1996).

Table 4. Recoveries and retention time of cypermethrin from spiked vegetables^a.

Name of insecticides	t _R (min)	Fortification level (µg/ml)	Mean recovery ±RSD ^b (%)		
			Yardlong bean	Carrot	Eggplant
Cypermethrin	49.6	0.001	94(6.6)	103(6.9)	90(6.9)
		0.005	98(6.2)	105(6.9)	91(7.1)
		0.05	93(6.7)	110(6.2)	93(6.9)
		0.1	97(6.6)	104(7.2)	95(7.0)
		0.5	101 (6.5)	106(7.0)	96(7.2)

^a n = 5, samples dry with Na₂SO₄, ^bRelative standard deviation.

Kabir et al. (2008) reported that sodium sulphate (Na₂SO₄) was also added (as required) with sample until water was removed from the sample. Furthermore, Na₂SO₄ has a solubility in water of 34-90% by mass (depending on hydrated state and temperature) (McGraw-Hill-Lange's, 1985) thus, available water is saturated with Na₂SO₄ which acts to salt out methamidophos from the water phase into the supercritical CO₂ phase. In the case of HMX, absorbed water is more accessible than water hydrated with Na₂SO₄ and due to its low solubility in water.

Method validation

The identification of the insecticides was performed by ECD, comparing the retention times of the standards and the peaks. Peak confirmation is necessary because the chromatograms of real samples can present peaks corresponding to other contaminants or endogenous compounds which elute at the same retention times as the compound studied.

Limits of detection and quantification

The lowest detectable limit (LDL) of cypermethrin insecticides was 0.02µg/ml with GC-ECD. The observed LDL undoubtedly viewing that maximum residue limit research could be successfully done in this laboratory.

Repeatability

The repeatability was determined by three injections of a standard solution at 1 µL, made with the auto injector. Table 4 shows that the relative standard deviation obtained for retention time and peak area. Therefore, the repeatability of the chromatographic method was acceptable.

Linearity

A calibration curve was prepared according to the above

working range of 0.001 to 0.5µg/ml with three replications. The linear regression (R²) was found to be 0.9925 and the equation derived from the data was:

$$y = 50538x + 794.86$$

where y is peak height and x is concentration of cypermethrin in µg/ml, which shown in Figure 2. The retention times and linearity of cypermethrin insecticides studied are shown in Table 4.

Recovery

Three types of vegetables sample matrix that is, yardlong bean, carrot and eggplant with cypermethrin were selected for this study to illustrate the efficiency of the extraction. Recovery studies of different samples fortified at 0.001, 0.005, 0.05, 0.1 and 0.5 µg/ml were performed (three replications). The recovery rate in yardlong bean matrices was 100%; a carrot matrix was 105 and 96% recovery from eggplant summarized in Table 4. These findings were excellent, because of the extraction method used and found recoveries of chlorpyrifos in tomato and potato matrices were 83 and 82%, respectively (Rissato et al., 2005).

Optimization of GC -ECD parameters

Gas chromatography consisted an HP-5 capillary column (30 m x 0.25 mm i.d., 0.25µm film thicknesses) with an electron capture detector set at 300°C. Injection mode was split less and injector temperature 280°C. The flow rate of carrier gas (helium) was 1.3 ml/min. Carrier and make up gas (nitrogen) pressure was 70 and 100 kPa, respectively. The column temperature was programmed as follows: the initial temperature of 100°C was increased at a rate of 10°C/min up to 240°C and held for 20 min, from 240 to 300°C at 50°C/min was used and held for 5 min at the final temperature. The total run time was 49.60 min. In all cases injection system was auto and injection volume was 1 µL.

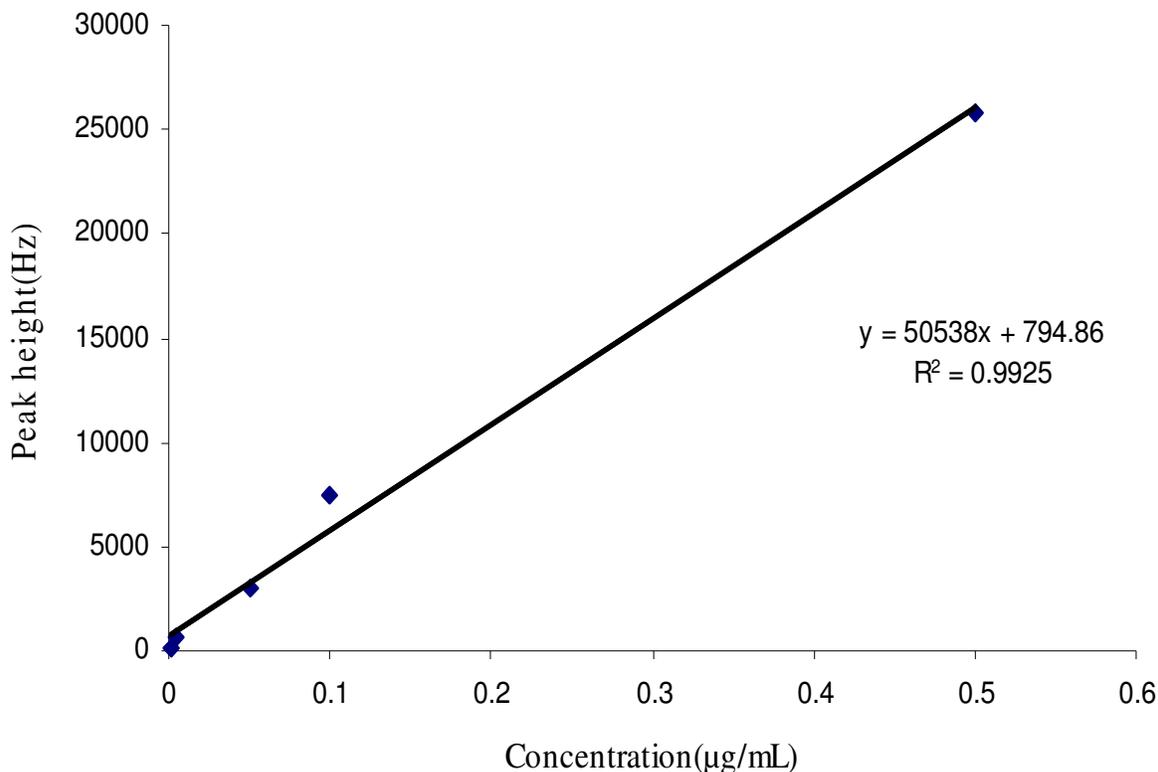


Figure 2. Calibration curve of Cypermethrin obtained with chromatograms of different concentrations.

Development of SFE conditions

The selection of the solvent for the SFE was based on several criteria. A volatile solvent must be used, as rapid evaporation of a large volume would be required in the sample preparation without causing loss of volatile pesticides. The most frequently applied supercritical fluid is carbon dioxide, a non-polar solvent. If polar compounds as pesticides have to be extracted, the addition of a polar solvent to the supercritical fluid, called cosolvent, may improve the extraction because of the increase in the polarity of the supercritical fluid and thus the recovery of pesticides (Abaroudi et al., 2002; King et al., 2006). It was found that the non-polar solvent, hexane, resulted in better recoveries for three insecticides of studied. Figure 1 shows a schematic view of the SFE instrument. The sample cartridge is filled with a sample and placed in a heated extraction chamber. Pressurized by a syringe pump, liquefied carbon dioxide enters the extraction chamber. CO₂ is heated and becomes supercritical. 2 ml eluting solvent was sufficient to condition the SFE and elute the analytes. A flow rate of 1 ml/min was sufficient to elute the analytes. In order to obtain good recoveries for the pesticides, parameter affecting the SFE conditions such as solvent polarity was optimised. The optimal conditions for the OP pesticides were found to be as follows: (1) hexane: matrices (1:10) and (2) flow rate: 1 ml/min.

Determination and Confirmation

Recording of chromatograms and quantitative measurements of peak areas were performed with a GC-ECD Chemstation. Cypermethrin recovered from yardlong bean and carrot with critical pressure, P_C at 20 lbs and critical temperature, T_C 40°C. Lehotay et al. (1995) also reported that 40°C, 200 bar of organochlorine pesticides in spiked green beans and carrots but methamidophos and acephate insecticides extracted from green beans with 60°C, 320 bar (Eller et al., 1997). On the other hand, the recovery rate of similar insecticides from eggplant was 96% with P_C at 31.5 lbs and 45°C in T_C. Wuchner et al. (1993) reported that diazinon was extracted from rice grain with CO₂ + 5% CH₃OH at 45°C, 315 bar.

The retention time (t_R) was 49.5 min of cypermethrin standard and similar t_R was found in formulated of yardlong bean and carrot matrices. Similarly, the t_R of cypermethrin standards was 49.6 in eggplant which was similar to both formulated and standard. Wuchner et al. (1993) observed a parallel finding of quinalphos in cereal products. The absorption spectrum of the detected peaks were confirmed by comparison with a standard of each pesticide. More over, from the above 03 (three) points of view, it was confirmed that the extracted fluids were cypermethrin from yardlong bean, carrot and eggplant matrices with 96 - 105% recoveries. Different insecticide extraction parameters of SFE such as critical pressure

Table 5. Extraction protocol of SFE for the determination of insecticides in vegetables.

Name of insecticides	Vegetable matrix	Critical		Extraction time (min)
		Pressure (lbs)	Temp. (°C)	
Cypermethrin	Yardlong Bean	20	40	40
	Carrot	20	40	40
	Eggplant	31.5	45	43

Temp. = Temperature.

(P_c), critical temperature (T_c) and extraction time of chlorpyrifos insecticide from yardlong bean, carrot and eggplant matrices were presented in Table 5.

Conclusion

SFE can be applied to high-water-content samples by the addition of a drying agent such as hydromatrix, magnesium sulfate, sodium sulfate and few minutes oven dry with sodium sulfate. The use of a drying agent (remove water) improved the recoveries of highly polar analytes. The SFE method has advantages of automation and simple operation compared to the solvent extraction methods, thus, it is a good alternative in practice within very short time, best recoveries and simplicity. The SFE sample preparation method, conditions and collection system are inexpensive and easy to perform, and may also be suitable for analysis without additional cleanup.

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Abbreviations

SFE, Supercritical fluid extraction; **P_c**, critical pressure; **T_c**, critical temperature; **t_R**, average retention time; **R²**, linear regression; **LDL**, lowest detectable limit; **GC-ECD**, gas chromatography-electron capture detection; **GC-ECD**, chromatography coupled to electron capture detector; **HPLC**, high performance liquid chromatography; **GPC**, gel-permeation chromatography.

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Supercritical fluid extraction (SFE) is a promising method of extraction for pesticide residues in fruits and vegetables. A rapid and high percentage of recoveries extraction of cypermethrin insecticides from fresh yardlong bean, carrot and eggplant vegetables matrixs using drying agent and SC-CO₂ was developed in this study. In vegetable samples, which typically contain 80 - 95% water, but samples dry with Na₂SO₄ showed 96 - 105% recoveries in SFE. Moreover, the performance of drying agents (Na₂SO₄) and their combination such as Na₂SO₄ -H₂O (6.7 + 1), Na₂SO₄ - H₂O (2 + 1) and Na₂SO₄ - H₂O (1 + 1) were evaluated. Na₂SO₄-H₂O (1 + 1) was shown moisture conveyanced by SFE/mg highest (93) performance. Cypermethrin recovered from yardlong bean and carrot with critical pressure, P_C at 20 lbs and critical temperature, T_C 40 °C. On the other hand, the recovery rate of similar insecticides from egg plant was 96% with P_C at 31.5 lbs and 45 °C in T_C. The recovery rate in yardlong bean matrices was 100%; 105% was in carrot matrix. The retention time (t_R) was 49.6 min of cypermethrin standard and similar t_R was found in formulated. The lowest detectable limit (LDL) of cypermethrin insecticides was 0.02 µg/mL with gas chromatography-electron capture detection (GC-ECD). GC-ECD temperature was of injection port and detector 280 and 300 °C, respectively, with split less. However, the method is useful to recover non-polar insecticides from the fresh vegetable; thus, further research could be continued with co-solvents and different drying agents to recover the polar residues from different types of fresh vegetables.

Key words: Lowest detectable limit, retention time, drying agents, recovery rate.

INTRODUCTION

The increase in the use of pesticides throughout the 20th century coincided with the development of industrial methods of cultivation and increased consumer demand. Consumers' aesthetic preferences have bolstered the use of pesticides, since most consumers avoid vegetables that

are less than perfect in color or shape, even if they are of equivalent nutritional value. Most vegetables are exposed to chemical pesticides. Part of these pesticides remain on the vegetables in the form of residues, a certain quantity of which penetrates the vegetable itself (systemic contamination), while others remain on the surface (topical contamination) but its residue not only pose problems for international trade but also damage the public health. For example, China's exports have suffered due to vegetable safety issues, as food safety standards in developed

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countries such as Europe, Japan and the US are more strictly enforced (Calvin et al., 2006; Jin et al., 2008). It has been reported that the substantive application of pesticides may cause pesticide residues in food crops, which is especially true for vegetables which among other crops receive the highest application of pesticides (Ngowi et al., 2007). Cypermethrin, (RS)-cyano (3-phenoxyphenyl) methyl (1RS)- cis-trans-3-(2,2-dichloroethenyl)-2,2-dimethyl-cyclopropane carboxylate was first synthesized in 1974 as a highly active synthetic pyrethroid insecticide effective against a wide range of pests in agriculture, public health and animal husbandry (Environment Agency, 2009). It is related chemically to natural insecticides called pyrethrins produced by certain sorts of plant, notably the pyrethrum extract (which comes from the chrysanthemum plant) but highly stable to light at temperatures below 220°C. In agriculture, its main use is against foliage pests and certain surface soil pests and residues do not accumulate in the environment when applied to crops, residues may occur in surface waters and soils. (Environment-agency, 2009).

The most frequently applied supercritical fluid is carbon dioxide, a non-polar solvent. If polar compounds as pesticides have to be extracted, the addition of a polar solvent to the supercritical fluid, called cosolvent, may improve the extraction because of the increase in the polarity of the supercritical fluid and thus the recovery of pesticides (Abaroudi et al., 2002; King et al., 2006). Even though supercritical CO₂ is a good solvent only for the extraction of non-polar to moderately polar compounds, the extraction of polar compounds can be improved by the addition of small quantities of polar organic solvents employed as modifiers (Rial-Otero et al., 2007). Limitations encountered in classical sample handling techniques such as SFE have been overcome by employing modern techniques that are faster and environmentally friendly (Rodil et al., 2007). The pressure and temperature of the supercritical fluid can be varied so as to effect selectivity during extraction (Fidalgo-Used et al., 2007). SFE is unapplicable to water samples directly (plant materials/tissues: fruit and vegetable typically consist of 80 - 95% water) and extreme moisture levels (high or low) can affect extraction of both the most polar and non-polar pesticide analytes. SFE faces two major limitations. Firstly, polar compounds require the addition of a modifier to either the fluid or the matrix, thereby decreasing the selectivity of the extraction; also, the range of pesticides that can be quantitatively extracted under the same conditions is limited. Secondly, results are strongly dependent on the matrix and typically small sample size, which leads to new optimizations each time a new matrix is considered. The physical structure of the matrix is of prime importance, as the extraction efficiency is strongly related to the ability of the supercritical fluid to diffuse within the matrix. For that reason, the extraction conditions of the same group of pesticides may differ from one matrix to another (Camel, 1998). Different chromatographic

methods for the determination of chlorpyrifos residues and its main metabolites in food and environmental samples have been developed (Carabias-Martínez et al., 2000; López-Roldán et al., 2004). Wilson et al. (2001) stated that gas chromatography (GC) coupled to electron capture detector (ECD) have been applied for the determination of cypermethrin. This work is aimed at i) Solving the moisture content problems with a quite simple preparation, ii) To establish a rapid and high percentages of recovery and iii) Find out an extraction protocol using SFE system of cypermethrin from yard long bean, carrot and eggplant matrixes..

MATERIALS AND METHODS

The present study has been undertaken to a rapid and high percentages of recoveries extraction protocol of cypermethrin from yardlong bean, carrot and eggplant matrixs using SFE system. It has been carried out at the Toxicology Laboratory of Department of Plant Protection, Faculty of Agriculture and Department of Food Science, Faculty of Food Science and Technology, University Putra Malaysia (UPM), Selangor, Malaysia.

Instruments

Supercritical fluid extraction (SFE), consists of an extraction vessel placed in a column oven (Model CO 1560, Jasco, Japan), back pressure regulator (Model BP1580-81, Jasco, Japan). The carbon dioxide was chilled to -5°C before a HPLC pump (Model PU-150, Jasco, Japan) and the liquefied CO₂ poured into the extractor at a constant flow of 4.0 mL min⁻¹.

Gas chromatography-electron capture detection (GC-ECD): Model G1540N, Agilent Technologies, USA.

Matrixes, pesticides and pesticide standards

The studied vegetables matrixes such as yardlong bean, carrot and eggplant were purchased from local market called Pasamalam. The cypermethrin insecticides standard is purchased from Sigma-Aldrich Labochemikalien GmbH, Germany supplied by local representative.

Fluid, solvent and drying agent

A special siphoned 99.995% pure carbon dioxide (CO₂) from Malaysian Oxygen Berhad (Selangor, Malaysia) was used in SFE. n-Hexane of a special grade (analytical grade) for pesticide residue analysis and drying agent were purchased from Mallinkrodt, Merck's local representative. Experiments were carried out to compare drying agent properties of Na₂SO₄ and HMX before performing SFE of produce samples. To determine the amount of material transported by the supercritical CO₂, the masses of the loaded vessels were measured before and after SFE. To determine the water retention capabilities of the drying agents, the vessel contents after SFE were transferred to a watch glass and were baked at 250°C for 24 h. The amount of water extracted during SFE was determined by subtracting the post-SFE water content in the sample from the original water content.

Preparation of cypermethrin standard solutions

A standard stock solution of cypermethrin was prepared by dissolving

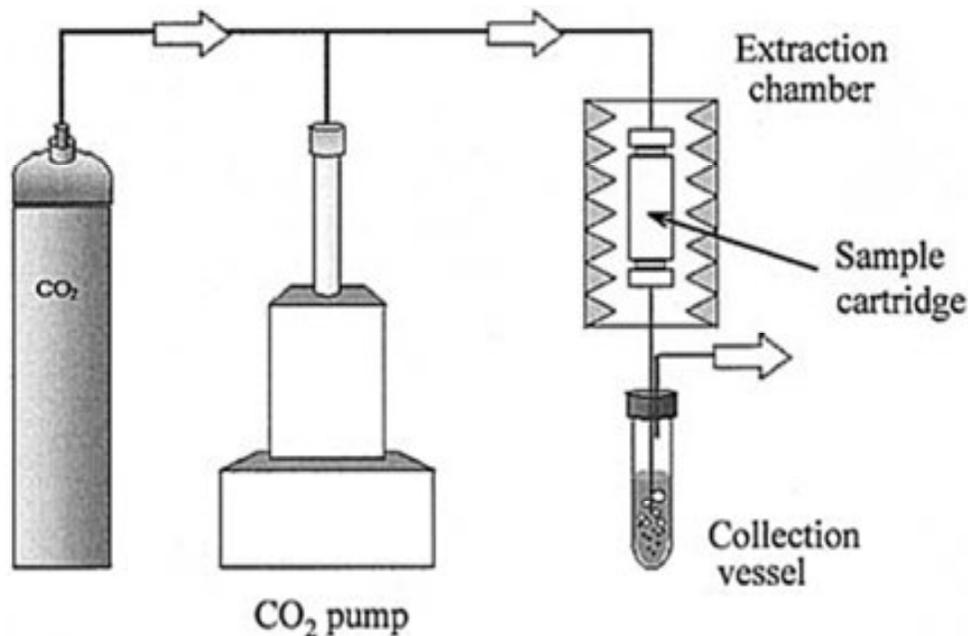


Figure 1. Schematic view of SFE instrument. Sample cartridge volume is approximately 10 ml (Yukiko et al., 2006).

100 mg of cypermethrin in 100 ml hexane in a 100 ml volumetric flask. Working standard solutions of 0.001, 0.005, 0.05, 0.1 and 0.5 $\mu\text{g/ml}$ were prepared by diluting the standard solution with appropriate amount of hexane.

Samples preparation with drying agents

The vegetable samples were purchased randomly, transported to the laboratory and stored for a short time at 4°C until analyzed. Before extraction procedure, the samples were checked for cypermethrin by GC-MS. Samples were washed by placing in a plastic strainer and sluice under normal tap water for 30 s, with gentle rotation by hand and chopped into small pieces as described by Walter et al. (2000). 20 g of chopped samples were taken and 20 g of $\text{Na}_2\text{SO}_4 \cdot \text{H}_2\text{O}$ in a chopper added well in that, a glass rod was used for removing moisture. Kabir et al. (2008) reported that sodium sulphate (Na_2SO_4) was also added (as required) with sample until water was removed from the sample. Similarly, Valverde-García et al. (1996) demonstrated that methamidophos, chlorpyrifos, endosulfans, methiocarb and chlorothalonil were extracted from vegetables when using anhydrous Na_2SO_4 as the drying agent. Filter paper, Whatman # 3 (Maidstone, Kent, UK) was cut into disks which were placed at both ends to keep particles from affecting sealing of the vessels. The vessels were kept cold before extraction in order to reduce losses of degradative pesticides (Lehotay et al., 1995). The homogenized mixtures, 6 g (2.4 g of sample), were loaded into the 7 ml vessels and extracted by SFE and mixing 12 g of sample (after fortification) with 12 g of $\text{Na}_2\text{SO}_4 \cdot \text{H}_2\text{O}$. The solvents were selected on the basis of the criteria described by Lehotay et al. (1995). Thus known and similar concentrated solutions of each of the standard and formulated cypermethrin were prepared. After this, cypermethrin-free samples were fortified with 10 ml of different levels (0-as blank, 0.001, 0.005, 0.05, 0.1 and 0.5 $\mu\text{g/ml}$) cypermethrin formulated solution in yardlong bean, carrot and eggplant after being kept at room temperature for 1 h.

Extraction through SFE

The spike sample was placed in the stainless steel extraction vessel (5.6 cm x 1.6-mm i.d.) and extraction vessel put in column/oven chamber. According to samples versus pesticide nature, put critical temperature (T_c) and critical pressure (P_c) in column-oven and back pressure regulator chamber, respectively. At last, software was switched on and waits for extraction. A piece of fused silica capillary tube (30 cm x 100 μ i.d.) was attached to the outlet of the extractor and the pesticides were directly collected in an aminopropyl cartridge at 10°C. The SFE collection screw capped test tubes were placed on an orbital shaker at 250 rpm for 1 h to desorb. Figure 1 shows the schematic flow diagram of SFE system. The color substance (if any) was removed by passing it through GPC (gel-permeation chromatography).

Fortification and GC calibration

Fortification was done by applying 100 μL of three fortification concentrations solutions (0.001, 0.005, 0.05, 0.1 and 0.5 $\mu\text{g/ml}$) over the surface of sampling media. Then the spiked samples were kept at 4°C inside a freeze drawer overnight. Next day the spiked sample were extracted and analyzed to gas chromatography-electron capture detection (GC-ECD). Three replications were considered for fortification.

For calibrating the GC, yardlong bean, carrot and eggplant samples were spiked with formulated insecticides solutions: 0.001, 0.005, 0.05, 0.1 and 0.5 $\mu\text{g/ml}$ drop-by-drop and stored at 4°C. Sample solutions (1 ml) of each were transferred into 2 ml screw capped GC vials and labeled. All vials placed in GC-auto sampler tray and analyzed with GC-ECD. GC-ECD was calibrated using standard solutions. The instrument parameters were temperature of injection port and detector 280 and 300°C, respectively. Carrier Gas: helium 1.3 ml/min; carrier and make up gas (nitrogen) pressure was 70 and 100 kPa, respectively; split less; mode current: 0.5 μV ; Injector: auto and injection volume 1 μL .

Table 1. Water content of three popular fresh vegetables^a.

S/N	Name of Vegetables	Food Weight (g)	Water Weight	Percent Water
01	Carrot	72	63	87
02	Yard long bean	72	57	79
03	Eggplant	41	38	92

^aJean A. T. (1998), Book: Bowes and Church's food values.

Table 2a. Performance of samples dry with Na₂SO₄ through supercritical fluid extraction.

S/N	Vegetable matrix	Oven Temp. (°C)	Drying time (min)	Recovery* (%)
01	Yard long Bean	48	5	100
02	Carrot		7	105
03	Eggplant		8.5	96

*Samples dried with Na₂SO₄.

Table 2b. Performance of samples dry without Na₂SO₄ through supercritical fluid extraction.

S/N	Vegetable Matrix	Oven Temp. (°C)	Drying Time (min)	Recovery* (%)
01	Yard long Bean	48	5	79
02	Carrot		7	86
03	Eggplant		8.5	71

*Samples dried without Na₂SO₄.

Table 3. Performance of drying agents through supercritical fluid extraction.

Sample matrix	size/g	Moisture in vessel before SFE/mg	Moisture in vessel after SFE/mg	Moisture conveyed by SFE/mg
Na ₂ SO ₄ , anhydrous	5	0	0	0
Na ₂ SO ₄ - H ₂ O (6.7 + 1)	5	664	643	21
Na ₂ SO ₄ - H ₂ O (2 + 1)	5	1044	988	56
Na ₂ SO ₄ - H ₂ O (1 + 1)	5	1410	1317	93

RESULTS AND DISCUSSION

Drying Agents

Vegetables typically consist of 80-95% water and extreme moisture levels (high or low) can affect extraction of both the most polar and non-polar pesticide analytes. Water content of three popular fresh vegetables is summarized in Table 1. Yardlong bean, carrot and eggplant were showed 96-105% recoveries oven dry with Na₂SO₄ through SFE is summarized in Table 2a. Similarly, 71 - 86% recoveries were found in oven dry without Na₂SO₄ through SFE is summarized in Table 2b. The solubility of H₂O in supercritical CO₂ is ≈ 0.3% (Kuk and Montagna, 1983), which corresponds to ≈110 mg of H₂O for the 38 g of CO₂ used in extractions and ≈70 mg of water were

required to dissolve methamidophos in CO₂ under the SFE conditions. The performance of drying agents and their combination were evaluated and the combination Na₂SO₄-H₂O (1 + 1) were shown in Table 3 that moisture conveyed by SFE/mg highest (93) performance. From Table 3, water were lost up to 93 mg from drying agents during SFE. After a series of extractions of samples containing Na₂SO₄, a build-up of fine particles appeared at the frit between the vessel and restrictor. When using Na₂SO₄ in routine applications, this frit should be changed frequently and a filter paper with pores < 2 mm should be used to contain the sample in the SFE vessel. Plugging of the automated variable restrictor is a potential problem with Na₂SO₄, especially if there is no filter placed before the restrictor, but with linear restrictors, plugging due to Na₂SO₄ is not a problem (Valverde-García et al., 1996).

Table 4. Recoveries and retention time of cypermethrin from spiked vegetables^a.

Name of insecticides	t _R (min)	Fortification level (µg/ml)	Mean recovery ±RSD ^b (%)		
			Yardlong bean	Carrot	Eggplant
Cypermethrin	49.6	0.001	94(6.6)	103(6.9)	90(6.9)
		0.005	98(6.2)	105(6.9)	91(7.1)
		0.05	93(6.7)	110(6.2)	93(6.9)
		0.1	97(6.6)	104(7.2)	95(7.0)
		0.5	101 (6.5)	106(7.0)	96(7.2)

^a n = 5, samples dry with Na₂SO₄, ^bRelative standard deviation.

Kabir et al. (2008) reported that sodium sulphate (Na₂SO₄) was also added (as required) with sample until water was removed from the sample. Furthermore, Na₂SO₄ has a solubility in water of 34-90% by mass (depending on hydrated state and temperature) (McGraw-Hill-Lange's, 1985) thus, available water is saturated with Na₂SO₄ which acts to salt out methamidophos from the water phase into the supercritical CO₂ phase. In the case of HMX, absorbed water is more accessible than water hydrated with Na₂SO₄ and due to its low solubility in water.

Method validation

The identification of the insecticides was performed by ECD, comparing the retention times of the standards and the peaks. Peak confirmation is necessary because the chromatograms of real samples can present peaks corresponding to other contaminants or endogenous compounds which elute at the same retention times as the compound studied.

Limits of detection and quantification

The lowest detectable limit (LDL) of cypermethrin insecticides was 0.02µg/ml with GC-ECD. The observed LDL undoubtedly viewing that maximum residue limit research could be successfully done in this laboratory.

Repeatability

The repeatability was determined by three injections of a standard solution at 1 µL, made with the auto injector. Table 4 shows that the relative standard deviation obtained for retention time and peak area. Therefore, the repeatability of the chromatographic method was acceptable.

Linearity

A calibration curve was prepared according to the above

working range of 0.001 to 0.5µg/ml with three replications. The linear regression (R²) was found to be 0.9925 and the equation derived from the data was:

$$y = 50538x + 794.86$$

where y is peak height and x is concentration of cypermethrin in µg/ml, which shown in Figure 2. The retention times and linearity of cypermethrin insecticides studied are shown in Table 4.

Recovery

Three types of vegetables sample matrix that is, yardlong bean, carrot and eggplant with cypermethrin were selected for this study to illustrate the efficiency of the extraction. Recovery studies of different samples fortified at 0.001, 0.005, 0.05, 0.1 and 0.5 µg/ml were performed (three replications). The recovery rate in yardlong bean matrices was 100%; a carrot matrix was 105 and 96% recovery from eggplant summarized in Table 4. These findings were excellent, because of the extraction method used and found recoveries of chlorpyrifos in tomato and potato matrices were 83 and 82%, respectively (Rissato et al., 2005).

Optimization of GC -ECD parameters

Gas chromatography consisted an HP-5 capillary column (30 m x 0.25 mm i.d., 0.25µm film thicknesses) with an electron capture detector set at 300°C. Injection mode was split less and injector temperature 280°C. The flow rate of carrier gas (helium) was 1.3 ml/min. Carrier and make up gas (nitrogen) pressure was 70 and 100 kPa, respectively. The column temperature was programmed as follows: the initial temperature of 100°C was increased at a rate of 10°C/min up to 240°C and held for 20 min, from 240 to 300°C at 50°C/min was used and held for 5 min at the final temperature. The total run time was 49.60 min. In all cases injection system was auto and injection volume was 1 µL.

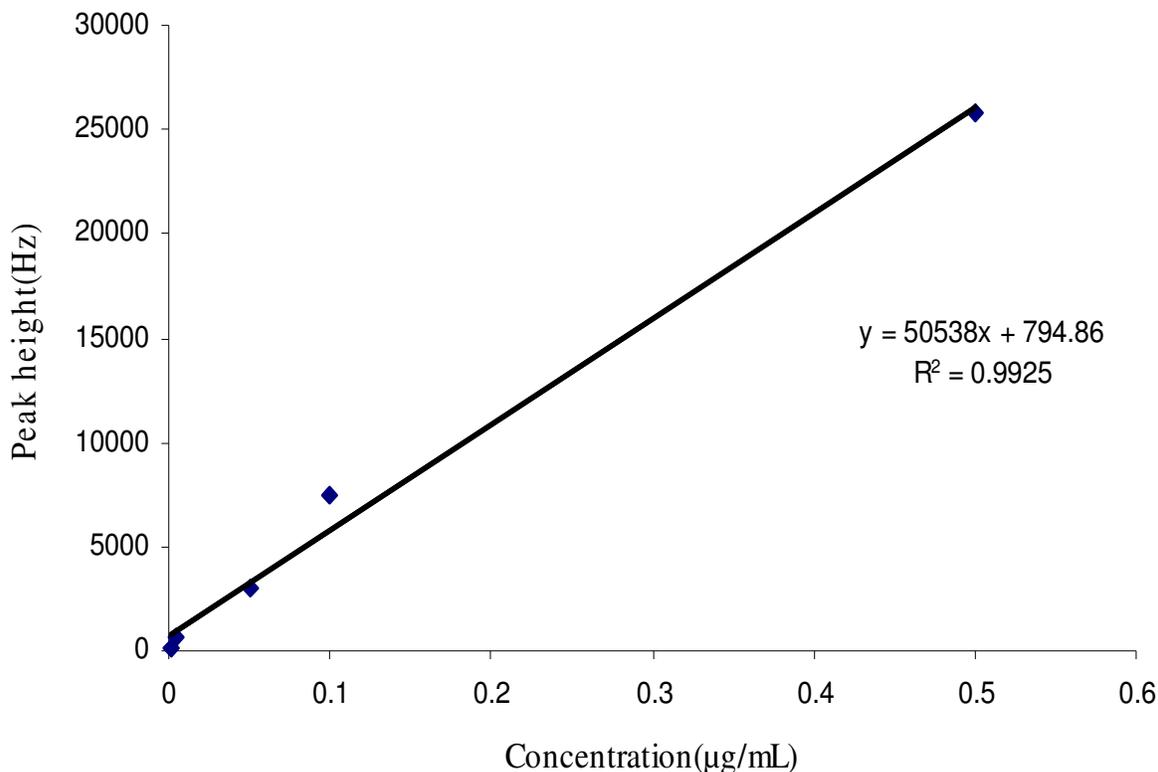


Figure 2. Calibration curve of Cypermethrin obtained with chromatograms of different concentrations.

Development of SFE conditions

The selection of the solvent for the SFE was based on several criteria. A volatile solvent must be used, as rapid evaporation of a large volume would be required in the sample preparation without causing loss of volatile pesticides. The most frequently applied supercritical fluid is carbon dioxide, a non-polar solvent. If polar compounds as pesticides have to be extracted, the addition of a polar solvent to the supercritical fluid, called cosolvent, may improve the extraction because of the increase in the polarity of the supercritical fluid and thus the recovery of pesticides (Abaroudi et al., 2002; King et al., 2006). It was found that the non-polar solvent, hexane, resulted in better recoveries for three insecticides of studied. Figure 1 shows a schematic view of the SFE instrument. The sample cartridge is filled with a sample and placed in a heated extraction chamber. Pressurized by a syringe pump, liquefied carbon dioxide enters the extraction chamber. CO₂ is heated and becomes supercritical. 2 ml eluting solvent was sufficient to condition the SFE and elute the analytes. A flow rate of 1 ml/min was sufficient to elute the analytes. In order to obtain good recoveries for the pesticides, parameter affecting the SFE conditions such as solvent polarity was optimised. The optimal conditions for the OP pesticides were found to be as follows: (1) hexane: matrices (1:10) and (2) flow rate: 1 ml/min.

Determination and Confirmation

Recording of chromatograms and quantitative measurements of peak areas were performed with a GC-ECD Chemstation. Cypermethrin recovered from yardlong bean and carrot with critical pressure, P_C at 20 lbs and critical temperature, T_C 40°C. Lehotay et al. (1995) also reported that 40°C, 200 bar of organochlorine pesticides in spiked green beans and carrots but methamidophos and acephate insecticides extracted from green beans with 60°C, 320 bar (Eller et al., 1997). On the other hand, the recovery rate of similar insecticides from eggplant was 96% with P_C at 31.5 lbs and 45°C in T_C. Wuchner et al. (1993) reported that diazinon was extracted from rice grain with CO₂ + 5% CH₃OH at 45°C, 315 bar.

The retention time (t_R) was 49.5 min of cypermethrin standard and similar t_R was found in formulated of yardlong bean and carrot matrices. Similarly, the t_R of cypermethrin standards was 49.6 in eggplant which was similar to both formulated and standard. Wuchner et al. (1993) observed a parallel finding of quinalphos in cereal products. The absorption spectrum of the detected peaks were confirmed by comparison with a standard of each pesticide. More over, from the above 03 (three) points of view, it was confirmed that the extracted fluids were cypermethrin from yardlong bean, carrot and eggplant matrices with 96 - 105% recoveries. Different insecticide extraction parameters of SFE such as critical pressure

Table 5. Extraction protocol of SFE for the determination of insecticides in vegetables.

Name of insecticides	Vegetable matrix	Critical		Extraction time (min)
		Pressure (lbs)	Temp. (°C)	
Cypermethrin	Yardlong Bean	20	40	40
	Carrot	20	40	40
	Eggplant	31.5	45	43

Temp. = Temperature.

(P_c), critical temperature (T_c) and extraction time of chlorpyrifos insecticide from yardlong bean, carrot and eggplant matrices were presented in Table 5.

Conclusion

SFE can be applied to high-water-content samples by the addition of a drying agent such as hydromatrix, magnesium sulfate, sodium sulfate and few minutes oven dry with sodium sulfate. The use of a drying agent (remove water) improved the recoveries of highly polar analytes. The SFE method has advantages of automation and simple operation compared to the solvent extraction methods, thus, it is a good alternative in practice within very short time, best recoveries and simplicity. The SFE sample preparation method, conditions and collection system are inexpensive and easy to perform, and may also be suitable for analysis without additional cleanup.

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Abbreviations

SFE, Supercritical fluid extraction; **P_c**, critical pressure; **T_c**, critical temperature; **t_R**, average retention time; **R²**, linear regression; **LDL**, lowest detectable limit; **GC-ECD**, gas chromatography-electron capture detection; **GC-ECD**, chromatography coupled to electron capture detector; **HPLC**, high performance liquid chromatography; **GPC**, gel-permeation chromatography.

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