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Determination of organophosphorus pesticide residues in vegetables using solid phase micro-extraction coupled with gas chromatography–flame photometric detector

Haizarul Aida Sapahin, Ahmad Makahleh *, Bahruddin Saad *

School of Chemical Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia

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Abstract An adequate and simple analytical method based on solid-phase microextraction (SPME) followed by gas chromatography–flame photometric detection (GC–FPD) for the determination of eleven organophosphorus pesticide residues (i.e., ethoprophos, sulfotep, diazinon, tolclofos-methyl, fenitrothion, chlorpyrifos, isofenphos, methidathion, ethion, triazophos, leptophos) in vegetables samples (cabbage, kale and mustard) was developed. Important parameters that influence the extraction efficiency (i.e., fibre type, extraction modes, extraction time, salt addition, desorption time and temperature) were systematically investigated. Four types of commercially available fibres (i.e., 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB), 100 μm polydimethylsiloxane (PDMS), and 85 μm polyacrylate (PA)) were evaluated. PA fibre exhibited the best performance and was used for the rest of the studies. The optimised extraction conditions were: extraction time, 30 min at room temperature; stirring speed, 1275 rpm; salt content, 10% NaCl; desorption time and temperature, 11 min at 260 °C; and no pH adjustment of the sample extract. The method was validated over the range 0.1–100 $\mu\text{g/L}$. Repeatabilities were satisfactory, ranging between 2.44% and 17.9% for all analytes. The limits of detection and quantitation ranged from 0.01 to 0.14 and 0.03 to 0.42 $\mu\text{g/L}$, respectively. The method was applied to twenty local vegetable (cabbage, kale

* Corresponding authors. Tel.: +60 4 6534047; fax: +60 4 6574857.

E-mail addresses: makahleha@yahoo.com (A. Makahleh), bahrud@usm.my (B. Saad).

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and mustard) products. Chlorpyrifos (0.22–1.68 µg/kg) was the most detected pesticide in the tested samples. The obtained values are however lower than the Maximum Residue Limits (MRLs) as stipulated in the Food Act & Regulations of Malaysia.

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1. Introduction

Organophosphorus pesticides (OPs) are the most widely used pesticides, amounting to more than several billion US dollars annually. This popularity is largely contributed to their favourable characteristics such as biodegradable and short persistence compared to the organochlorine pesticides (Chen et al., 2010). OPs protect crops from pests by inhibiting acetylcholinesterase enzyme activity in insects. They are sprayed over crops or soils, causing residues to be found in surface and groundwaters, fruits, vegetables and in drinking water (Yao et al., 2001).

A challenge in the analysis of pesticide residues is in sample preparation, procedures that are mandatory prior to the instrumental analysis. The pesticides are traditionally extracted using liquid–liquid extraction (LLE) (Fenoll et al., 2007; Wang et al., 2008; Hassan et al., 2010; Pirard et al., 2007). The LLE procedures consume large amounts of solvents, involve several steps, time consuming and difficult to be automated. Alternatively, solid-phase extraction (SPE) involving the use of different types of sorbents (e.g., amine, PSA, C18) has been used (López-Blanco et al., 2006; Alberio et al., 2005; Wang et al., 2009). Other extraction techniques such as accelerated solvent extraction (Conte et al., 1997; Bidari et al., 2011; Wu et al., 2011), matrix solid phase dispersion (Navarro et al., 2002; Torres et al., 1996; Radišić et al., 2009; Muccio et al., 1997) and QuEChERS with dispersive solid-phase extraction (d-SPE) technique using different type of sorbents such as PSA, C18, silica gel, graphitised carbon black, florisil and amine modified graphene (Anastassiades and Lehotay, 2003; Walorczyk et al., 2011; Guan et al., 2013; Chai and Elie, 2013; Ciešlik et al., 2011) were also reported. Although these techniques provide many benefits such as the reduction of matrix interferences and minimisation of solvent consumption and save time, they generally lack selectivity and sometimes low recoveries of analytes are obtained (Navarro et al., 2002; Yang et al., 2011).

Solid phase microextraction (SPME), pioneered by Pawliszyn and co-workers in 1990s (Sang et al., 2013), offers interesting options to overcome limitations of the SPE and LLE techniques (Kin and Huat, 2010). It consists of a coated fibre and syringe-like handling device that are used to isolate and concentrate analytes of interest. SPME is recognised not only as a solvent-free technique but also offers savings of 70% of the sample preparation time (Chen et al., 2010). SPME coating materials used to analyse pesticide residues in various matrices such as polydimethylsiloxane/divinylbenzene (PDMS/DVB), polyacrylate (PA), polydimethylsiloxane (PDMS) and carbowax divinylbenzene (CW-DVB) are commercially available. Several methods have been reported for the determination of OPs in different matrices (e.g., mangoes, fruit juices, honey and vegetables) using direct-immersion mode (Filho et al., 2010; Cortés-Aguado et al., 2008; Campillo et al., 2006) or head-space mode

(Kin and Huat, 2010). Other new microextraction approaches such as air-assisted liquid–liquid microextraction (You et al., 2013), stir bar (Hu et al., 2013) and cloud point (Zhou et al., 2011) have also been reported.

Since the majority of the OPs are volatile and thermally stable, they are amenable to gas chromatography (GC) analysis. The determination of OPs in fruits, vegetables and water using GC with either electron capture (ECD) (Kin and Huat, 2009), flame photometric (FPD) (Yu et al., 2004; Pappas and Kyriakidis, 2003; Liu et al., 2013; Ahmadi et al., 2006; Khalili-Zanjani et al., 2008; Berijani et al., 2006), nitrogen phosphorus (NPD) (Wang et al., 2008) or mass spectrometry (MS) (Nguyen et al., 2008; Melo et al., 2012) detectors has been reported.

The Chemistry Department and the Ministry of Health are the main regulatory agencies entrusted with the monitoring of food items in Malaysia. A survey on pesticide residues carried out by the Department of Agriculture Sarawak reported that 95% of the total residue violation is caused by organophosphate pesticides (Lian and Seng, 2003). To meet the challenge of analysing more samples and matrices that widely produced in Malaysia and have not been previously reported using SPME (i.e., mustard and kale), it is important that new approaches in the determination be introduced. Thus, the main purpose of the present studies is to evaluate the viability of the SPME technique for the selective extraction of the common OPs in Malaysia (namely ethoprophos, sulfotep, diazinon, tolclofos-methyl, fenitrothion, chlorpyrifos, isofenphos, methidathion, ethion, triazophos, leptophos) in these local vegetables samples. The proposed method was validated and applied for the analysis of these pesticides in several samples found in the market.

2. Materials and methods

2.1. Reagents and chemicals

Certified individual pesticide standards, namely ethoprophos (93.1%), sulfotep (97.4%), tolclofos-methyl (99.4%), diazinon (98.3%), fenitrothion (95.2%), chlorpyrifos (99.9%), isofenphos (95.6%), methidathion (95.8%), ethion (97.8%), triazophos (96.5%) and leptophos (96.7%) were obtained from Fluka (Buchs, Switzerland). Individual stock standard solutions (500 mg/L) were prepared by dissolving an accurate weight of each pesticide in 100 mL of acetone. Working standard solutions (10 mg/L) for the eleven OPs were prepared by serial dilution of the individual stock with acetone. All standards were stored under refrigeration at 4 °C. Acetone and methanol (analytical grade) were supplied by QRec (>99%, Chonburi, Thailand) and sodium chloride (>99.5%) was obtained from Merck (Darmstadt, Germany). The properties and the chemical structures of the studied OPs are shown in Table 1 and Fig. 1, respectively.

Table 1 Chemical formula and physical properties of the studied pesticides.

Pesticides	Chemical formula	Molecular mass (g mol ⁻¹)	log <i>K</i> _{ow} ^a	Solubility in water (mg L ⁻¹)	PV ^b (mPa)
Ethoprophos	C ₈ H ₁₉ O ₂ PS ₂	242.3	2.99	1300	78
Sulfotep	C ₈ H ₂₀ O ₃ P ₂ S ₂	322.3	3.99	10	14
Tolclofos-methyl	C ₉ H ₁₁ Cl ₂ O ₃ PS	301.1	4.56	0.708	0.877
Diazinon	C ₁₂ H ₂₁ N ₂ O ₃ PS	304.3	3.69	60	11.97
Fenitrothion	C ₉ H ₁₂ NO ₃ PS	277.2	3.32	19	0.676
Chlorpyrifos	C ₉ H ₁₁ Cl ₃ NO ₃ PS	350.8	4.70	1.05	1.43
Isofenphos	C ₁₅ H ₂₄ NO ₄ PS	345.3	4.04	24	0.53
Methidathion	C ₆ H ₁₁ N ₂ O ₄ PS ₃	302.3	2.57	240	0.25
Ethion	C ₉ H ₂₂ O ₄ P ₂ S ₄	384.4	5.07	2	0.20
Triazophos	C ₁₂ H ₁₆ N ₃ O ₃ PS	313.3	3.55	35	1.33
Leptophos	C ₁₃ H ₁₀ BrCl ₂ O ₂ PS	412.07	6.31	2.4	1.99

^a Octanol–water partition coefficient at pH 7, 20 °C.

^b Vapour pressure at 25 °C (mPa).

2.2. Instrumentation and apparatus

A HP5890 series II plus GC unit (Hewlett–Packard, PA, USA) with split/splitless injector coupled with flame photometric detector (FPD) was used. The data were analysed using Chemstation software (Ver. A.10.02). A capillary column, DB-5 (5% phenyl methylpolysiloxane, 30 m × 0.25 mm id × 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA) was used to separate the pesticides using the following temperature program: Initial temperature, 100 °C; held for 1 min; heated to 200 °C at 20 °C/min and held for 8 min; heated to a final temperature of 250 °C at 10 °C/min and held for 7 min. The injector temperature was maintained at 260 °C for effective fibre desorption and detector temperature was at 275 °C. Nitrogen (CP grade, 99.999%) was used as carrier gas and supplied by SGS (Malaysia). The flow of carrier gas was 1.0 mL/min and the injection was performed using splitless mode.

Detected OPs were confirmed using a GC–MS system (HP7890/MSD 5975C, Agilent, CA, USA) operated in the splitless mode. A capillary column DB-5MS (5% phenyl methylpolysiloxane, 30 m × 0.25 mm id × 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA) was used with helium carrier gas at 1.5 mL/min. The oven temperature program was set as previously mentioned for GC–FPD system. The mass spectrometer was operated in the electron impact ion (EI) mode with a source temperature of 230 °C. The chromatographic resolution was performed in scan mode from *m/z* 50 to 400. The confirmation of pesticides peaks has been done by comparing the fragment ions with NIST library and the standard reference with similarity index more than 70%. All prepared reagents were kept in a dispenser (BRAND, Germany) with capacity of 1–10 mL.

2.3. Sample preparation

Pesticide-free samples (cabbage, kale and mustard) were collected from Agro Technology Park, Malaysian Agricultural Research Development Institute (MARDI), Cameron Highlands, Malaysia. A representative sample (0.5 kg) was finely chopped into small pieces, combined and homogenised using a Robot coupe food processor R3 (Jackson, MS, USA). After the sample was homogenised, 0.5 g of the sample was accurately weighed in 20 mL vial followed by the addition of

2 mL of methanol/acetone (1:1, v/v) as extraction solvent and 1 mL of NaCl solution (10%, w/v). The sample was then diluted to 10 mL with water and sealed with septum cap for the SPME analysis. Pesticides-free vegetables were obtained from Malaysian Agriculture and Development Institute (MARDI) and used for recovery studies. Vegetable samples (eight mustard, seven kale and five cabbage samples) were purchased from six local supermarkets in Ipoh, Perak. All the samples are products of Cameron Highland, Malaysia.

2.4. SPME procedure

SPME sampling stands, fibre holder and commercial fibre assembly coated with 100 μm polydimethylsiloxane (PDMS), 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB), 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) and 85 μm polyacrylate (PA) were obtained from Supelco (Bellefonte, PA, USA). The fibre was attached to the holder for manual sampling and was pre-conditioned before use according to the manual given by the manufacturer. The fibre inside the holder was attached to the SPME sampling stand and was pushed out from the needle and immersed into the sample solutions (prepared as described in Section 2.3) for 30 min at ambient temperature. The solution was stirred at a constant speed (1275 rpm) during the extraction using a magnetic stirrer. After the extraction, the fibre was immediately transferred to the GC injector and desorbed for 11 min at 260 °C. The extraction efficiency is based on total peak areas of the OPs.

2.5. Method validation

Method validation (linearity, limits of detection (LOD) and quantitation (LOQ), repeatability and recovery) was performed using the proposed SPME–GC–FPD method according to ICH and SANCO 2013 guidelines. Linearity was investigated over the range of 0.1–100 μg/L. The LOD and LOQ values were calculated based on the standard deviation of the response of the standard blank and the slope of calibration curve at signal-to-noise ratio of 3 and 10, respectively. Repeatability, expressed as relative standard deviation (% RSD), was done by extracting six replicates at three concentration levels (5, 50 and 100 μg/L). Recovery studies were carried

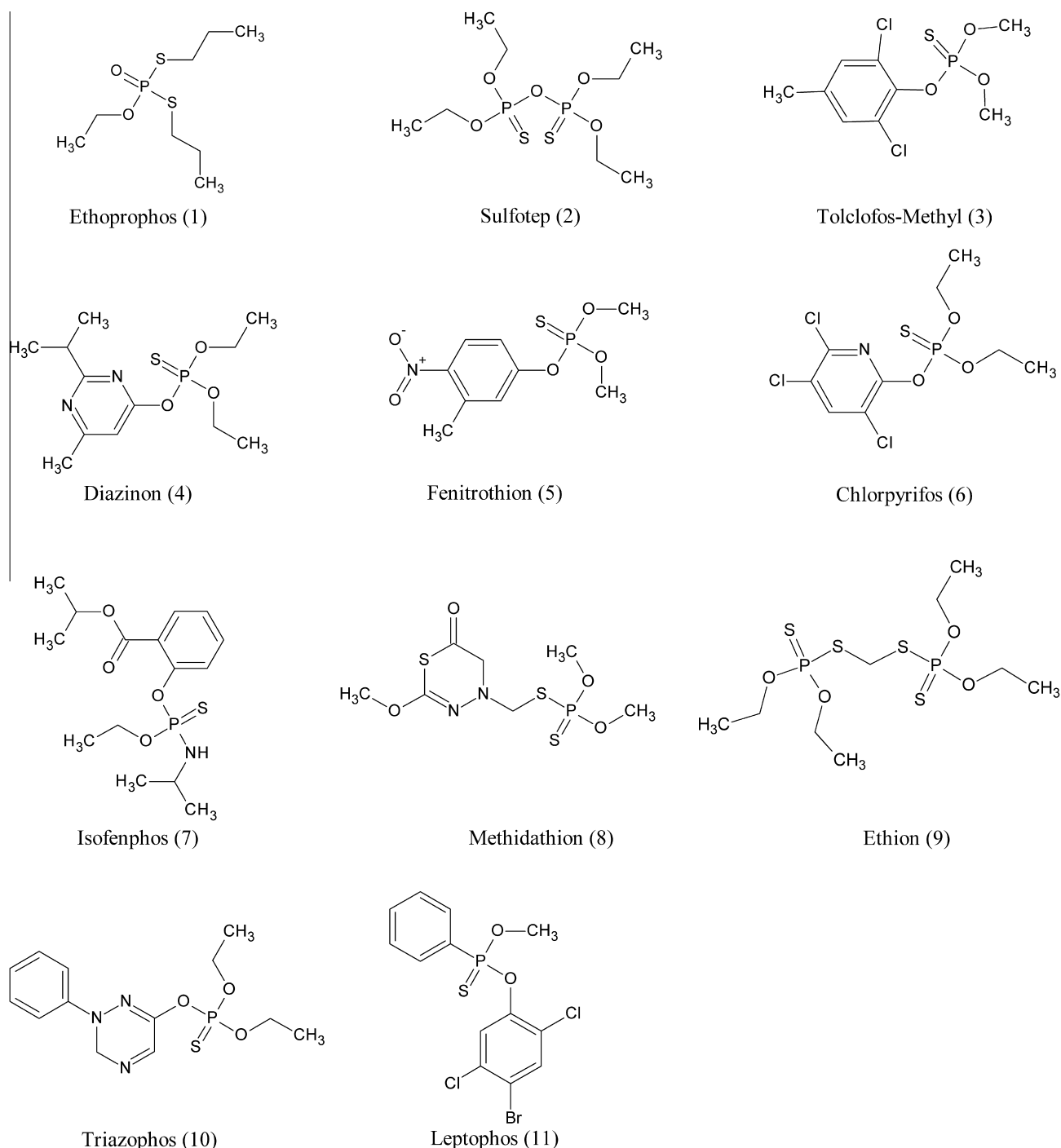


Figure 1 Chemical structures of the studied organophosphorus pesticides.

out using three vegetable matrices (cabbage, kale and mustard) spiked at three fortification levels (5, 50 and 100 µg/L). All spiked samples were extracted in triplicates.

3. Results and discussion

3.1. Gas chromatography (GC) separation

Preliminary separation conditions were based on the work of Yao et al. (2001). Injector and detector temperatures used were

230 and 250 °C, respectively. The oven temperature was programmed as follows: initial temperature 60 °C held for 1 min, heated to 150 °C at 20 °C/min and held for 1 min, heated to a final temperature of 230 °C at 10 °C/min. Under these conditions, only six peaks were obtained with a total run time of 14.50 min. In order to achieve separation of all analytes that were injected, the oven temperature program was modified. The holding time was increased to obtain adequate retention times for the eleven OPs. The initial temperature (60, 80, 100 and 120 °C) was investigated, with 100 °C

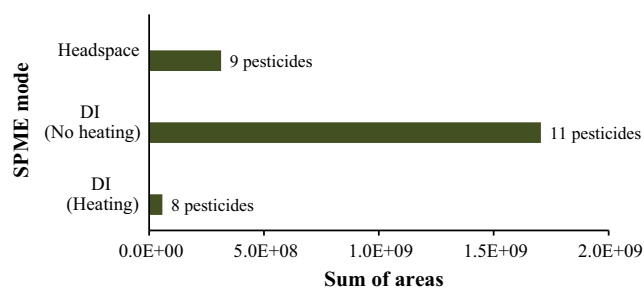


Figure 2 Extraction efficiency by three modes of SPME: headspace (HS), direct immersion (DI) at ambient temperature and direct immersion (DI) at 60 °C.

giving the best results. Two different temperature ramps were also optimised to achieve full separation of the analytes in short analysis time. The best ramps were 100 to 200 °C at 20 °C/min and held for 8 min; heated to 250 °C at 10 °C/min (hold for 7 min). Under the optimum chromatographic conditions, all the peaks were separated in 26 min.

3.2. Extraction mode and fibre coating

The extraction efficiency for each fibre is related to the logarithm octanol–water partition coefficient ($\log K_{ow}$) and volatility (PV) (Table 1). The $\log K_{ow}$ indicates the polarity strength and their tendency towards the hydrophilic and lipophilic layers. $\log K_{ow}$ of the OPs was between 2.57 (methidathion) and 6.31 (leptophos) which reflect their tendency towards lipophilic layers. Furthermore, these compounds are volatile with vapour pressures between 0.20 mPa (ethion) and 78 mPa (ethoprophos).

Three extraction modes, i.e., headspace (HS), direct immersion (DI) at 60 °C and ambient temperature were investigated by comparing the sum of the peak areas of the extracted OPs. DI mode at ambient temperature exhibited the highest extraction efficiency (the highest total peak areas) for all compounds

studied (Fig. 2). It is anticipated that the DI–SPME will provide more sensitive results compared to the HS–SPME due to the direct contact between the coating materials and the analytes. The HS mode was able to extract all the pesticides except methidathion and triazophos. The least satisfactory is using direct immersion mode at 60 °C, which is unable to extract three pesticides namely fenitrothion, methidathion and triazophos. This is due to the possible degradation of these compounds under heating condition (Kin and Huat, 2010). Thus, DI mode without heating was selected for further experiments.

Four types of commercial SPME fibre coatings: 100 μm PDMS (85 μm PA, 65 μm PDMS–DVB and 50/30 μm DVB/CAR/PDMS (tripolar phase) were used to extract eleven OPs (50 $\mu\text{g/L}$) in aqueous solution. 85 μm PA fibre exhibited the higher extraction efficiency for the eleven OPs used, especially for chlorpyrifos and ethion (Fig. 3). This fibre was used for further work. The good ability of PA fibre in extracting pesticide residues was previously reported by Tankiewicz et al. (2013).

3.3. Optimisation of DI–SPME method

Factors affecting the SPME process such as absorption time, stirring speed, salting out effect, desorption temperature and desorption time were evaluated. An extraction procedure was established with the initial conditions: extraction time, 30 min; stirring rate, 800 rpm, 10% NaCl solution, in accordance with data obtained from literature (Kin and Huat, 2009).

The influence of extraction time (20–40 min) was studied using 85 μm PA fibre directly immersed in deionised water containing 50 $\mu\text{g/L}$ OPs with the addition of NaCl solution (Fig. 4(A)). It was observed that the signal for all of the OPs increased as the extraction time increased up to 30 min and dropped thereafter, especially for triazophos and fenitrothion. Thus 30 min exposure was therefore selected for the following experiments.

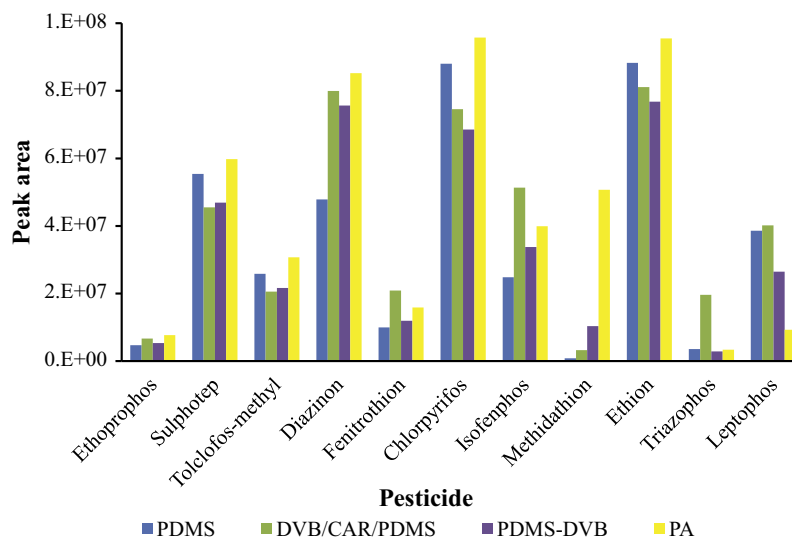


Figure 3 Comparison of different SPME fibres in extracting OPs at concentration of 50 $\mu\text{g/L}$. Extraction time, 30 min; stirring speed, 800 rpm; desorption time, 10 min and desorption temperature 240 °C in direct immersion mode.

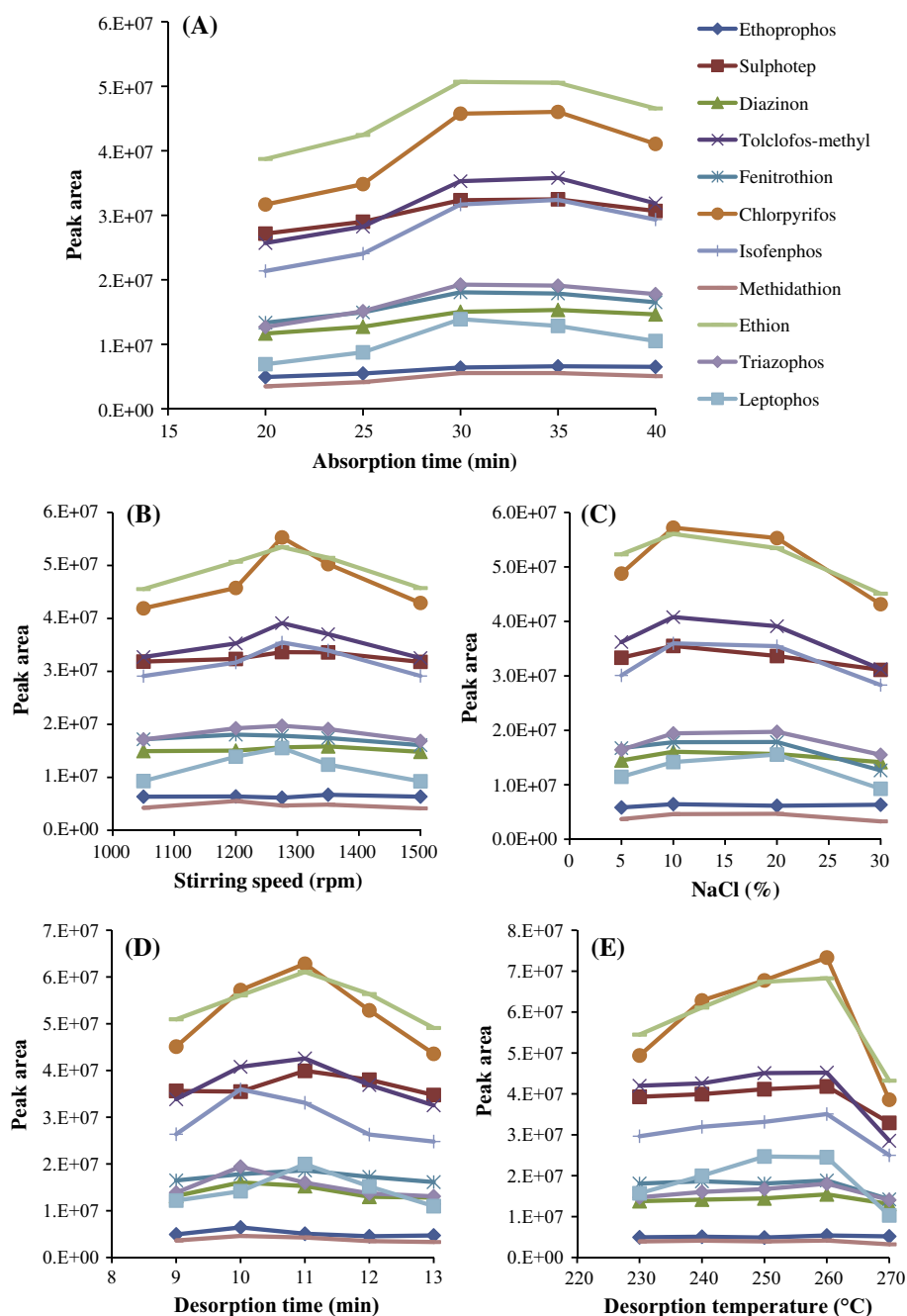


Figure 4 Optimisation parameters for extraction of OPs (50 $\mu\text{g/L}$) with 85 μm PA coating: (A) Absorption time, (B) stirring speed, (C) effect of NaCl, (D) desorption time, and (E) desorption temperature.

In order to achieve faster extraction equilibrium and improve the mass transfer of the analytes from solution to the fibre, different stirring speeds (1050, 1200, 1275, 1350 and 1500 rpm) were studied. The signal for most of the pesticides decreased as the stirring velocity was increased, starting at 1350 rpm (Fig. 4(B)). Thus, 1275 rpm was used for the rest of the studies.

The effect of ionic strength of the solution on the SPME procedure was studied using different concentrations (5, 10, 20 and 30% (w/v)) of sodium chloride. Generally, the addition of suitable amount of salt will decrease the solubility of the compounds in the liquid phase, thus enhancing the extraction

of the compounds to the fibre coating. It was observed that the peak areas increase with the addition of salt up to 10% NaCl especially for chlorpyrifos, ethion, tolclofos-methyl and isofenphos (Fig. 4(C)). The use of higher salt content (more than 10%) will decrease the instrumental response due to the deposition of salt. Further experiments were conducted using 10% (w/v) NaCl.

Duration of desorption (9, 10, 11, 12 and 13 min) was investigated, high sensitivity was obtained when desorbed for 11 min (Fig. 4(D)). Five desorption temperatures (230–270 °C) were also studied. The best result was obtained at 260 °C (Fig. 4(E)). Long exposure periods of the PA fibre at high tem-

Table 2 Method validation parameters for each pesticide by using the DI-SPME-GC-FPD method.

Pesticides	Retention time (min)	Linearity range ($\mu\text{g/L}$)	Regression equation	r^2	LOD ($\mu\text{g/L}$)	LOQ ($\mu\text{g/L}$)
Ethoprophos	6.4	0.5–100	$y = 2 \times 10^7 + 39,313$	0.996	0.14	0.42
Sulfotep	6.8	0.1–100	$y = 1 \times 10^8 + 122,130$	0.999	0.03	0.08
Diazinon	7.9	0.3–100	$y = 4 \times 10^7 + 56,499$	0.998	0.07	0.21
Tolclofos-methyl	9.5	0.1–100	$y = 9 \times 10^7 + 134,633$	0.998	0.03	0.09
Fenitrothion	10.2	0.5–100	$y = 2 \times 10^7 + 38,437$	0.997	0.14	0.42
Chlorpyrifos	11.1	0.1–100	$Y = 1 \times 10^8 + 208,339$	0.996	0.03	0.08
Isofenphos	13.1	0.3–100	$y = 7 \times 10^7 + 100,724$	0.999	0.04	0.12
Methidathion	13.9	0.3–100	$y = 1 \times 10^7 + 28,258$	0.996	0.06	0.17
Ethion	17.1	0.1–100	$y = 1 \times 10^8 + 49,142$	0.993	0.03	0.08
Triazophos	17.5	0.1–100	$y = 5 \times 10^7 + 55,500$	0.998	0.01	0.03
Leptophos	21.3	0.5–100	$y = 2 \times 10^7 + 80,798$	0.991	0.14	0.42

perature can cause thermal degradations, resulting in lower efficiency.

3.4. Adopted extraction conditions

The adopted extraction conditions were: absorption time, 30 min; stirring speed, 1275 rpm; ionic strength, 10% NaCl; desorption temperature, 260 °C; desorption time, 11 min. The proposed method shows some advantages over the previous reported methods in terms of heating requirement and

types of analytes. The proposed method was performed at ambient temperature, compared to the previously DI-SPME methods, where the sample was heated in the range 40–75 °C (Filho et al., 2010; Yao et al., 2001; Campillo et al., 2006). The procedure (ambient temperature) was purposely used to provide good pre-concentration for the extracts and prevent any degradation of the analytes. Two of these OPs, tolclofos-methyl and leptophos, are for the first time reported using SPME. The proposed method showed considerable advantages over the previously reported methods such as LLE, SPE, DLLME and MAE in terms of solvent consumption and waste

Table 3 Repeatabilities in aqueous solution and recoveries in three vegetable matrices using DI-SPME-GC-FPD method.

Pesticide	Spiking level ($\mu\text{g/L}$)	Repeatability (% RSD, $n = 6$)	% Recovery (average \pm SD, $n = 3$)		
			Cabbage	Mustard	Kale
Ethoprophos	5	8.72	110 \pm 4.78	85.8 \pm 3.84	109 \pm 4.49
	50	2.44	74.6 \pm 1.32	96.2 \pm 14.5	117 \pm 17.2
	100	6.16	80.4 \pm 2.52	78.1 \pm 8.04	83.5 \pm 12.2
Sulfotep	5	7.34	105 \pm 13.3	93.0 \pm 5.23	91.5 \pm 9.50
	50	9.75	102 \pm 7.58	91.5 \pm 10.3	92.3 \pm 10.5
	100	4.99	98.0 \pm 7.28	101 \pm 5.48	104 \pm 11.0
Diazinon	5	4.10	106 \pm 10.7	91.5 \pm 6.80	101 \pm 3.49
	50	5.28	94.9 \pm 6.80	90.8 \pm 4.00	96.1 \pm 6.78
	100	3.70	102 \pm 5.38	96.2 \pm 13.2	96.6 \pm 2.18
Tolclofos-methyl	5	11.4	87.1 \pm 10.9	87.2 \pm 8.21	100 \pm 3.95
	50	11.7	90.9 \pm 5.68	96.7 \pm 7.79	92.8 \pm 3.66
	100	8.25	104 \pm 9.88	97.5 \pm 7.04	94.8 \pm 13.5
Fenitrothion	5	9.40	87.3 \pm 9.69	91.4 \pm 10.1	92.8 \pm 7.06
	50	9.94	96.5 \pm 16.7	97.4 \pm 8.50	90.4 \pm 9.43
	100	3.99	94.0 \pm 5.05	96.3 \pm 5.48	92.2 \pm 9.48
Chlorpyrifos	5	10.3	102 \pm 9.66	89.1 \pm 5.28	81.4 \pm 5.39
	50	12.3	91.3 \pm 7.96	98.4 \pm 6.94	89.8 \pm 13.0
	100	11.2	93.2 \pm 7.62	95.1 \pm 10.3	103 \pm 17.8
Isofenphos	5	8.51	96.2 \pm 8.76	86.0 \pm 2.57	81.1 \pm 6.16
	50	9.95	101 \pm 7.65	94.2 \pm 6.86	96.2 \pm 7.10
	100	6.04	108 \pm 9.63	88.5 \pm 5.71	90.9 \pm 1.05
Methidathion	5	14.2	95.6 \pm 9.35	92.6 \pm 5.69	87.4 \pm 7.54
	50	12.8	80.8 \pm 11.4	84.2 \pm 4.86	95.6 \pm 10.3
	100	6.16	84.1 \pm 8.17	102 \pm 5.18	80.2 \pm 2.13
Ethion	5	10.7	97.4 \pm 14.4	85.3 \pm 2.56	95.5 \pm 7.72
	50	12.6	113 \pm 15.3	96.4 \pm 6.24	97.0 \pm 5.20
	100	14.2	97.4 \pm 5.50	92.9 \pm 16.8	93.8 \pm 10.9
Triazophos	5	9.89	95.8 \pm 16.4	101 \pm 4.30	100 \pm 7.15
	50	3.82	94.1 \pm 7.05	84.2 \pm 6.15	93.2 \pm 4.74
	100	7.77	92.7 \pm 7.33	76.8 \pm 2.27	79.9 \pm 7.12
Leptophos	5	9.49	23.3 \pm 2.62	19.1 \pm 1.71	24.4 \pm 3.27
	50	14.5	24.9 \pm 3.16	16.7 \pm 3.04	21.3 \pm 2.29
	100	17.9	28.5 \pm 5.63	48.7 \pm 7.25	29.9 \pm 5.96

generated (Fenoll et al., 2007; Bidari et al., 2011; Wang et al., 2009; Zhao et al., 2012).

3.5. Method validation

The linearity range, limits of detection (LOD) and quantitation (LOQ), repeatability and recovery of the DI-SPME method were investigated under the optimised conditions. The linearity range was studied by varying the concentration of the standard solution from 0.1 to 100 µg/L. Calibration study was tested by spiking the working standard solutions into water containing NaCl solution. The calibration curves were illustrated by plotting the peak area versus concentration of each pesticide. Over the studied range (0.1–100 µg/L), the calibrations were linear only for sulfotep, tolclofos-methyl, chlorpyrifos, ethion and triazophos. Diazinon, isofenphos and methidathion were linear over 0.3–100 µg/L, while the linearity range for ethoprophos, fenitrothion and leptophos was 0.5–100 µg/L (Table 2). All calibrations were well correlated with determination coefficients, $r^2 > 0.99$.

The LOD values obtained were in the range from 0.01 (triazophos) to 0.14 µg/L (ethoprophos, fenitrothion, leptophos), meanwhile the LOQs ranged from 0.03 to 0.42 µg/L, which is lower than the MRL (1 mg/kg). The values obtained were found to be comparable to the previously reported method using GC-FPD (0.05–0.30 µg/L) as reported by Yao et al. (2001) but lower than the other reported methods using GC-MS (2–109 µg/L) (Zambonin et al., 2004), (1–33 µg/L) (Filho et al., 2010), and (0.01–50 µg/L) (Cortés-Aguado et al., 2008).

To evaluate the precision of the developed method, intra-day repeatability ($n = 6$) was performed using three concentration levels (5, 50 and 100 µg/L) of the studied pesticides. % RSD values varied between 2.44% (ethoprophos) and 17.9% (leptophos) (Table 3). Overall, the repeatabilities obtained for each pesticide in all concentration levels indicated satisfactory extraction.

The effect of different sample weight (0.5, 1.0, 2.0 and 3.0 g) on the recoveries was also investigated. The recoveries were found to increase as the mass of the sample decreased. The best recoveries were obtained when 0.5 g sample was used. Centrifugation step was not applied due to the lower recoveries. Table 3 shows recovery results for samples that were spiked with different levels of pesticides. Satisfactory recoveries (74.6–117%) for most of the studied pesticides were obtained except leptophos (16.7–48.7%), probably due to its high molecular weight (412.7) and low polarity ($\log K_{ow} = 6.31$), making it difficult to extract when spiked into real samples. Despite leptophos, the obtained recoveries were within the acceptable range (70–120%) and comparable with the previous reported works using DI-SPME (72–117%) (Filho et al., 2010), HS-SPME (71–98%) (Kin and Huat, 2009), matrix solid phase dispersion (62–102%) (Navarro et al., 2002), accelerated solvent extraction (62–107%) (Wu et al., 2011), vortex-assisted liquid-liquid microextraction (72–106%) (Jia et al., 2010), microwave-assisted extraction (76.5–109%) (Zhao et al., 2012) and stir bar sorptive-extraction (77–119%) (Hu et al., 2013) and better than the obtained recoveries using solid phase extraction (63–137%) (Yang et al., 2011). Typical chromatogram for the spiked cabbage sample is shown in Fig. 5(A).

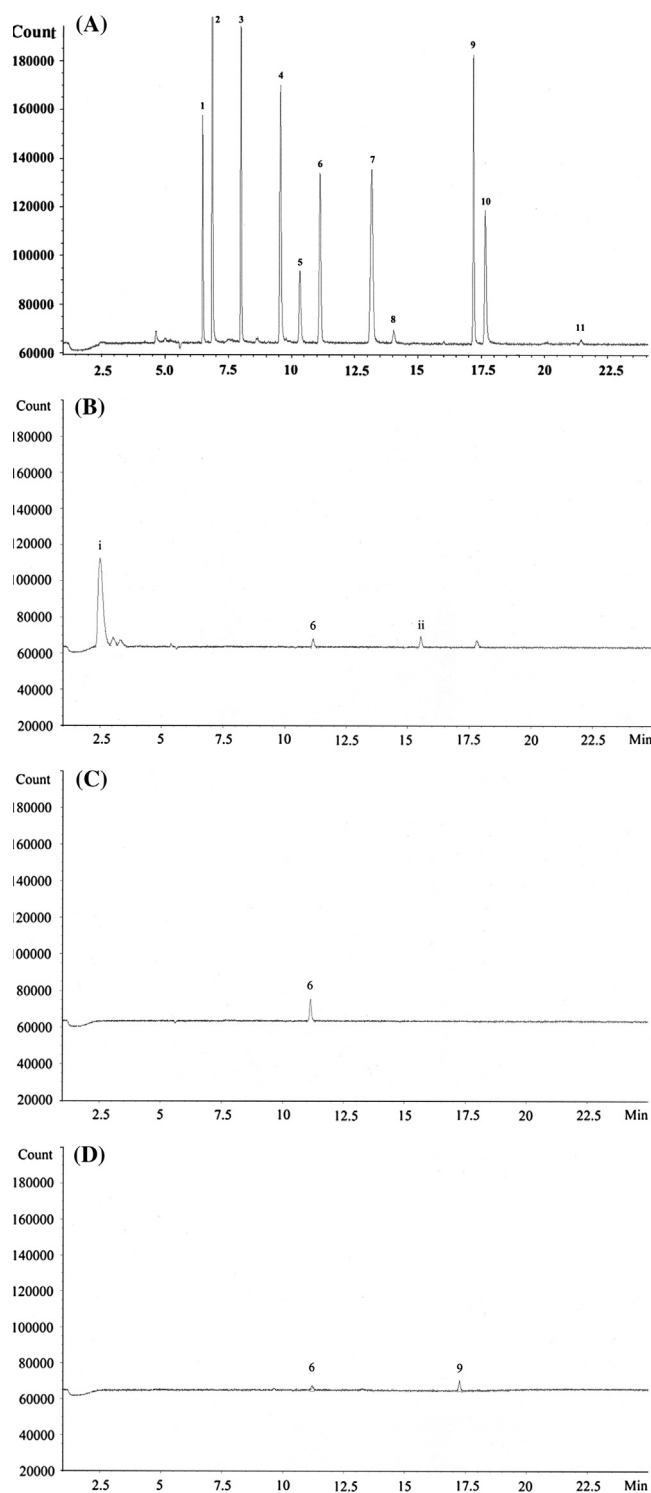


Figure 5 Typical chromatograms obtained for: (A) spiked cabbage with OPs (5 µg/L), (B) mustard, (C) kale and (D) cabbage samples using DI-SPME-GC-FPD with 85 µm PA in three different locations. Peak label: (1) ethoprophos, (2) sulfotep, (3) diazinon, (4) tolclofos-methyl, (5) fenitrothion, (6) chlorpyrifos, (7) isofenphos, (8) methidathion, (9) ethion, (10) triazophos, (11) leptophos, (i) trichlorfon, and (ii) Demephion.

Table 4 Pesticide concentrations in the positive vegetables samples ($n = 3$).

Pesticides	Concentration ^a , (average \pm SD)					
	Kale	Cabbage	Mustard1	Mustard2	Mustard3	Mustard4
Ethoprophos	n.d	n.d	n.d	n.d	n.d	n.d
Sulfotep	n.d	n.d	n.d	n.d	n.d	n.d
Diazinon	n.d	n.d	n.d	n.d	n.d	n.d
Tolclofos-methyl	n.d	n.d	n.d	n.d	n.d	n.d
Fenitrothion	n.d	n.d	n.d	n.d	n.d	n.d
Chlorpyrifos	0.82 \pm 0.06	0.22 \pm 0.03	1.14 \pm 0.06	1.83 \pm 0.23	0.31 \pm 0.01	1.68 \pm 0.28
Isofenphos	n.d	n.d	n.d	n.d	n.d	n.d
Methidathion	n.d	n.d	n.d	n.d	n.d	n.d
Ethion	n.d	0.29 \pm 0.04	n.d	n.d	n.d	n.d
Triazophos	n.d	n.d	n.d	n.d	0.72 \pm 0.06	n.d
Leptophos	n.d	n.d	n.d	n.d	n.d	n.d

^a Values are given as (μg of analyte per kg vegetables); n.d, non detectable.

3.6. Application to commercial samples

The developed DI-SPME-GC-FPD method using 85 μm PA fibre was applied for the determination of OPs in twenty vegetable samples. The samples were immediately processed according to the sample preparation procedure as mentioned before. The analysis was performed in triplicate and between each run of the sample, a blank was carried out to avoid carryover and possible contamination from the extraction process.

The results obtained for the positive samples are summarised in Table 4. Of the twenty samples that were analysed, six samples were found to contain pesticides residues. All positive samples contained chlorpyrifos (0.22–1.83 $\mu\text{g}/\text{kg}$) and were mostly detected in mustard samples. The residue levels of the pesticides determined were lower than the MRLs (1 mg/kg) specified by the International Food Act & Regulation 1985 and Codex Alimentarius Commission. Chlorpyrifos is one of the most widely used pesticides and commonly found in vegetables sold by retailers in the Ipoh area. The results are in agreement with the data compiled from 2004 to 2010 by the Pesticide Analytical Centre (Department of Chemistry Malaysia) which found chlorpyrifos to be the highest OPs detected in vegetables for nine districts in Ipoh.

The presence of chlorpyrifos, ethion and triazophos was confirmed by GC-MS analysis on the suspected sample. Under the selected extraction conditions, co-extraction for some compounds was observed. These compounds were also identified using GC-MS. Two of OPs that were outside the scope of the studies (demephion and trichlorfon) were successfully identified in mustard samples. These findings suggest that the SPME method can be applied to a wide range of compounds in combination with various types of detectors. Typical chromatograms for mustard, cabbage and kale samples subjected to DI-SPME-GC-FPD method are shown in Fig. 5(B–D).

In the current study, the standard deviations (SDs) obtained from the samples show the possibility of using only a small mass (0.5 g) to represent the sample. This is considered advantageous when compared to the previous reported methods where large amounts of sample are required. This is made possible by the reproducibility of the homogenisation and extraction processes.

4. Conclusions

A direct immersion SPME using 85 μm PA fibre followed by GC-FPD method for the determination of eleven OPs including tolclofos-methyl and leptophos was successfully developed. This method is adequate, simple, offers good sensitivity to meet the regulatory requirements and significantly reduced solvent consumption. Another distinct advantage of the proposed method is that centrifugation step is not required in the sample preparation. The proposed method was successfully used to determine organophosphorus pesticide residues in new vegetable matrices such as kale and mustard. Due to its many positive features, the method is therefore recommended for the routine analysis of organophosphorus residues in vegetables.

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