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Headspace Solid Phase Microextraction in Combination with Gas Chromatography-Mass Spectrometry for the Rapid Screening of Pesticide Residues in Vegetables and Fruits

Chai Mee Kin[1], Tan Guan Huat[2] and Asha Kumari[3]

[1]*Dept. of Science and Mathematics, College of Engineering,Universiti Tenaga Nasional, Km 7, Jalan Kajang-Puchong, 43009 Kajang, Selangor.*

Mkchai@uniten.edu.my Fax:03-89263506

[2,3]*Dept. of Chemistry, Faculty of Science, Universiti Malaya, Lembah Pantai, 50603 Kuala Lumpur.*

Abstract : A method for the determination of 5 organophosphorus (diazinon, malathion, quinalphos, chlorpyrifos and profenofos) and 2 organochlorine (alpha endosulfan and beta endosulfan) pesticides in vegetables (cucumber, tomato, bakchoy) and fruits (star fruit, strawberry, guava) samples was developed. The samples were diluted with water and extracted with 2% vol/weight (v/w) of a mixture of methanol/acetone (1:1). The analytes in samples were extracted by headspace solid-phase microextraction (HS-SPME) and analyzed by gas chromatography-mass spectrometry (GC-MS) using a selected ion-monitoring (SIM) mode. Limit of detection of the method was between 0.001 to 0.01 mg/kg and limit of quantification was between 0.005-0.05 mg/kg for all investigated pesticides. Relative standard deviations for duplicate analyses of sample fortified at 3 levels were not higher than 10%. Recovery test were performed for concentration between 35-0.25 mg/kg. Mean recoveries for each pesticide were between 77.0-96.4% for vegetables and between 79.7-97.5% for fruits with RSD below 5%. Therefore, the proposed method is applicable in the analysis of pesticides in vegetable and fruit matrices.

Keywords: Headspace Solid-Phase Microextraction, GC-MS, pesticides residues.

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Introduction

During the past decade, special attention has been paid to sample preparation method that ensures reduction of the amount of organic solvent, or even their complete elimination, and also keeps the number of operation and process to a minimum. Prior to chromatographic separation, pesticides are extracted and preconcentrated from the aqueous medium and interfering compounds in the matrix are removed at the same time. Liquid-liquid extraction (LLE) is the classical approach for pesticide extraction but this technique is time consuming and requires large volumes of expensive and toxic solvent [1-3]. A substantial improvement for aqueous sample preparation techniques resulted from the development of solid phase extraction (SPE), using bonded silica sorbents. SPE offers the advantages of a shorter analysis time, lower cost, and the consumption of very low volumes of organic solvent. SPE is less time-consuming than LLE but still needs column conditioning and elution with organic solvents; another drawback of SPE is its disposable [1-3]. Therefore, simple, rapid, environmental friendly and inexpensive sample preparation methods are needed in environmental analysis.

Solid-phase microextraction (SPME) is an advance in the samples preparation for trace analysis. Because organic compounds are adsorbed directly from an aqueous or gaseous sample onto a fiber coated with an appropriate stationary phase, and then the fiber needle can be directly injected into a GC injector port for analysis. SPME has been extensively used for direct extraction of pesticide from aqueous sample [4-7]. On the other hand, food like fruit and vegetable are mostly in a solid and heterogeneous form, not allowing a direct extraction. Then, SPME fiber can also be suspended in the headspace above the aqueous or solid sample. This option, named headspace SPME (HS-SPME), eliminated interference problem because the fiber is not in contact with the complex matrices of fruits and vegetables.

Due to the success of HS-SPME as a method for quantitative determination of some pesticides in different matrixes [8-16], the present study was performed as an attempt to couple HS-SPME sampling and GC-MS analysis for the determination of five organophosphorus (diazinon, malathion, quinalphos, chlorpyrifos and profenofos) and two organochlorine (alpha endosulfan and beta endosulfan) pesticides in vegetables (cucumber, tomato, bakchoy) and fruits (guava, star fruit and strawberry) sample.

Methodology

1.0 Chemicals and Reagents

Pesticide analytical standards used were obtained from Dr. Ehrenstorfer (Augsburg, Germany): diazinon, malathion, chlorpyrifos, quinalphos, profenofos, alpha endosulfan and beta endosulfan. Pesticides were used without further purification (degree of purity were > 95%) for all pesticides.

2.0 Sample preparation

Stock solution of each pesticide at different concentration level 0.25-1.75 g/kg were prepared in methanol and stored at 4 ^{o}C. Preparation of different concentration levels of stock solution is due to their sensitivity to the MS detector. Working standard solutions of a mixture of pesticides were freshly prepared daily by volume dilution in distilled water. Tetracosane ($C_{24}H_{50}$,2 mg/kg) as an internal standard was added to the vial prior to GC analysis.

Fresh organic pesticide free vegetables and fruits (100g) were weighed and chopped. 30 g of chopped samples were placed in a 150 mL beaker. Calculating aliquots 0.3 mL (low), 1.8 mL (medium) and 6.0 mL (high) of stock solution at three concentration levels respectively were spiked into the samples drop by drop. After being kept at room temperature for 1 hour, the spiked vegetable and fruits were added with 30 g of distilled water and blended and homogenized in a food blender. Then, the samples were place in separate vials and analyzed following the recommended procedure.

3.0 HS-SPME Analysis

All determinations were performed using a 100 µm film thickness poly dimethylsiloxane (PDMS) coated fiber mounted in a manual syringe holder (Supelco, Bellefonte, PA, USA). Initially 1.0 g of blended sample, 0.5 g of NaCl, 100 µL of methanol/acetone (1:1), were introduced in a 15 mL clear glass vial and topped up with distilled water until 5.00g. The sample was added with the internal standard. The vial was capped with a PTFE-faced silicon septum. PDMS fiber was exposed to the headspace above the sample for 30 min at 60 ^{o}C. Each sample was stirred constantly during the sorption step using a stir bar and a stirring plate. Thermal desorption of the analytes was achieved by inserting the sorbent fiber into the injection port (held at 260 ^{o}C) for 10 min.

4.0 Gas Chromatography – Mass Spectrometry

Gas chromatographic analysis was carried out using a Hewlett-Packard system 6890 gas chromatography coupled with a HP model 5972 A quadrupole mass spectrometer. Data acquisition and processing were provided by Vectra VL 5/90 Series 3 computer equipped with HPG 1030 A Chemstation data system was used. The pesticides were separated on CB5-MS 30m x 0.25 mm, 0.25 µm capillary column, and contained 5% phenyl-methylpolysiloxane. A silanized narrow-bore injected liner (0.75 mm ID) for the SPME injections was installed and the fiber was inserted into this injector using the splitless mode. Positive identification of compounds was based on comparison of GC retention times and mass spectra of authentic compounds. The column temperature was held at 80 ^{o}C for 2 min, then heated to 180 ^{o}C at a heating rate of 30 ^{o}C/min, then heated to 200 ^{o}C at a heating rate of 1.5 ^{o}C/min. Finally temperature increased to 280 ^{o}C at a rate of 20 ^{o}C/min which was held for 8 min. Helium gas used as the carrier gas with a flow rate 1.3 mL/min (linear velocity = 42 cm/sec). The solvent delay time was set at 8 min. Selected ion monitoring (SIM) mode was used in quantitation. The most abundant and characteristic mass fragment was chosen for quantification and two others for confirmation (Table 1).

Table 1: Pesticides analyzed, retention times and typical fragment ions (m/z) of the target pesticides in GC-MS (SIM)

Pesticides	Retention Time (min)	Quantitation ion (m/z)	Confirmation Ion (m/z)
Diazinon	9.96	304	179, 152
Malathion	13.34	285	173, 125
Chlorpyrifos	13.83	314	197, 258
Quinalphos	16.39	298	146, 241
Alpha-endo	17.40	339	195, 263
Profenofos	19.19	374	208, 339
Beta-endo	21.95	207	239, 339
Internal Std	24.75	98	322, 66

Result and Discussion

1.0 Method Optimization

Some factors affecting HS-SPME of pesticides from vegetables and fruits have been studied already. The use of a PDMS fiber produces best extraction efficiency for pesticides [10, 13-19] than other types of fiber. The 100 μm PDMS fiber (a non-polar phase) is recommended in the literature because it is a rugged liquid coating that is able to withstand high injector temperature up to 300 ^{o}C. Fibers coated with thicker films required a longer time to achieve extraction equilibrium, but might provide higher sensitivity due to the greater mass of the analytes that can be extracted. An increase in extraction temperature caused an increased in extraction rate and a simultaneous decrease in the analyte distribution constant between solution and fiber. The optimum temperature for extracting the selected pesticides was 60 ^{o}C. At higher temperature, evaporation of sample was important and losses of analytes seemed to take place. Equilibrium times for PDMS are typically more than 1 hour, but 30 min is usually enough for most analytical purposes. A 6 minute-period was shown to be sufficient to desorb pesticides in the GC injector port; with the fiber remaining for another 4 min to eliminate any possible residues on the fiber to ensure a reproducible desorption. Finally, a large desorption time (10 min) and high injector temperature (260 ^{o}C) was selected as an optimum desorption time and temperature for the further experiments. Salting out effect is positive for improving extraction of pesticide [14-17, 19-21].

The presence of endogenous substances and solid particles affects the equilibrium fiber/water and the integrity of the fiber. It was observed that if the primary sample slurry is directly extracted without further dilution, recoveries were lower than 20 % and the fiber deteriorates quickly. Dilution of the primary sample improved extraction and protected the fiber from deterioration but at the same time reduced sensitivity. A dilution of 1/5 was sufficient to reduce matrix interference and to reach the required sensitivity. Dilution was also chosen by other authors who extracted aqueous matrices with suspended matter such as fruits [16,18,22], vegetable [12,15,20-21,23] and honey [24-25], must and wine sample [19,24].

In order to increase the extraction efficiency, the effect of adding organic solvent to the sample was tested and considered by other authors [16,19-20,25]. The organic solvents tested were methanol, acetone, acetonitrile and ethyl acetate. In all experiments, the volume of organic solvent added to the slurry was 100 μL (2%). All of the organic solvent used can increase the extraction efficiency. An average percentage recovery (%) obtained using a mixture of methanol/acetone (1:1) was much higher compared to that using the other organic solvent. When compared with the extraction without dilution and an organic solvent, the increase in percentage recovery extracted was from 20 % up to 99%. Besides the extraction efficiency, a mixture of methanol/acetone (1:1) was selected because it is relatively non-toxic, easy to volatilize and readily obtainable in the laboratory. Thus, the final recommended procedure used the addition of 100 μL methanol/acetone (1:1) to the 1.00 g sample slurry containing 10% NaCl and then topped up to 5.00 g with distilled water.

2.0 Analytical Performance

The dynamic range of the developed HS-SPME-GC-MS procedure (SIM mode) in six different levels (two replicated for each level) were linear for all the analytes over at least two concentration decades with correlation coefficients better than 0.9833. Theoretical limits of detection and quantification were determined taking into account the usual definition: the concentration that originated for each pesticide, a signal equal to three times the noise signal was considered the limit of detection. The concentration that originated, for each pesticide, a signal equal to ten times the noise level was considered the limit of quantification.

Limit of detection and quantification (listed in Table 2) were evaluated for each pesticide as follow:

a) Retention times were determined running the chromatogram of a standard solution.

b) The fiber was dipped above the distilled water and a blank was run. From this chromatogram, average noise levels were measured.

c) The concentration that led to signals three or ten times the noise level were evaluated using the average of the peak areas obtained in two injections of the standard solution and taking into account the values of the noise level.

Table 2: Analysis of standard solution: linear range, limit of quantification (LOQ), limit of detection (LOD) and Maximum Residue Level (MRL) in vegetables and fruits followed European Union standard.

Pesticide	Linear range (mg/kg)	Correlation coefficient (r^2)	LOQ (mg/kg)	LOD (mg/kg)	MRL (mg/kg)
Diazinon	0.075 – 15	0.9960	0.05	0.01	0.5
Malathion	0.125 – 25	0.9957	0.05	0.01	0.5
Chlorpyrifos	0.050 – 10	0.9840	0.02	0.005	0.2
Quinalphos	0.150 – 30	0.9955	0.03	0.01	0.05
Alpha-endo	0.025 – 5	0.9833	0.005	0.001	0.05
Profenofos	0.175 – 35	0.9966	0.03	0.01	0.05
Beta-endo	0.015 - 30	0.9916	0.01	0.002	0.05

Compare to the liquid-liquid extraction method which showed the LOD ranged from 0.02 – 0.15 mg/kg [26], the values above are much better. Besides, the values of LOQ and LOD which got from HS-SPME above are acceptable because they are lower than the values of MRL which set by European Union [27].

3.0 Recovery Study

The optimized method was examined, for the extraction and determination of seven pesticides in three vegetables and three fruits samples. SPME is a non exhaustive extraction procedure and for this reason the relative recovery, defined as the ratio of the concentration found in samples and working solution, spiked with the same amount of analytes, instead of the absolute recovery (used in exhaustive extraction procedures) was employed. Figure 1 shows the chromatogram on relative recovery of a spiked cucumber sample with the selected pesticides and extracted by SPME.

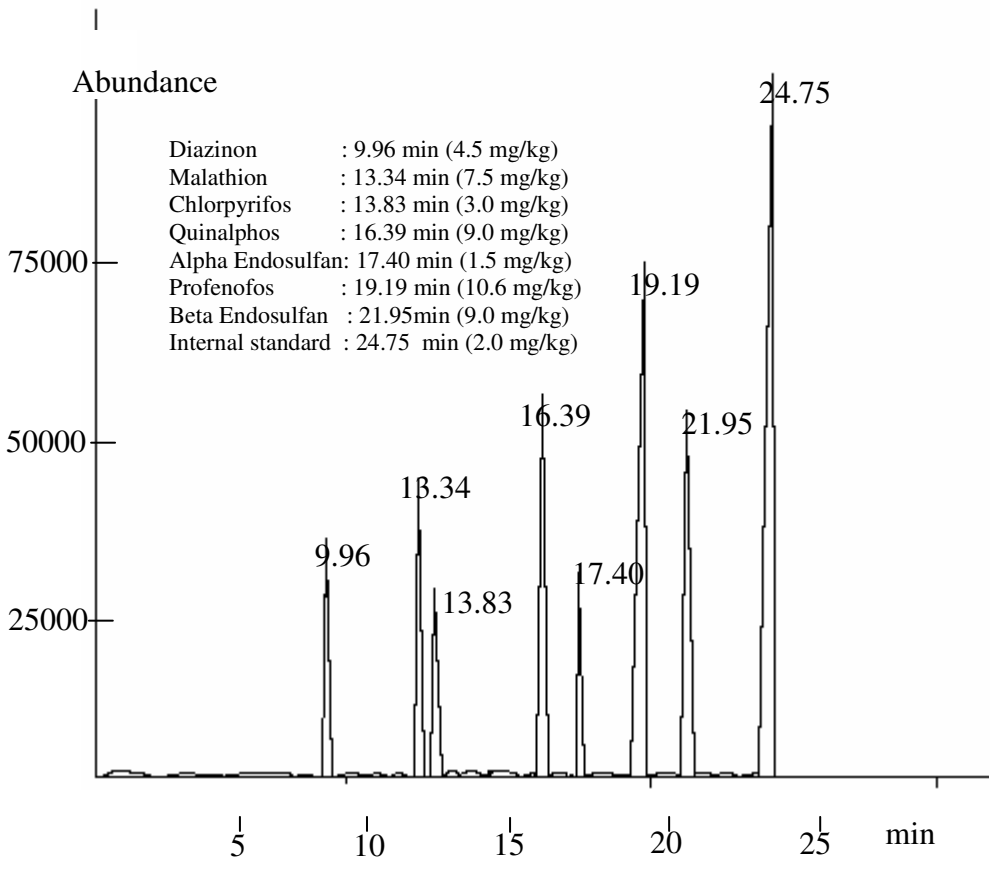

Figure 1: Chromatogram on relative recovery of a spiked cucumber and extracted by SPME.

Recoveries of seven pesticides were obtained at three levels of fortification. Three replicates of each fortification level were prepared. The mean recoveries from spiked samples are shown in Table 3. The acceptable relative recoveries were obtained, ranging between 75.0-98 % for the vegetables samples (RSD: 0.3 – 12.5%), 77-99% for the fruit samples (RSD: 0.0 – 7.2%). The percentage of relative recovery and RSD values obtained for the fruit samples are clearly better than those obtained for the vegetable samples. This is probably due to the higher total suspended solids present in these samples. When all the vegetable and fruit samples were compared, it appeared that the relative recoveries obtained in bakchoy were lower than the other samples. This could be due to the water content of the bakchoy is the lowest among the samples. As can be seen, matrix had little effect on the developed headspace SPME method. The HS-SPME process is affected by the suspended matter and dissolved compounds contained in the vegetable and fruit samples, which could adsorb the analytes, forming micelles and thus making it difficult for the analytes to reach the fiber [16,20].

A coating lifetime is important for practical application (changes of efficiency with number of analyses). The coating is damaged mainly during the extraction due to interference between the matrix of samples and the fiber. This effect of more pronounced when the sampling preformed directly from the aqueous solution (immersion SPME). On the contrary, in the HS-SPME the fiber is suspending in the headspace above liquid layer of the samples and there is no interference between the matrix of samples and the coating. Thus the coating is protected and the lifetime is increased. In conventional SPME process (immersion technique) each fiber can be re-used around 30 times for surface water samples and 27 times in run-off water [28]. As it is observed in this study using headspace technique the fibers can be re-used 100 – 120 times.

Table 3: Spiked concentration levels and relative recoveries over fortified vegetables

Pesticide	Spiking levels (mg/kg)	Recovery,% (RSD %, n=3)					
		Cucumber	Tomato	Bakchoy	Guava	Starfruit	Straw berry
Diazinon	0.75	89 (2.7)	95 (4.2)	94 (6.6)	79 (4.5)	91 (0.2)	83 (2.1)
	4.5	90 (4.5)	97 (1.5)	95 (1.7)	84 (5.4)	91 (6.8)	87 (0.3)
	15	93 (0.3)	91 (6.2)	94 (4.9)	87 (4.4)	88 (2.1)	86 (2.4)
Malathion	1.25	80 (6.3)	91 (2.2)	94 (9.9)	95 (1.1)	89 (3.6)	95 (4.8)
	7.5	83 (7.5)	92 (1.8)	97 (2.2)	98 (3.6)	87 (4.2)	91 (5.0)
	25	84 (0.2)	89 (5.2)	97 (4.1)	96 (2.4)	90 (1.3)	96 (0.1)
Chlorpyrifos	0.5	88 (1.7)	89 (2.4)	77 (3.3)	95 (6.4)	84 (4.5)	81 (7.0)
	3.0	91 (3.0)	88 (4.3)	80 (12.5)	96 (3.1)	82 (3.9)	81 (1.8)
	10	88 (1.1)	80 (5.5)	80 (1.2)	94 (2.5)	78 (1.1)	81 (1.7)
Quinalphos	1.5	84 (3.2)	97 (9.1)	85 (3.0)	92 (0.3)	89 (7.2)	96 (7.1)
	9.0	88 (6.5)	95 (8.0)	84 (5.3)	93 (6.3)	89 (1.9)	90 (3.6)
	30	83 (1.3)	96 (1.4)	92 (11.3)	92 (1.5)	88 (0.9)	92 (7.1)
α-Endo	0.25	93 (3.4)	93 (4.3)	76 (2.6)	92 (6.5)	77 (0.8)	91 (3.1)
	1.5	97 (1.5)	93 (8.0)	76 (5.2)	92 (2.5)	82 (0.6)	92 (5.1)
	5.0	93 (2.6)	95 (1.6)	79 (4.0)	92 (0.6)	80 (0.9)	90 (2.3)
Profenofos	1.75	86 (5.1)	88 (0.8)	87 (7.7)	97 (5.1)	91 (2.7)	96 (4.7)
	10.6	89 (0.9)	89 (0.6)	87 (5.0)	97 (2.4)	92 (1.3)	92 (2.3)
	35	87 (0.7)	92 (1.7)	93 (8.1)	97 (4.6)	87 (1.3)	95 (0.2)
β-Endo	1.50	97 (0.9)	89 (4.1)	76 (1.2)	98 (2.6)	85 (6.9)	97 (2.4)
	9.0	95 (2.7)	86 (7.7)	75 (2.0)	99 (1.1)	86 (0.2)	92 (3.9)
	30	98 (5.4)	83 (1.0)	81 (8.8)	95 (4.8)	85 (1.4)	96 (0.0)
Range	0.25-35	Vegetables: 75-98 (0.3-12.5)			Fruits: 77-99 (0.0-7.2)		

Conclusion

The potential for using HS-SPME as a sample preparation technique prior to GC-MS for pesticide analysis in vegetable and fruit samples has been demonstrated. Taking into account the high sensitivity of the SPME technique and the effect of the matrix interference on the extraction process, sample dilution and the use of small amount of organic solvent have proven to improve the extraction efficiency of the SPME technique with biological samples. This study shows SPME is a simple, fast and solvent-free method and will be an alternative sample preparation method to the traditional LLE and SPE.

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