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Headspace Solid Phase Microextraction (HS-SPME) coupled to Gas Chromatography-Mass Spectrometry (GC/MS) for the Analysis of Multi-Class Pesticides

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Abstract

The development of a method based on headspace solid phase microextraction (HS-SPME) technique, for the simultaneous determination of 7 multiclass and multiresidue pesticides (fenobucarb, diazinon, chlorothalonil, thiobencarb,chlorpyrifos,endosulfan I and endosulfan II) in two species of apple, using gas chromatography-mass spectrometry is discussed. The different parameters affecting the SPME technique were evaluated. The extraction capacity of three fiber coatings; polydimethylsiloxane (100µm PDMS), polyacrylate (65µm PA), and polydimethylsiloxane-divinylbenzene (85µm PDMS-DVB), were studied and compared. Validation of the method using two apple species spiked with standard solution yield better linear range, accuracy, precision, detection and quantification limits. The linearity was between 5 to 500 µg.kg^{-1} with good correlation coefficients (R) greater than 0.989. The average recoveries for all pesticides investigated were between 89–100% in green apple and 94–103% in red apple with RSD ranging from 0.67–3.32% and 1.00 - 2.69 % respectively. The LOQs were between 6.71 and 14.56 µg.kg^{-1} and LODs ranged from 2.23 to 11.11 µg.kg^{-1}.

Keywords: SPME, headspace analysis, GC-MS, fruit samples, pesticide residues

1. Introduction

Fruits and vegetables provide the body with micronutrients (minerals and vitamins) that are very vital to the body but are required in small quantities (Abdulra'uf et al., 2012). This has led to their production in large quantities to meet the ever growing demand. To achieve this, pesticides was introduced to protect the fruits and vegetables on the farm and during storage. Pesticides, including organophosphorous pesticides (OPP), organochlorine pesticides (OCP)

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and carbamate pesticides (CP) have been used effectively in controlling pests, fungi and weeds, thereby beneficial to the steady increase in agricultural production (Chai & Tan, 2010; Vázquez et al., 2008). However, they persist in the food chain, due to their penetrating effect into the tissues of fruits and vegetables. This results in contamination and possible health risk to both animal and human health (Araoud et al., 2007). Due to their potential risk, there is need to strike balance between their expected benefits and possible risk, and therefore the need for an extensive analysis and evaluation of fruits and vegetables for monitoring and safety purposes.

The introduction of solid phase microextraction (SPME) technique, in 1990 (Arthur and Pawliszyn, 1990), has helped to overcome the problems inherent in the solvent-based sample preparation techniques. It is a solvent-free technique which integrates sampling, isolation, concentration,(Ouyang and Pawliszyn, 2008) and enrichment into one step . It can easily be automated, thus, integrating sampling, extraction, pre-concentration, and sample introduction into the analytical instrument in a single and uninterrupted sampling step, resulting in high throughput analysis (Pawliszyn, 1997; Risticevic et al., 2009). Its automation has helped to avoid sample contamination and loss of analytes. The technique is based on the partition of analyte and establishment of equilibrium between the sample matrix and the stationary phase made of coated fused silica or metal alloy (Arthur et al., 1992; Aulakh et al., 2005). The technique is compatible with LC-MS, GC-MS and CE. (Blasco et al., 2003; Falqui-Cao et al., 2001; Lambropoulou and Albanis, 2007; Rodriguez et al., 2003) The headspace mode, avoids direct contact with the sample matrix (Liu et al., 2005), thus reducing matrix interference.

This paper describes a headspace SPME procedure for simultaneous determination of multiresidue pesticides of different classes (OPP, OCP and CP) in apple samples. The procedure was then applied to two species of apple samples purchased from Malaysian local wet market.

2. Materials and Methods

2.1 Standard Reagents and Solutions

Pesticide standards (fenobucarb, diazinon, chlorothalonil, thiobencarb, chlorpyrifos, endosulfan I and endosulfan II) were purchased from Accustandard Inc., New Haven CT, USA, with percentage purity greater than 97%. All the solvents used in this study were pesticide grade and were purchased from Fisher Scientific, Longhborough, UK.

2.2 Sample Preparation and Fortification

Standard solutions of each pesticide were prepared by diluting the stock standards (100 $mg.L^{-1}$) in methanol to 10 $\mu g.mL^{-1}$ and stored at 4 ^0C. The working standard solution containing the 7 pesticides was prepared daily in methanol and the working standard was used to spike the matrix to a required concentration for the optimization of extraction parameters. Calibration standards with concentrations of 5 to 500 $\mu g.kg^{-1}$, were prepared by spiking a calculated amount of the working standard directly in the sample matrix. A 100 g of chopped apple sample were weighted and homogenized in food processor and 5g aliquot was placed in sample vial, diluted with appropriate amount of water and was subjected to the following HS-SPME procedure.

2.3 Headspace - Solid Phase Microextraction Procedure

The SPME fiber holder for autosampler and several replaceable fibers coated with polydimethylsiloxane (PDMS, 100µm), polydimethylsiloxane/divinylbenzene (PDMS/DVB, 65µm) and Polyacrylate (PA, 85µm) purchased from Supelco (Bellefonte, PA, USA) were compared. The fibers were conditioned prior to their first use as recommended by the manufacturer. All analysis was performed in 20 mL amber glass vial with headspace volume of 10 mL. For the HS-SPME, 5 g of previously homogenized sample was weighed in 20 mL amber glass vial, spiked with known amount of the standard mixture and allowed to rest for 2 hrs. Optimum dilution was made with 5 mL of distilled water containing 10 % NaCl and the mixture was shaken ultrasonically for 10 min. The analytes were then extracted with 100 µm PDMS in the headspace mode at 60 ^0C for 30 min. After the extraction, the fiber was placed in the GC injector for desorption at 270 ^0C for 7 min.

2.4 Equipment

Extraction and analysis of pesticides were performed with CTC combiPAL autosampler, coupled to a GC-MS (Shimadzu QP2010 Series) and operated in the splitless mode at 270 ^0C. The capillary column was fused silica DB5-MS column (30 m x 0.25 mm x 0.25 µm i.d). The GC oven temperature program was as follows: 60 ^0C (2 min), ramped to 180 ^0C (0 min) at 30 $^0Cmin^{-1}$, then to 210 ^0C (0 min) at 5 $^0Cmin^{-1}$, and finally increased to 270 ^0C at 5 $^0Cmin^{-1}$, where it was held for 5 min. The MS transfer line was 290 ^0C, ion source 200 ^0C and ionization model at 70 eV. The analyses were done in selected ion monitoring (SIM) mode. The following target and reference ions were monitored for the selected pesticide: Fenobucarb (121, 150 and 107); Diazinon (179, 137 and 152); Chlorothalonil (266, 264 and

268); Thiobencarb (100, 72 and 125); Chlorpyrifos (197, 98 and 125); Endosulfan I (241, 195 and 207); Endosulfan II 195, 159 and 207) each at 10µgkg

3. Results and Discussion

The optimization of various parameters was carried out with pesticide standards in a matrix-matched solution. A univariate analysis was performed to have a better understanding of the behaviour of each parameter.

3.1 Optimization of Desorption Conditions

The analytes can be desorbed effectively under high temperature at a shorter time, when used within the manufacturer specified temperature range. The complete desorption of analytes into the injector from the fiber improves detector response and eliminates carry over and memory effect (Lord and Pawliszyn, 2000; Menezes Filho et al., 2010). Optimum desorption conditions for desorption time and injector were then studied. The injector temperature was varied between 240 and 300 ^{0}C and desorption time between 2 and 10 min. It was observed that peak area response increased up to 270 ^{0}C beyond which peak area it remained constant and there was no significant difference between the peak area at 270 to 300 ^{0}C and the desorption of all pesticides was completed at 7 min, and these conditions (270 ^{0}C and 7 min) were chosen.

3.2 Fiber Selection.

Different coating materials with different fiber volumes were used for SPME fiber. Therefore, it is necessary to compare the performance of different fiber in extracting the pesticides from aqueous and matrix - matched solutions. For these reasons, three fibers (Section 2.3) were tested by extracting the target analytes in the sample matrix. As shown in Fig. 1, the best results were obtained with 100 µm PDMS and, thus, it was selected for further method development.

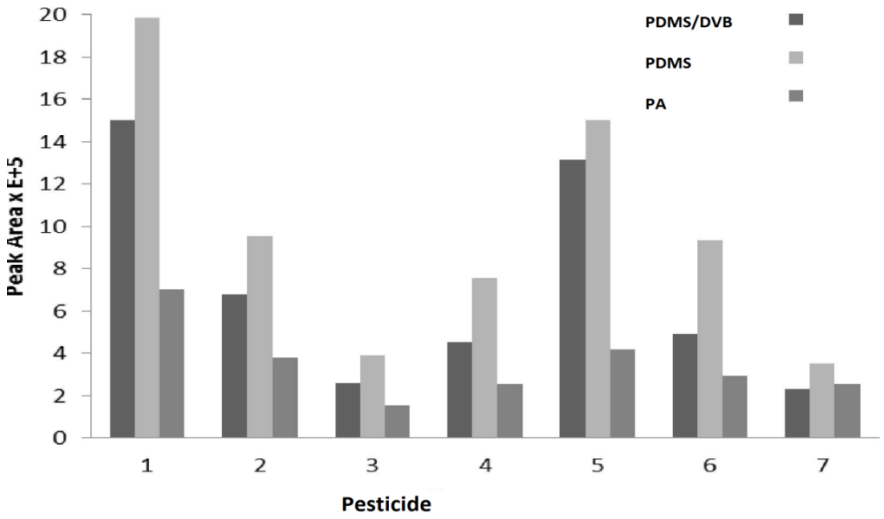

Fig 1: Comparison of extraction efficiency of the selected fibers. Peak identification 1. Fenobucarb; 2. Diazinon; 3. Chlorothalonil; 4. Thiobencarb; 5. Chlorpyrifos; 6. Endosulfan I; 7. Endosulfan II, each at 10 µgkg^{-1}. Extraction temperature, room temperature; extraction time, 20 min; stirring rate 250 rpm; no salt, desorption temperature/time, 270 ^{0}C for 7 min

3.3 Optimization of Extraction Condition

To obtain the maximum HS-SPME extraction efficiency of PDMS coated fibers for the investigated analytes, several extraction conditions such as extraction time, extraction temperature, stirring rate and salting out effect were studied.

3.3.1 Extraction Time

The time required for the extraction of the selected pesticides to reach equilibrium in the fiber stationary phase was evaluated at ambient temperature for 10, 20, 30, 40, 50, 60, 70, 80 and 90 min (Fig. 2). Basically, SPME extraction is considered to reach equilibrium when the analytes adsorbed by the fiber coating is independent of further increase in extraction time. The result indicated that the peak area response was improved for all analytes up to 30 min, and was chosen for subsequent analysis.

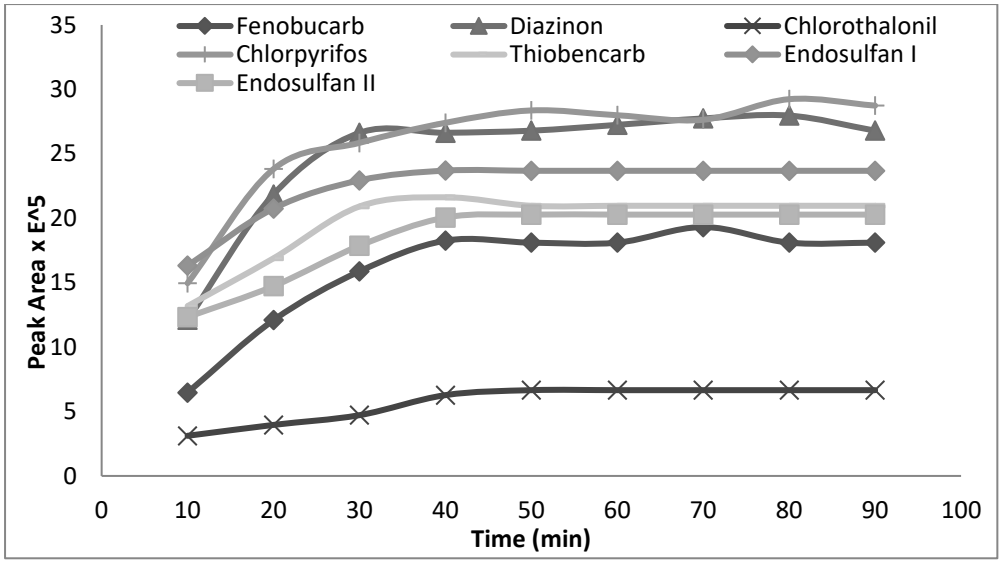

Fig 2: HS-SPME extraction time curve. See Fig 1 for Peak identification. Extraction temperature, room temperature; extraction time (10 - 90 min); stirring rate 250 rpm; no salt, desorption temperature/time, 270 ^{0}C for 7 min.

3.3.2 Extraction temperature

The effect of temperature was also investigated, since it can have an opposing effect on the extraction efficiency (Kudlejova et al., 2012; Risticevic et al., 2010). Increase in temperature of the sample matrix increases extraction efficiency and consequently reduces distribution constant, therefore, must be carefully optimized (De Fatima Alpendurada, 2000; Abdulra'uf and Tan, 2012). The extractions were carried out at 30, 40, 50, 60 70 80 and 90 ^{0}C. All the investigated pesticide showed increase in peak area at temperature up to 60 ^{0}C (Fig. 3), and it can be inferred that the increase in temperature favoured the mass transfer of the analytes from the sample matrix to the headspace and then to the fiber. The extraction temperature of 60 ^{0}C was chosen, since it yielded the best peak area for all the pesticides under investigation.

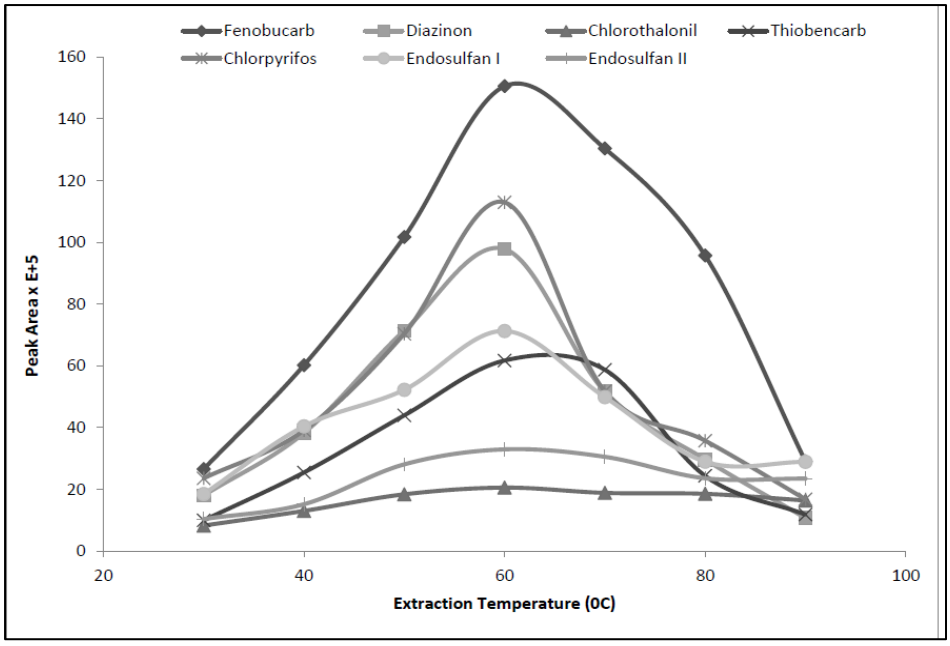

Fig. 3; Effect of temperature on extraction efficiency. See Fig 1 for Peak identification. Extraction temperature, (30 - 90 ^{0}C); extraction time, 30 min; stirring rate 250 rpm; no salt, desorption temperature/time, 270 ^{0}C for 7 min.

3.3.3 Stirring rate

Stirring reduces the diffusion layer in the sample matrix and improve the transfer of analytes to the fiber (Beltran et al., 2000; Zambonin et al., 2002; Zeng et al., 2008). The extraction was performed at 250, 300, 350, 400, 450, 500 and 600 rpm. As shown in Fig. 4, the peak area for the investigated pesticides increased as stirring rate increased up to 500 rpm and was chosen. Higher stirring rate has been observed to cause magneton flutter and formation air bubble in the solution (Zeng, et al., 2008), leading to swelling of the fiber and reducing the extraction efficiency and fiber life time.

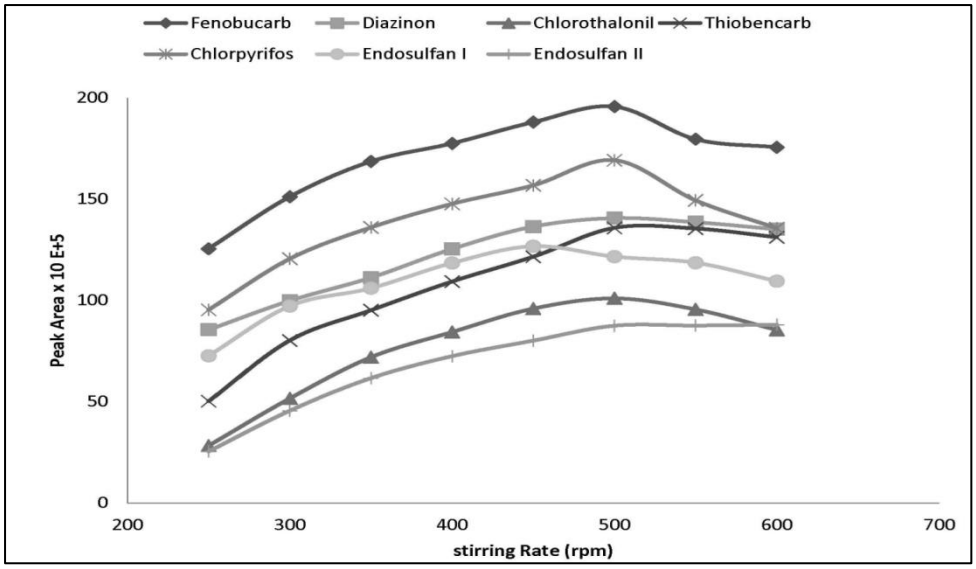

Fig. 4; Effect of stirring rate. See Fig 1 for Peak identification. Extraction temperature, 60 ^{0}C; extraction time 30 min; stirring rate (250 - 600 rpm); no salt; desorption temperature/time, 270 ^{0}C for 7 min.

3.3.4 Ionic Strength

Salting out weakens interaction between the analytes and the sample matrix (Menezes Filho, et al., 2010) and allows the analyte molecules to diffuse more readily from the sample matrix to the headspace (Risticevic, et al., 2010), by reducing the solubility of the pesticides in water. The effect of ionic strength was investigated by adding different amounts of NaCl to the matrix solution (0, 5, 10, 15, 20 25 and 30 %). The 10 % addition (not shown) yielded the best results for all pesticide investigated and was chosen (not shown). It was observed that, extraction efficiency decreased at higher salt concentration of salt.

Subsequently the following optimized conditions were selected for method validation and analysis of real samples (100 µm PDBS, 60^{0}C, 30 min, 500rpm, 10% NaCl, 270^{0}C, 7 min, as fiber type, extraction temperature, extraction time, stirring rate, salt addition, desorption temperature and desorption time respectively.

3.4 Validation of the Analytical Method

The characteristics of the developed method was validated using the optimized conditions by determining the figures of merit of the analytical methodologies, such as repeatability, limits of detections (LOD) and quantifications (LOQ), the linearity and relative recovery at two different levels of fortification (10 and 50 µg.kg^{-1}). The external standard calibration curve was constructed with 6 point concentrations, and each was analyzed in triplicate using the optimized conditions.

The repeatability of the proposed method was evaluated based on the experimental results obtained by analyzing two apple samples each spiked at 10µg.kg^{-1} (Fig 5). The relative standard deviation obtained was lower and were between 0.67 and 3.32 %. The method linearity was validated at six concentration levels, and the linearity ranges, correlation coefficients, LODs and LOQs are as summarized in Table 1. As shown in Table 2, the relative recoveries obtained for the two apple samples spiked at two concentration levels of each pesticides (5 and 50µg.kg^{-1}) and analyzed in triplicate were good for all the investigated pesticides. It ranges from 89 to 100 % in green apple and from 94 to 103 % in red apple with the RSD values ranging from 0.67 to 3.32 % and 1.00 to 2.69 respectively. The best recoveries were obtained at lowest concentration, this may be due to the fact that saturation of the fiber may occur at higher concentration (Simplicio and Boas, 1999). The LOD values obtained were lower than the maximum residue levels as stipulated in the European Union regulations (EC/149/2000, EC/396/2005 and EC/839/2008) for fruits and vegetables (European Union (EU), 2011). The LOQ for the methods were 3 order of magnitude lower than the MRL which show the sensitivity of the developed method.

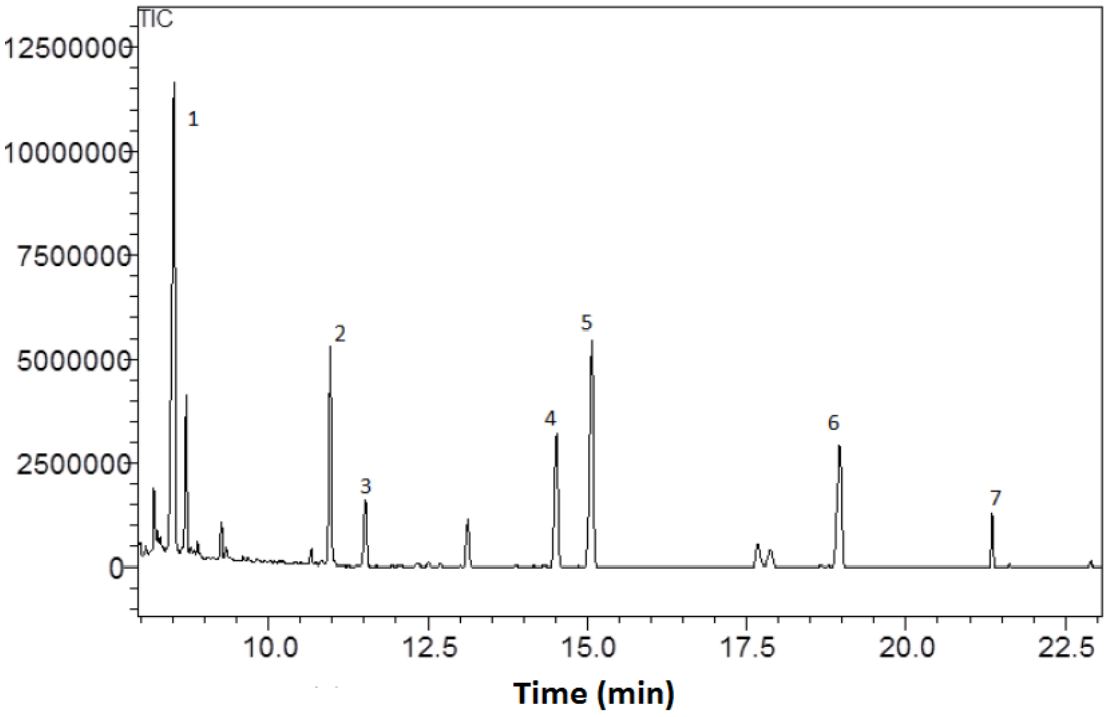

Fig. 5: GC-MS(TIC) Chromatogram of an apple sample spiked at 10 µgkg^{-1} and extracted by HS- SPME. (60^{o}C, 30 min, 500 rpm and 10 % NaCl) and desorbed at 270 °C for 7 min.

Table 1: Analytical figure of merit of the proposed method

S/N	Pesticides	Linearity ($\mu g.kg^{-1}$)	R^2	LOD ($\mu g.kg^{-1}$)	LOQ ($\mu g.kg^{-1}$)	MRL ($\mu g.kg^{-1}$)
1	Fenobucarb	50–500	0.9998	15.16	5.05	1000
2	Diazinon	5–500	0.9985	4.48	2.39	10
3	Chlorothalonil	50–500	0.9987	14.56	4.85	1000
4	Thiobencarb	10–500	0.9999	6.71	2.23	100
5	Chlropyrifos	50–500	0.9999	8.86	2.95	500
6	Endosulfan 1	10–500	0.9997	17.71	5.90	50
7	Endosulfan II	10–500	0.9992	33.37	11.11	50

Table 2: Average recovery of spiked pesticides at 10 μgkg^{-1} from apple samples

S/N		Green apple		Red apple	
	Pesticides	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
1	Fenobucarb	99.70	1.50	103.26	1.30
2	Diazinon	89.50	1.80	102.80	2.10
3	Chlorothalonil	97.80	1.49	98.33	2.69
4	Thiobencarb	100.13	0.67	94.87	1.62
5	Chlorpyrifos	99.82	0.89	101.6	1.00
6	Endosulfan 1	99.65	1.78	90.33	1.40
7	Endosulfan II	11.65	3.32	101.93	1.23

3.5 Analysis of Real Samples

The HS-SPME method developed in this study was subsequently applied to the analysis of apple sample obtained from Malaysian local wet market located at section 17, Selangor. Samples were analyzed in triplicate, in order to ascertain the applicability of the developed

method (Abdulfra'uf and Tan, 2013). A total of 22 red and 22 white apple were analyzed, and the chlorpyrifos was detected at a concentration below the limit of quantification and thus were not quantified. This shows that the apple samples are save for consumption, since the target pesticides were shown to be at concentration lower than the maximum residue level (EU, 2011).

4. Conclusion

The developed method was successfully applied for the extraction of seven multiclass pesticides in apple sample obtained in the Malaysian night market. The recoveries, relative standard deviation, LOQ and LOD obtained were comparable with official method for pesticide residue analysis. The proposed HS-SPME method demonstrates it ability for an effective screening of multiclass pesticides in fruit samples. The use of headspace sampling technique allows for the variation of sample matrix related conditions, which increases fiber life time and also ensures effective extraction. The extraction method is characterized by the absence of any clean-up step, which ensures that loss of analytes and contamination are completely eliminated.

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References

Abdulra'uf, L. B., Sirhan A. Y., andTan G. H. (2012) Recent developments and applications of liquid phase microextraction in fruits and vegetables analysis. Journal of Separation Science,**35**(24), 3540-3553

Abdulra'uf, L. B., Chai, M. K. and Tan, G. H. (2012): Applications of solid-phase microextraction for the analysis of pesticide residues in fruits and vegetables: A review. *Journal of Association of Official Analytical Chemistry International*, **95**(5), 1272-1290.

Abdulra'uf, L. B., and Tan, G. H. (2013): Multivariate study of parameters in the determination of pesticide residues in apple by headspace solid phase microextraction coupled to gas chromatography–mass spectrometry using experimental factorial design. *Food Chemistry*, **141**(4), 4344-4348

Araoud, M., Douki, W., Rhim, A., Najjar, M. F., & Gazzah, N. (2007): Multiresidue analysis of pesticides in fruits and vegetables by gas chromatography-mass spectrometry. *Journal of Environmental Science Health, Part A*, **42**, 179-187.

Arthur, C. L., Killam, L. M., Buchholz, K. D., Pawliszyn, J., and Berg, J. R., 1992. Automation and optimization of solid-phase microextraction. *Analytical Chemistry*, **64**, 1960-1966.

Arthur, C. L., and Pawliszyn, J. (1990): Solid phase microextraction with thermal desorption using Fused silica optical fibers. *Analytical Chemistry*, **62**, 2145-2148.

Aulakh, J. S., Malik, A. K., Kaur, V., and Schmitt-Kopplin, P. (2005): A review on solid phase microextraction—high performance liquid chromatography (SPME-HPLC) analysis of pesticides. *Critical Review in Analytical Chemistry*, **35**, 71-85.

Beltran, J., Lopez, F. J., and Hernandez, F. (2000): Solid-phase microextraction in pesticide residue analysis. *Journal of Chromatography* A, **85**, 389-404.

Blasco, C., Font, G., Manes, J., and Pico, Y. (2003): Solid-phase microextraction liquid chromatography/tandem mass spectrometry to determine postharvest fungicides in fruits. *Analytical Chemistry*,**75**, 3606-3615.

Chai, M. K., and Tan, G. H. (2010): Headspace solid-phase microextarction for the evaluation of pesticide residue contents in cucumber and strawberry after washing treatment. *Food Chemistry*, **123**, 760-764.

De Fátima Alpendurada, M. (2000): Solid-phase microextraction: A promising technique for sample preparation in environmental analysis. *Journal of Chromatography A*, **889**, 3-14

European Union (EU) (2011): EU pesticides database, established in directives EC/148/2008 and EC/839/2008. (148/2008. 396/2005, 839/2008). Retrieved 22/10/2011, from Directorate General for Health & Consumers http://ec.europa.eu/sancopesticides/public/index.cfm

Falqui-Cao, C., Wang, Z., Urruty, L., Pommier, J.-J., and Montury, M. (2001): Focused microwave assistance for extracting some pesticide residues from strawberries into water before their determination by SPME/HPLC/DAD. Journal of Agriculture and *Food Chemistry*, **49**(11), 5092-5097.

Kudlejova, L., Risticevic, S., and Vuckovic, D. (2012): *Solid-phase microextraction method development.* in: Pawliszyn, J. (ed.). Handbook of solid phase microextraction. Elsevier, Waltham, USA, 201-249.

Lambropoulou, D. A., and Albanis, T. A. (2007): Methods of sample preparation for Determination of pesticide residues in food matrices by chromatography-mass spectrometry-based techniques: A review. *Analytical and Bioanalytical Chemistry*, **389**, 1663-1683.

Liu, M., Zeng, Z., and Tian, Y. (2005): Elimination of matrix effects for headspace solid-phase microextraction of important volatile compounds in red wine using a novel coating. *Analytical Chimica Acta*, ***540***,341-353.

Lord, H., and Pawliszyn, J. (2000): Evolution of solid-phase microextraction technology. *Journal of Chromatography A*, **885**(1-2), 153-193

Menezes Filho, A., dos Santos, F. N., and de Paula Pereira, P. A. (2010): Development, validation and application of a methodology based on solid-phase microextraction followed by gas chromatography coupled to mass spectrometry (SPME/GC-MS) for the determination of pesticide residues in mangoes. *Talanta*, **81**(1-2), 346-354.

Ouyang, G., and Pawliszyn, J. (2008): A critical review in calibration methods for solid-phase microextraction. *Analytical Chimica Acta*, **627**, 184-197

Pawliszyn, J. (1997): *Solid phase microextraction: Theory and practice:* VCH, New York.

Risticevic, S., Lord, H., Gorecki, T., Arthur, C. L., and Pawliszyn, J. (2010): Protocol for solid-phase microextraction method development. *Nature Protocol*, **5**, 122-139.

Risticevic, S., Niri, V. H., Vuckovic, D., and Pawliszyn, J. (2009): Recent developments in solid-phase microextraction. *Analytical and Bioanalytical Chemistry*, **393**, 781-795.

Rodriguez, R., Manes, J., and Pico, Y. (2003): Off-line solid-phase microextraction and Capillary electrophoresis mass spectrometry to determine acidic pesticides in fruits. *Analytical Chemistry,* **75**(3), 452-459.

Simplicio, A. L., and Boas, L. V. (1999): Validation of a solid-phase microextraction method for the determination of organophohporus pesticides in fruits and fruit juices.*Journal of Chromatography A,* **883**, 32-42.

Vazquez, P. P., Mughari, A. R., and Galera, M. M. (2008): Solid-phase microextraction (spme) for the determination of pyrethroids in cucumber and watermelon using liquid chromatography combined with post-column photochemically induced fluorimetry derivatization and fluorescence detection. *Analytical Chimica Acta,* **607**, 74-82.

Zambonin, C. G., Cilenti, A., and Palmisano, F. (2002): Solid-phase microextraction and gas chromatography-mass spectrometry for the rapid screening of triazole residues in wine and Strawberries. *Journal of Chromatography A*, ***967***, 255-260.

Zeng, J., Chen, J., Lin, Z., Chen, W., Chen, X., and Wang, X. (2008): Development of polymethylphenylsiloxane-coated fiber for solid-phase microextraction and its analytical application of qualitative and semi-quantitative of organochlorine and pyrethroid pesticides in vegetables. *Analytical Chimica Acta,* **619**(1), 59-66