Optimization of Headspace Solid-Phase Microextraction for the Determination of Pesticide Residues in Vegetables and Fruits

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A headspace solid-phase microextraction method has been developed for the determination of 8 pesticides in vegetables and fruits by using gas chromatography with an electron capture detector. Two types of fibers (polyacrylate, 85 μm and polydimethylsiloxane, 100 μm) have been assayed and compared. The main factors: extraction and desorption parameters, ionic strength, and the effects of dilution and organic solvents, were studied and optimized. The optimized procedures resulted in more than 80% recovery for all the investigated vegetable and fruit samples with RSD values below 10%.

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Pesticides, including organochlorine pesticides (OCPs) and organophosphorous pesticides (OPPs) are examples of well-known environmental contaminants. OPPs are used as a substitute for OCPs in many countries nowadays because they can degrade more easily in the environment. Although OPPs as a whole are not the most toxic pollutants, they can be traced to a wide range of surface water, fruits, vegetables and foodstuff.¹ For fast monitoring of the environmental contamination caused by OCPs and OPPs and to accomplish risk assessment, more sensitive, rapid and simple analytical methods are paramount.

Liquid-liquid extraction (LLE) and solid-phase extraction (SPE) are the most popular methods for extracting pesticides. However, they are time consuming and labor-intensive, and require large volumes of sample and solvents. In addition, because pesticides generally are present at ppt levels, mixed with other contaminants at higher concentrations, interfering compounds can produce extraneous peaks. LLE and SPE can carry contaminants into the final sample, producing a high background.²⁻⁴

Solid-phase microextraction (SPME), developed by Kataoka *et al.*,⁵ has received an increasing amount of attention at the analytical level in numerous scientific disciplines.⁶ It is simple^{6,7} and, as will be discussed later, offers great flexibility. In comparison with direct SPME, headspace (HP) SPME can shorten the time of extraction significantly because the diffusion rate of the analytes in gaseous phase is faster than that in the aqueous phase.¹ Only a few references on the application of HS-SPME for the determination of OCPs and OPPs in fruit and vegetable samples can be found.^{8,9} Most applications are based on the preparation of fruit or vegetable juices and analysis of target compounds by directly dipping of the SPME fibers into the slurry.⁹⁻¹⁴

However several disadvantages related to fiber stability

and sensitivity have been pointed out. Such problems can be overcome if headspace SPME is applied. Since the fiber is not in contact with the sample, background adsorption and matrix effects can be reduced, which also enhances the life expectancy of the SPME fibers.

The objectives of this study are to evaluate the HS-SPME process by comparing two coating materials and to optimize the main parameters affecting the HS-SPME. Finally, we intended to develop an efficient multi-residue method on the basis of HS-SPME to extract each selected pesticide in fruit and vegetable samples.

Experimental

Chemicals and reagents

Eight pesticides standards which are popular used by local farmers in vegetable and fruit cultivation¹⁵ were > 95% pure and were purchased from AccuStandard Inc., USA. Stock solutions of each pesticide were prepared. Working standard solutions were prepared daily by volume dilution in distilled water. In the calibration and quantitation studies, an internal standard, 1-chloro-4-fluorobenzene, 200 $\mu\text{g/L}$ which is effective as a surrogate to compensate the data of all 8 kinds of pesticides was added to each sample prior to GC analysis.

SPME procedure

The SPME kits were provided from Supelco (Bellefonte, PA, USA) and used without modification. The fiber coatings assayed were polydimethylsiloxane (PDMS, 100 μm) and polyacrylate (PA, 85 μm). Before measurements, both fibers were conditioned to fully remove any contaminant.

Preliminary experiments were carried out to evaluate the HS-SPME process by comparing two coating materials and optimizing the main parameters, *i.e.*, extraction time and temperature, desorption time and temperature, and ionic

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strength. In these studies, the spiked distilled water samples were used, whereas the spiked vegetable and fruit samples were used for the study of the effects of dilution and organic solvent.

Recovery tests were performed using a PDMS 100 μm fiber. A 1.0-g portion of the homogenized vegetable and fruit sample was mixed with 100 μL of a mixture of methanol/acetone (1:1), and topped up with distilled water containing 10% NaCl to 5.0 g. Then, the samples were mixed with the internal standard. The PDMS fiber was exposed to the headspace for 30 min at 60°C. Quantification of pesticides in vegetable and fruit samples was carried out by a five point-calibration in the matrix using spiked samples by comparing the ratio of the peak area of the analyte against the peak area of the internal standard *versus* the concentration of the analytes. Every sample was analyzed in triplicate.

Gas chromatography-electron capture detector (GC-ECD)

A Shimadzu GC 17A Ver. 2.21 gas chromatograph with an

electron capture detector (ECD) was used. An SGE BPX5, 30 m $\times$ 0.32 mm i.d. capillary column with a 0.25- μm film was used with the following oven temperature program: 120°C, then 7°C/min ramped to 250°C, and held for 4.5 min. The detector temperature was at 300°C with splitless mode. Nitrogen gas (99.999%) was used as the carrier gas with a gas flow at 24.4 cm/s linear velocity and the pressure at 94 kPa.

Results and Discussion

Selection of SPME coating

The fibers, 100 μm PDMS and 85 μm PA, are the most sensitive fiber coatings for the analysis of OCPs and OPPs.¹⁶⁻¹⁹ From Tables 1 and 2, one can observe that compounds with higher octanol-water partition coefficient ($\log K_{ow}$) and low solubilities in water, such as chlorpyrifos, α -endosulfan and β -endosulfan, were the more extensively adsorbed when the PDMS fiber is used due to the higher affinity to the non-polar fiber coating. Compounds with higher polarities such as malathion and diazinon were adsorbed at a higher percentage (65 – 80%) by PA in relation to PDMS fiber. Generally, PDMS fiber showed a high extraction efficiency, lower detection limits and more sensitivity for the investigated compounds if one compares with the PA fiber. α -Endosulfan presents the best limit of detection due to the high ECD response as a consequence of having six chlorine atoms in its molecule.

Effect of extraction time and temperature

Adsorption time profiles are presented in Figs. 1A and 1B. Each data point is the average of two independent measurements. For the PDMS fiber, the equilibrium time of most analytes is shorter (60 min), whereas all the analytes need 90 min to reach

Table 1 Physicochemical properties of the selected pesticides²⁰

Name	Water solubility/ mg L^{-1} at 25°C	Vapor pressure/ mmHg	$\log K_{ow}$
Diazinon	40	9.02×10^{-5}	3.30
Chlorothalonil	0.6 – 1.2	5.7×10^{-7}	3.05
Malathion	130	3.94×10^{-5}	2.75
Chlorpyrifos	2	2.02×10^{-5}	4.69
Quinalphos	22	2.6×10^{-6}	4.44
Profenofos	28	6.23×10^{-6}	4.74
α -Endosulfan	0.32	3.0×10^{-6}	3.83
β -Endosulfan	0.32	5.96×10^{-7}	3.83

Table 2 Mixture concentration, peak area, linear range and LOD of the selected pesticides

Compound	Conc./ $\mu\text{g L}^{-1}$	Peak area ($\times 10^4$)		Percent of peak area PA/PDMS	Linear range/ $\mu\text{g L}^{-1}$		LOD/ $\mu\text{g L}^{-1}$	
		PA	PDMS		PA	PDMS	PA	PDMS
Diazinon	160	5.16	7.79	66.28	5000 – 0.5	2000 – 0.3	0.25	0.05
Chlorothalonil	80	2.09	5.96	35.09	5000 – 1.0	2000 – 0.3	0.25	0.05
Malathion	160	3.24	4.07	79.53	15000 – 15	10000 – 10	0.6	0.3
Chlorpyrifos	4	21.37	81.31	26.29	500 – 0.1	100 – 0.02	0.025	0.005
Quinalphos	160	2.99	4.34	68.92	15000 – 15	10000 – 10	0.6	0.3
α -Endosulfan	2	15	69.52	21.58	500 – 0.05	50 – 0.01	0.005	0.001
Profenofos	20	2.86	5.35	53.41	1000 – 0.2	350 – 0.05	0.025	0.005
β -Endosulfan	4	10.9	41.92	26.01	1000 – 0.2	300 – 0.05	0.025	0.005

Fig. 1 A, Extraction time of PDMS. B, Extraction time of PA. ♦, Diazinon; ■, chlorothalonil; ▲, malathion; ×, chlorpyrifos; ✕, quinalphos; ○, α -endosulfan; +, profenofos; ▀, β -endosulfan.

Table 3 Recovery test on vegetable and fruit sample by using optimized developed procedure

Pesticide	Linear range/ $\mu\text{g L}^{-1}$	LOD/ $\mu\text{g L}^{-1}$	Recovery, % ($n = 3$)	
			Tomato	Guava
Diazinon	1000 – 10	0.2	91 ± 1.3	82 ± 3.3
Chlorothalonil	1000 – 10	0.2	92 ± 1.4	84 ± 0.8
Malathion	5000 – 50	1.0	94 ± 1.8	95 ± 0.8
Chlorpyrifos	50 – 0.5	0.02	82 ± 2.5	94 ± 0.5
Quinalphos	5000 – 50	1.0	95 ± 0.3	92 ± 1.9
α -Endosulfan	20 – 0.1	0.01	93 ± 0.7	92 ± 1.2
Profenofos	100 – 1	0.1	90 ± 1.4	94 ± 0.8
β -Endosulfan	100 – 1	0.1	81 ± 0.8	97 ± 0.9

the equilibrium when the PA fiber is used. This is because the PDMS coating is a viscous liquid polymer and the diffusion coefficient of the analyte in it will be orders of magnitude higher than its diffusion coefficient in a solid polymer such as the PA fiber. Therefore, since the dynamics of mass transport in a well-stirred solution is controlled by the diffusion coefficients of analytes in the coating, the extraction time required with a liquid polymer coating will be considerably less than that required with a solid phase polymer.¹⁷ Another limitation of the PA fiber for the extraction of OCPs and OPPs is the more polar character of its coatings. Various authors^{18–20} have shown that the diffusion coefficient of an analyte is higher in the 100 μm PDMS fiber than in the 85 μm PA fiber. As has been reported, for quantitative analysis it is not necessary for the analytes to reach equilibrium as long as the extractions are carefully timed and the mixing conditions and volumes remain constant.²¹ So, an adsorption time of 30 min has been selected for the extraction for both the PDMS and PA fibers to keep the total extraction time comparable with the chromatographic run time.

As for the physical properties of the pesticides, increasing the temperature improved the mobility of the pesticides through the liquid and gas phases and better recoveries were obtained up to 60°C. At higher temperatures, the ability of the SPME fiber to adsorb the tested pesticides begins to decrease. This is because adsorption is an exothermic process and therefore not favorable at a high temperature. Thus increasing the temperature will make the distribution constant at equilibrium decrease.¹⁷ Moreover, the decrease of the extraction yield could be due to the enhanced hydrolysis of OPPs at elevated temperatures. An increase in water vapor pressure is another cause of decrease in sensitivity of HS-SPME when the extraction temperature exceeded 60°C.

Effects of desorption temperature and time

The temperature of the GC injector and desorption time were tested in order to ensure the complete desorption of pesticides to avoid a carryover. Results showed that all the analytes were only completely removed from the coating at 240 – 300°C. Hence a temperature of 240°C for the PDMS fiber and 260°C for the PA fiber were selected, since high temperatures can shorten the coating lifetime and can result in the bleeding of the polymer, causing problems in the separation and quantification.⁸

After performing several experiments using desorption times between 1 to 15 min, we found that a 6-min period was sufficient to desorb pesticides in the GC injector port; with the fiber remaining for another 4 min, one can eliminate any possible residues on the fiber to ensure a reproducible desorption.

Effects of ionic strength

In SPME procedure the salting out effect can be employed to modify the matrix by adding salts such as sodium chloride (NaCl) to increase the ionic strength of the water so as to decrease the solubility of analytes and release more analytes into the headspace, thereby contributing to enhanced adsorption on the fiber.^{22,23} Thus, with reference to the PDMS fiber the compounds with higher water solubilities such as diazinon and malathion showed an increase in the extraction yield with addition of increasing concentrations of NaCl until 30% (w/v) is reached. However, no effect or even a decrease in extraction yield was observed for compounds with a low water solubility after 10% (w/v). For the PA fiber, a similar behavior was observed. Salt contents of 10% were selected for the PDMS and PA fibers. Besides, Berrada *et al.*²⁰ found that, when working at a high salt concentration, the fiber would be blocked by the formation of the salt crystal.

Effects of dilution

In the attempt to reduce the matrix interference, we established the effects of adding water on the samples in order to favor the release of the analyte from the matrix by using different amounts of water ranging from a dilution factor of 1 to 10. Recoveries were calculated by comparing the peak ratios of the relevant chromatographic peak to the spiked sample and an aqueous solution at the same pesticide concentration level that was progressively subjected to the same dilution.

The results showed that the detection response of all pesticides was enhanced with the addition of water and decreased when the amount of water added exceeded a certain level. The optimum dilution factors for cucumber and tomato samples were 2 and 3. For the other samples it was a factor of 5. This could be due to the water content of the vegetables and fruits. The HS-SPME process is affected by the suspended matter and dissolved compounds (sugar, pectins *etc.*) 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 (interfering with diffusion).²⁴ Since the analytes were analyzed by HS-SPME, the addition of higher amounts of water would dilute the concentration of the analytes and increase the diffusion barrier of pesticides from the aqueous phase to the gaseous phase.

Moreover, the increase or decrease in average recovery (%) obtained was compound and structure-dependent. The average recovery of chlorpyrifos was significantly increased (14 to 75%) when the amount of water was increased. This could be caused by chlorpyrifos having a low water solubility (2 mg/L) and a high vapor pressure (2.02×10^{-5} mmHg). The desorbed pesticides will be easily released from aqueous solution to the gaseous phase. However, malathion which has a relatively high water solubility (130 mg/L) and a low vapor pressure (3.94×10^{-5} mmHg) when released from the sample matrix, will be retained in the aqueous solution, resulting in not much increase of the average recovery (25 to 58%) compared to chlorpyrifos.

Effects of organic solvent

The addition of an organic solvent could also promote the release of organic compounds from the vegetable and fruit samples. However, the presence of a high concentration of an organic solvent would lead to a significant decrease in the extraction efficiency of the analytes.²⁴ The results showed the extracting solvent consisting 2% (v/v) of a mixture of methanol/acetone (1:1) with optimum dilution showed the best recovery, from 82 to 97% of the pesticides for all the investigated vegetable and fruit samples with RSD between

Fig. 2 Chromatogram of a spiked tomato and one extracted by HS-SPME. Internal standard, 2.69 min (200 $\mu\text{g/L}$); diazinon, 13.56 min (160 $\mu\text{g/L}$); chlorothalonil, 14.72 min (80 $\mu\text{g/L}$); malathion, 16.42 min (160 $\mu\text{g/L}$); chlorpyrifos, 16.65 min (4 $\mu\text{g/L}$); quinalphos, 18.28 min (160 $\mu\text{g/L}$); α -endosulfan, 19.37 min (2 $\mu\text{g/L}$); profenofos, 19.76 min (20 $\mu\text{g/L}$); β -endosulfan, 21.83 min (4 $\mu\text{g/L}$).

0.3 – 3.3% if compared to the percentage of recoveries obtained from condition without dilution and when organic solvent amounts added were very small, the majority of them being lower than 30%.

Table 3 shows the linear range, LOD, mean relative recoveries and RSD value obtained by using the optimized developed HS-SPME procedure.

The developed method was also tested on actual vegetable (tomato) and fruit (guava) samples. We found that the pesticide residues of these two types of samples were below standard maximum residue level, which is only 1 to 50 $\mu\text{g/L}$. Figure 2 shows the chromatogram of a spiked tomato and one extracted by HS-SPME.

Conclusion

A simple method which used the addition of 100 μL methanol/acetone (1:1) to the 1.00 g sample slurry containing 10% NaCl and topped up to 5.00 g with distilled water and followed by GC-ECD has been developed for the determination of eight pesticides. This optimized HS-SPME procedure could be adopted as a fast and accurate method for analyzing pesticides in vegetable and fruit samples and could be used as an alternative to the conventional extraction technique, which involves high volumes of solvents, clean up procedures and several time-consuming steps. The convenience, simplicity and reliability of the SPME technique should undoubtedly make it a valuable tool for the environmental screening of pesticides in the future.

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