



Short communication

Headspace solid-phase microextraction in combination with gas chromatography–mass spectrometry for the rapid screening of organophosphorus insecticide residues in strawberries and cherries

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Abstract

A headspace solid-phase microextraction (HS-SPME) method in combination with GC–MS was used for the extraction and quantification of diazinon, fenitrothion, fenthion, parathion ethyl, bromophos methyl, bromophos ethyl and ethion. The method was developed using a 100- μm poly(dimethylsiloxane) fiber. The obtained results showed higher responses of the insecticides after addition of aliquots of water and solvent to the fruit samples. Calibration curves that were constructed for the analytes spiked into strawberry and cherry samples followed linear relationships with good correlation coefficients ($R^2 > 0.986$). Linearity range was between 50 and 500 $\mu\text{g}/\text{kg}$ and the precision was found to be lower than 15% when applying the optimized HS-SPME procedure to fruit samples. Limits of detection in both strawberry and cherry samples using GC–MS (selected ion monitoring mode) were below 13 $\mu\text{g}/\text{kg}$. Moreover, the HS-SPME method was applied to the analysis of fruit samples and compared with liquid–liquid extraction. Results obtained in this study were in good agreement with those obtained using liquid–liquid extraction demonstrating that the recommended procedure was a fast, accurate and stable sample pretreatment method obtaining good efficiency for the extraction of organophosphorus insecticides from strawberries and cherries.

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1. Introduction

Organophosphorus pesticides (OPPs) are widely used in agriculture as insecticides and contaminate to variable extents agricultural products such as fruits. Due to their toxic properties [1,2] and potential risk to consumers the control of their presence in food commodities is a topic of public concern.

The current developments of analytical technologies to detect pesticide residues in fruits and vegetables have mostly focused on the simplification, miniaturization and improvement of the sample extraction and clean-up methods with universal microextraction procedures [3,4]. A clear example is the sample treatment reduction by Louch et al. in the solid-phase microextraction (SPME) technique [5]. This technique has become more and more popular in the extraction of organic compounds [6], and its potential compared to conventional extraction methods has been recognized. It is an inexpensive,

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solvent-free and reliable technique with excellent sensitivity and good selectivity.

Although direct SPME applications for the determination of pesticides in food samples, such as juices [7–9], wine [10–12], honey [13,14] and fruit samples [4,15] have been reported, few references on the headspace (HS) SPME approach for the determination of pesticides in fruit samples can be found [16–18]. Due to the success of HS-SPME as a method for quantitative determination of organophosphorus insecticides in fruit juices [16], the present study was performed as an attempt to couple HS-SPME sampling and GC–MS analysis for the determination of seven selected OPPs in fruit samples.

2. Experimental

2.1. Chemicals, reagents and samples

Pesticide standards were obtained from Riedel-de Haën (Seelze, Germany) (Table 1), HPLC-grade water and methanol from Pestiscan (Labscan, Dublin, Ireland) and anhydrous sodium sulfate from Merck (Darmstadt, Germany). Stock standard solutions of 1000 mg/l of each compound were prepared in methanol. Working standard solutions of analyzed insecticides were prepared by diluting the stock solutions with methanol.

Fresh strawberry and cherry samples (50 g) were weighed separately into a 150-ml beaker. Calculating aliquots (250 μ l) of diluted working standards solutions at 10, 20, 50, 70 and 100 mg/l, respectively, were spiked into the fruits, drop-by-drop to yield concentrations ranging from 50 to 500 μ g/kg. After

being kept at room temperature for 3 h the spiked strawberries and cherries were sliced and homogenized in Ultra Turax for 30 s at 8000 rpm. Then fruit samples were placed in vials separately and analyzed following the recommended procedure.

2.2. HS-SPME analysis

All determinations were performed using a 100- μ m film thickness poly(dimethylsiloxane)-coated fiber mounted in a manual syringe holder (Supelco, Bellefonte, PA, USA). The choice of fiber coating as well as the sampling (stirring rate, extraction time, salt addition and temperature) and desorption conditions was the same as those adopted previously [16]. The HS-SPME procedure developed was very simple. Initially, 5 g of the strawberry and cherry sample containing 15% (w/v) Na_2SO_4 and an adequate amount of water (water/pulp ratio 1/2 for strawberries and 1/3 for cherries), were combined in a 20 and 25-ml vial, respectively. After equilibration at 75 °C for 10 min, the fiber was exposed to the headspace above the sample for 45 min. Each sample was stirred vigorously 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 240 °C) for 5 min [16–19]. All desorptions were performed in the splitless mode.

2.3. Gas chromatography–mass spectrometry

Gas chromatographic analysis was carried out using a Shimadzu model QP 5000 gas chromatograph and mass spectrometer. The insecticides were separated on a DB-5-MS, 30 \times 0.25 mm, 0.25 μ m

Table 1
Insecticides analyzed, retention times and typical fragment ions (m/z) of the target insecticides in GC–MS(SIM)

Insecticide	t_R (min)	Linearity	Quantitation ions (m/z)	Confirmation ions (m/z)	Ion set
(1) Diazinon	12.19	0.999	137	304 179	1
(2) Fenitrothion	16.06	0.999	277	260 109	
(3) Fenthion	17.37	0.997	278	125 109	2
(4) Parathion ethyl	17.59	0.995	109	291 139	
(5) Bromophos methyl	18.55	0.993	331	329 125	
(6) Bromophos ethyl	22.17	0.997	97	359 301	3
(7) Ethion	31.01	0.991	97	231 153	

capillary column, contained 5% phenyl–methylpoly-siloxane. The column was initially maintained at 55 °C; subsequently, the temperature was increased to 200 °C at a rate of 5 °C/min, which was held for 20 min and finally increased to 270 °C at a rate of 10 °C/min. Helium was used as the carrier gas with a flow rate of 1.0 ml/min. The temperatures of the ion source and the interface were set at 240 and 290 °C, respectively. The splitless mode was used for injection. The insecticides were detected by mass spectrometry with electron impact (70 eV) selected ion monitoring (SIM) mode at 1.75 kV. For each analyte, the most abundant and characteristic mass fragment was chosen for quantification and two others for confirmation (Table 1).

3. Results and discussion

3.1. Effect of water and solvent content

The influence of adding water on the samples in order to favor the release of analyte from the matrix was established by using different amounts of water ranging from 5 to 20 ml into the 5-g strawberry and cherry sample system (Fig. 1a and b). The responses of all insecticides were enhanced with the addition of water and decreased when the amount of water added exceeded 10 and 15 ml for strawberry and cherry samples, respectively. The HS-SPME process seems to be affected by the suspended matter and dissolved compounds (sugars, pectins etc) contained in fruit samples because they can adsorb the analytes, forming micelles and/or making it difficult for analytes to reach the fiber (interfering with diffusion) [7,20]. 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 OPPs from aqueous phase to gaseous phase.

The addition of hydrophilic solvents can also promote the release of organic compounds from fruit samples. Nevertheless, it is well known that the presence of a high concentration of organic solvent led to a significant decrease in extraction efficiency of analytes [21]. Therefore, only a small amount of solvent was recommended for use as amendment. Based on previous works related to the analysis of

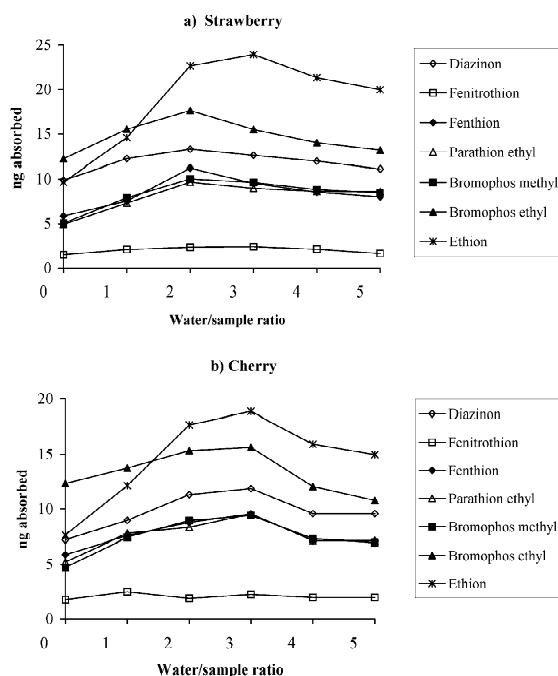


Fig. 1. Effect of water addition on extraction efficiency: (a) in strawberry; (b) in cherry samples at concentration level 200 $\mu\text{g}/\text{kg}$, $n = 3$.

pesticides in complex matrices [21,22] the addition of methanol was chosen in order to enhance the sensitivity of the procedure. The influence of water and organic solvent addition in strawberry samples are shown in Fig. 2. The ratio of solvent/water at 2.5% (v/v) was selected since when the solvent concentration was increased at higher values a

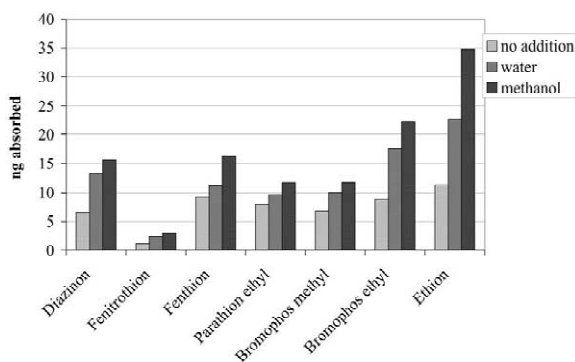


Fig. 2. Effect of water and solvent addition (methanol) on extraction efficiency in strawberry samples at concentration level of 200 $\mu\text{g}/\text{kg}$, $n = 3$.

Table 2
Analytical parameters of analyzed insecticides in the GC–MS system using SPME in strawberry and cherry samples

Insecticide	Strawberry samples				Cherry samples				
	Linearity	LOD ^a ($\mu\text{g}/\text{kg}$)	RSD ^b (%)	LOQ ^c ($\mu\text{g}/\text{kg}$)	Linearity	LOD ^a ($\mu\text{g}/\text{kg}$)	RSD ^b (%)	LOQ ^c ($\mu\text{g}/\text{kg}$)	MRLs ^d (mg/kg)
Diazinon	0.994	8.4	7.8	28.0	0.991	10.2	9.7	34.0	0.5
Fenitrothion	0.992	5.2	7.2	17.3	0.989	6.3	8.8	21.0	0.5
Fenthion	0.989	8.1	8.3	27.0	0.988	9.9	9.2	33.0	0.5
Parathion ethyl	0.990	8.9	11.8	29.7	0.987	12.3	13.9	41.0	1.0
Bromophos methyl	0.993	6.3	9.4	21.0	0.991	10.2	11.7	34.0	0.5
Bromophos ethyl	0.991	7.1	10.2	23.7	0.988	9.3	12.0	31.0	0.5
Ethion	0.987	10.7	12.7	35.7	0.986	12.7	14.6	42.3	0.5

^a LOD=Limit of detection. Calculated from the chromatogram of the sample spiked at 40 $\mu\text{g}/\text{kg}$ concentration level.

^b RSD=Relative standard deviation.

^c LOQ=Limit of quantitation. Calculated from the chromatogram of the sample spiked at 50 $\mu\text{g}/\text{kg}$ concentration level.

^d Maximum residue limits of selected OPPs in fruits [23].

decrease on the extraction efficiency of analytes was observed. In addition, the presence of high concentrations of organic solvents in the fruit matrix led to less clean chromatograms.

3.2. Linearity, precision and detection limits

Linearity experiments were carried out with fruit samples, spiked with each of the OPPs in a concentration range from 50 to 500 $\mu\text{g}/\text{kg}$. The results from three different experiments showed acceptable linearity with regression coefficients ranged from 0.986 to 0.994 (Table 2). Lower coefficients were observed for fenthion, which is not easily separated from ethyl parathion as well as for the late eluting compounds such as bromophos ethyl and ethion. The low correlation of bromophos ethyl and ethion may

be attributed to the complexity of the matrix as well as to low water solubilities and long equilibrium times of these compounds with the 100- μm PDMS fiber. At concentrations $>500 \mu\text{g}/\text{kg}$, the absorbed amount of analyte was lower than expected probably due to fiber saturation and for this reason a higher concentration was not used for regression.

The precision of the HS-SPME was determined by performing five measurements with a single extracting solution from spiked strawberry samples. The relative standard deviations (RSDs) were in the range from 7 (diazinon) to 15 (ethion) (Table 2).

The limits of detection and the limits of quantitation were defined as the signal-to-noise ratios of about 3:1 and 10:1, respectively, in the analysis of spiked fruit samples (expressed as $\mu\text{g}/\text{kg}$). The data of Table 3 demonstrate that the method allows

Table 3
Mean recoveries (%) and RSD ($n = 3$) of the selected insecticides from strawberry and cherry samples

Insecticide	Spiked concentration ($\mu\text{g}/\text{kg}$)											
	Strawberries						Cherries					
	75	RSD (%)	150	RSD (%)	300	RSD (%)	75	RSD (%)	150	RSD (%)	300	RSD (%)
Diazinon	87	9	84	8	88	8	75	8	75	10	79	7
Fenitrothion	91	8	94	9	87	8	88	8	87	9	90	9
Fenthion	88	10	89	9	90	10	85	9	87	10	89	11
Parathion ethyl	81	11	85	12	86	9	79	10	77	10	78	9
Bromophos methyl	78	9	81	8	79	12	78	11	77	11	80	9
Bromophos ethyl	76	12	79	13	81	13	77	11	80	9	81	11
Ethion	76	13	78	14	77	13	74	14	76	12	77	15

detection of the insecticides in both strawberry and cherry samples at concentrations lower than 13 $\mu\text{g}/\text{kg}$. Thus, the maximum residue limits (MRLs) required by European and international regulations for all selected compounds can be verified without difficulty [23].

3.3. Recoveries

The recoveries of the extraction procedure were studied by spiking strawberry and cherry samples ($n = 3$) at three concentration levels (75, 150 and 300 $\mu\text{g}/\text{kg}$). The calibration curves generated from the spiked samples were used for the quantification. The results obtained for the overall procedure led to the extraction recoveries that ranged between 74 and 91% for all insecticides studied (Table 3). Typical HS-SPME–MS(SIM) chromatogram of the OPPs from extraction of strawberries spiked at 250 $\mu\text{g}/\text{kg}$ is presented in Fig. 3.

3.4. Comparison between HS-SPME and liquid–liquid extraction

Both the optimized HS-SPME procedure and liquid–liquid extraction were used to determine the selected OPPs in the fruit samples. For HS-SPME analysis, triplicate analyses of fruit samples were carried out by standard addition, spiking the samples at a concentration level (200 $\mu\text{g}/\text{kg}$) between 25 and 500 $\mu\text{g}/\text{kg}$. The standard addition method was selected in order to take the matrix into account. Liquid–liquid extraction was performed at the same concentration level (200 $\mu\text{g}/\text{kg}$) following the procedure described by Agüera et al. [24] and using 3 μl as final injection volume in the GC–MS system. Concerning the determined amount of OPPs, comparable standard deviations were obtained for both methods, which ranged from 5 to 19% and 4 to 17% for HS-SPME and liquid–liquid extraction procedures, respectively (Table 4). These results indicate that HS-SPME can be considered a quicker and

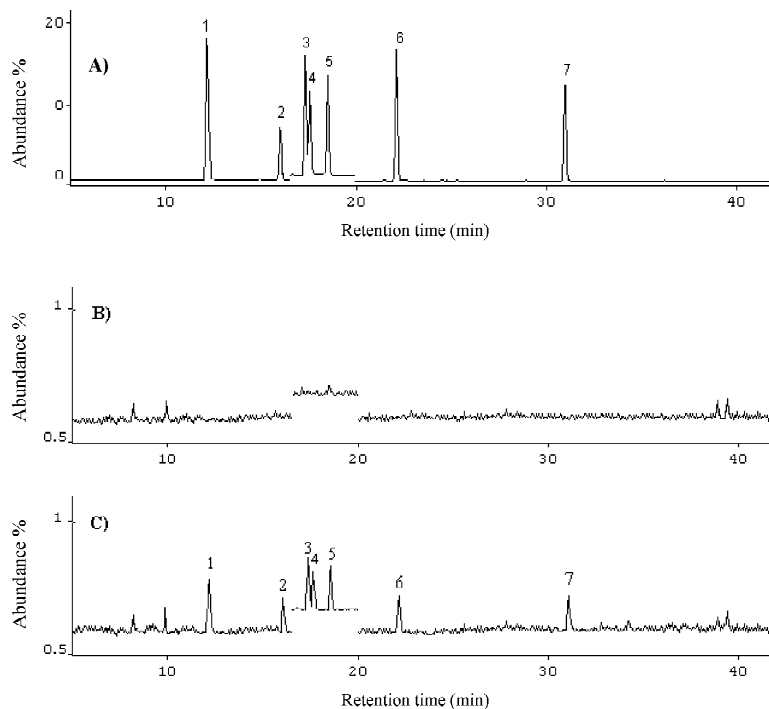


Fig. 3. GC–MS(SIM) chromatograms of spiked strawberry samples obtained by SPME: (A) at 250 $\mu\text{g}/\text{kg}$, (B) blank, (C) 40 $\mu\text{g}/\text{kg}$. Peaks: (1 = diazinon, 2 = fenitrothion, 3 = fenthion, 4 = parathion ethyl, 5 = bromophos methyl, 6 = bromophos ethyl, 7 = ethion).

Table 4
Analysis of OPPs in strawberry and cherry samples using HS-SPME and liquid–liquid extraction (LLE) procedures

Insecticide	Concentration ($\mu\text{g}/\text{kg}$ of fruits)			
	Strawberries		Cherries	
	Mean	RSD (%)	Mean	RSD (%)
HS-SPME				
Diazinon	172	5	168	7
Fenitrothion	180	6	172	8
Fenthion	173	8	170	8
Parathion ethyl	171	10	148	9
Bromophos methyl	158	12	152	12
Bromophos ethyl	153	11	158	13
Ethion	147	13	146	15
LLE				
Diazinon	168	4	176	6
Fenitrothion	174	6	180	7
Fenthion	176	7	166	9
Parathion ethyl	167	9	160	11
Bromophos methyl	162	12	160	8
Bromophos ethyl	155	9	164	10
Ethion	152	12	158	13

cheaper alternative for the determination of OPPs in fruit samples, such as strawberries and cherries.

4. Conclusions

Optimized HS-SPME procedure can be proposed as a fast and accurate method for analyzing OPPs in fruit samples and can be used instead of the liquid–liquid extraction technique which involves high volumes of solvents, clean-up procedures and time-consuming steps. In terms of sensitivity this method, with detection limits at low $\mu\text{g}/\text{kg}$ levels ($<13 \mu\text{g}/\text{kg}$), can satisfy the requirements set by European and international regulations for the MRLs which are usually at $0.5 \text{ mg}/\text{kg}$ for most of the selected OPPs in fruits such as strawberries and cherries.

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