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Headspace Solid Phase Microextraction Applied to the Analysis of Organophosphorus Insecticides in Strawberry and Cherry Juices

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A method based on a headspace solid phase microextraction (HS-SPME) technique followed by gas chromatography with flame thermionic and mass spectrometric detection was developed for the determination of seven organophosphorus (OPs) insecticide residues in strawberry and cherry juice samples. The extraction capacities of four fiber coatings, polyacrylate (PA 85 μm), poly(dimethylsiloxane) (PDMS 100 μm), carbowax−divinylbenzene (CW−DVB 65 μm), and poly(dimethylsiloxane)−divinylbenzene (PDMS−DVB 65 μm), have been studied and compared. The method was developed using spiked strawberry and cherry juices in a concentration range of 0.5−50 μg/L. The PDMS 100 μm fiber showed good extraction efficiency for the target compounds. An increase in the extraction efficiency of OP insecticides was observed when the parameters affecting the HS-SPME process such as temperature, extraction time, salt additives, stirring rate, pH, and effect of dilution were optimized. Good linearity of compounds was observed in the tested concentration range. The relative standard deviations were found to be <20%. The limits of detection were between 0.025 and 0.050 μg/L. The mean relative recoveries ranged from 82 to 102%.

KEYWORDS: Fruit juice analysis; OP insecticides; HS-SPME; gas chromatography

INTRODUCTION

Pesticide residue analysis in food is nowadays a priority objective in pesticide research in order to get an extensive evaluation of food quality and avoid possible risks to human health. Organophosphorus insecticides (OPs) constitute a class of pesticides widely used in agriculture to combat a high number of pests in a great variety of crops. The utilization of this class of pesticides is favored over their more persistent organochlorine counterparts because of their ability to degrade more readily in the environment. OP insecticides demonstrate rather low environmental persistence but high toxicity. As a consequence, the determination of OP insecticide residues in crops has been strictly regulated by governments in all countries, with two basic aims, namely, to detect the presence of forbidden pesticides on a particular commodity and to determine whether the concentrations of the pesticides used exceed their maximum residue limits (MRLs) (*1*, *2*).

To achieve a practical and reliable method for the determination of pesticide residues in complex matrices such as food samples, several sample preparation methods have been developed including liquid−liquid extraction (LLE) (*3*), supercritical fluid extraction (SFE) (*4*), solid phase extraction (SPE) (*5*, *6*), and solid phase microextraction (SPME) (*7*−*8*). However, in LLE and SPE large quantities of solvent waste are generated, multiple operation steps are needed, preconcentration of the extract prior to analysis is required, and interfering compounds are more likely to be coextracted. On the other hand, headspace sampling and purge and trap methods are simpler, less laborious, faster, and solvent-free techniques. Nevertheless, these methods have some disadvantages, such as the risk of cross-contamination and leaks, and the use of high flow rates can sometimes be incompatible with on-line operation.

SPME constitutes a convenient alternative to other commonly used extraction methods because it integrates sampling, extraction, concentration, and sample introduction into a single step without the use of solvents. This technique is of increasing interest in the field of pesticide residue analysis and has been applied for the determination of several classes of pesticides in aqueous media (*9*, *10*) or in other sample matrices (*11*−*13*). Results of the analysis showed that SPME was an accurate and fast method for sample preparation and analysis in several matrices. However, several disadvantages related to fiber stability and sensitivity have been pointed out (*14*). Sometimes, the small sample volume used in accordance with the matrix of the sample may affect the HS-SPME precision, eliminating the advantages of this technique.

Recently, headspace SPME (HS-SPME) has also been used to determine pesticides in water (*15*) and biological fluid samples (*16*, *17*). Compared with direct SPME, headspace SPME can shorten the time of extraction significantly because of the faster diffusion rate of the analytes in the gaseous phase than in the liquid phase (*18*). Because the fiber is not in contact with the

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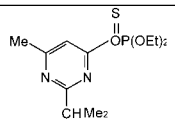
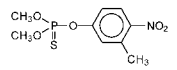
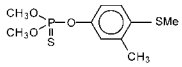
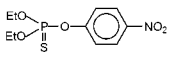
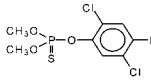
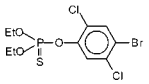
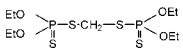
Table 1. Physicochemical Properties of the Selected Insecticides, Chemical Structure, Molecular Weight, Solubility in Water, Vapor Pressure, and Henry Constants

Insecticides	Chemical Structure	Molecular Weight	Water Solubility (mg/L)	Vapour Pressure mPa	Henry Constants (m^3Atm/mol)[a] (K_H = Pi/S)	LogK_{ow}[b]
Diazinon		304.3	40	8 at 20 ^{0}C	0.60	3.30
Fenitrothion		277.2	30	18 at 20 ^{0}C	1.64	3.40
Fenthion		278.3	55	4 at 20 ^{0}C	0.20	4.09
Parathion Ethyl		291.3	24	5 at 20 ^{0}C	0.60	3.76
Bromophos Methyl		366.1	40	17 at 20 ^{0}C	1.54	4.88
Bromophos Ethyl		394.0	2	6.1 at 30 ^{0}C	11.87	5.68
Ethion		384.5	1	0.2 at 25 ^{0}C	0.76	5.07

[a] Henry constants were estimated by using the equation K_H = Pi/S (where *S* is the solubility in mg/L and Pi the vapor pressure). [b] Log K_{ow}, water–octanol partition coefficients (*27*).

sample, matrix effects can be reduced, enhancing the life expectancy of the fiber. Furthermore, HS-SPME eliminates the possibility of introducing trace-level water caused by the wick effect in direct SPME (*19*).

The determination of pesticides in food samples by SPME has received only limited attention. Most of the SPME works to date have focused on liquid samples, such as wine (*13*, *20*, *21*) and honey samples, which are usually analyzed after dilution with water (*22*, *23*). Applications of HS-SPME for the analysis of OP insecticides in fruit juices have not been well documented. The aim of the present study is to investigate the applicability of the HS-SPME for the determination of OP insecticides in strawberry and cherry juice samples. Parameters such as fiber type, temperature, extraction time, salt content, and dilution were optimized. Seven insecticides, diazinon, fenitrothion, fenthion, parathion-ethyl, bromophos-methyl, bromophos-ethyl, and ethion, were selected due to their widespread use in agriculture in Greece (*24*) and other Mediterranean countries (*25*) and are also included in the European Union's list (*26*) for control of their residues in juice samples (**Table 1**). The analysis was performed by gas chromatography with flame thermionic detection (FTD) and electron ionization mass spectrometry detection (EI-MS).

MATERIALS AND METHODS

Reagents and Standards. Pesticide standards (diazinon, fenitrothion, fenthion, parathion-ethyl, bromophos-methyl, bromophos-ethyl, and ethion) were purchased from Riedel-de Haën (Seelze, Germany). All pesticide standards were of 98−99% purity. Methanol was purchased from Pestiscan (Labscan, Ltd., Dublin, Ireland). Anhydrous sodium sulfate was purchased from Merck (Darmstadt, Germany). Stock standard solutions of 1000 mg/L of each compound were prepared in methanol. Working standards solutions of analyzed insecticides at concentration levels of 5, 10, and 50 mg/L were prepared by diluting the stock solutions with methanol. Juice solutions were prepared by spiking the juice with an appropriate amount of the working solutions to yield concentrations ranging from 0.5 to 50 μg/L. In this way, a methanol content of <0.1% v/v was present in the juice samples, thus not affecting the extraction performance. Each concentration level was run in triplicate under optimum extraction conditions. The samples were analyzed by GC-FTD and GC-MS (SIM) to obtain the calibration curves and the linear range of the method.

Sample Preparation. The strawberries and cherries were grown by local farmers and collected in May 2000 at the optimum stage of ripeness from crops on which synthetic compounds such as fertilizers, pesticides, or additives were not used. The use of ecological samples does not guarantee the absence of interfering compounds, because various natural endogenous substances of persistent pesticide residues can remain in the ecosystems. For this reason blank samples were analyzed for OP insecticide residues by GC-MS (SIM) with the method described by Bolles et al. (*28*) before being spiked, and none of the target analytes were detected.

Two hundred grams of whole fresh strawberry and cherry fruits was sliced and homogenized in an Ultra Turax for 30 s at 8000 rpm. The mixture was centrifuged at 2268*g*, and the supernatant liquid portion after appropriate dilution was spiked with various amounts of the standard solutions. Before analysis, the samples were prepared by pipetting 5 mL of the spiked strawberry and cherry juice, respectively, into a 10 mL amber vial along with Na_2SO_4 and magnetic stirrer.

SPME Fibers. SPME holder and fiber assemblies for manual sampling were provided from Supelco (Bellefonte, PA) and used without modification. The fiber coatings assayed were as follows: polyacrylate (PA 85 μm), poly(dimethylsiloxane) (PDMS 100 μm), carbowax−divinylbenzene (CW−DVB 65 μm), and poly(dimethylsiloxane)- divinylbenzene (PDMS−DVB 65 μm). Before measurements, the fibers were conditioned in the injector for 3 h at 240 °C, with the

split vent open, and the PA fiber was conditioned overnight at the same conditions to fully remove any contaminant that might cause high baseline noise and large ghost peaks. Then the fiber was repeatedly injected into the GC until interfering peaks disappeared. During this desorption process the GC column oven temperature was maintained at 240 °C.

Headspace Solid Phase Microextraction Procedure. The HS-SPME extractions after optimization were performed by placing 5 mL of spiked diluted juice (30% water for strawberry and 50% water for cherry) into 10 mL crimp-top headspace vials, capped with PTFE−gray butyl-coated septa and mixed with 0.75 g of anhydrous sodium sulfate. The samples were heated by supporting them with a clamp in a water bath on top of the hot plate stirrer. After 10 min, the needle of the SPME device pierced the septum of the vial and the fiber was immersed to the headspace of the sample for 45 min, 1 cm above the spiked juice, which was kept at (75 ± 1 °C). Magnetic stirring with a 0.8 cm long PTFE-coated stir bar was used to agitate the sample at 960 rpm. After extraction, the fiber was inserted into the hot injector of the GC systems for analysis.

Gas Chromatographic Conditions. Chromatographic analysis was performed using a Shimadzu 14A capillary gas chromatograph equipped with a flame thermionic detector (FTD) at 250 °C. The DB-1 column, 30 m × 0.32 mm i.d., used contained dimethylpolysiloxane (J&W Scientific, Folsom, CA). The temperature was programmed as follows: initial temperature was kept at 150 °C for 2 min, which was increased to 200 °C, at 5 °C/min, held for 8 min, then raised to 210 °C at 1 °C/min, and kept for 2 min. The temperature was finally increased to 270 °C at 20 °C/min and held for 4 min. The injection temperature was 240 °C. Helium was used as the carrier (1.5 mL/min) and makeup gases (40 mL/min). The detector gases were hydrogen and air, and their flow rates were regulated at 4 ml/min and 120 ml/min, respectively. The SPME fiber was desorbed for 2 min in the GC split/splitless injection port, held at 240 °C. The injection port was in splitless mode, the splitter opening after 2 min.

A Shimadzu GC 17A gas chromatograph, coupled to a QP-5000 mass spectrometer, was used for analysis. Chromatographic separation of the insecticides was accomplished with a DB-5MS (J&W) fused-silica capillary column (30 m, 0.32 mm i.d., 0.25 mm) coated with a 5% biphenyl−95% dimethylsiloxane stationary phase. Helium was the carrier gas at a flow rate of 1.0 mL/min. Sample injection was in the splitless mode at 240 °C. The GC oven temperature program was as follows: initial temperature, 55 °C, ramped at 5 °C/min to 200 °C followed by another ramp of 1 °C/min to 210 °C, held for 2 min, and finally ramped to 270 °C at 20 °C/min (held for 3 min). The temperatures of the ion source and the interface were set at 240 and 290 °C, respectively. The mass spectrometer was operated in the electron impact (70 eV) selected ion monitoring (SIM) mode at 1.75 kV. The sum of two ions (*m*/*z*) was selected from the spectrum of each compound to quantify the response under sthe SIM mode with a dwell time of 100 ms and 1.44 cycles/s (scan range = *m*/*z* 40−450): 137 (100) and 304 (26) for diazinon, 109 (100) and 277 (38) for fenitrothion, 278 (100) and 125 (76) for fenthion, 109 (100) and 291 (49) for parathion-ethyl, 125 (79) and 331 (100) for bromophos-methyl, 97 (100) and 357 (60) for bromophos-ethyl, and 97 (100) and 231 (67) for ethion. The values in parentheses are the relative abundances (percent) of each peak in the spectrum. Peak areas for each compound were plotted against the insecticide concentration to obtain standard curves for each analyte. The insecticide concentration of the samples was then calculated by using the linear regression equation. All GC-MS identifications were based on the comparison of mass spectra and GC retention times of the insecticides analyzed with those of standards.

RESULTS AND DISCUSSION

Optimization of Headspace Solid Phase Microextraction. The different parameters that influence the partition of analytes between the headspace and the solution (the extraction time, temperature, ionic strength, stirring rate, and dilution effect) were optimized by analyzing spiked juice samples containing 1 μg/L of target compounds in the FTD system.

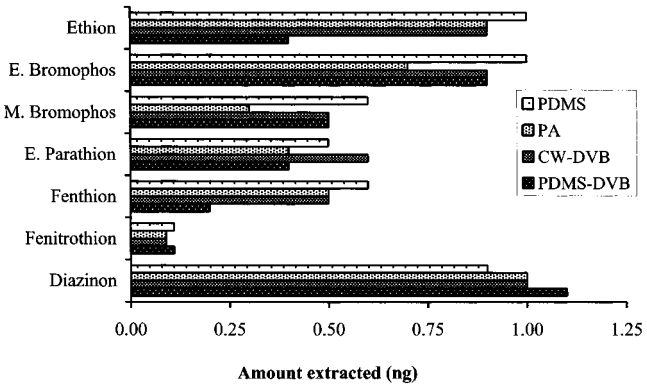


Figure 1. Amount extracted (nanograms) by four types of SPME coatings at 1 μg/L.

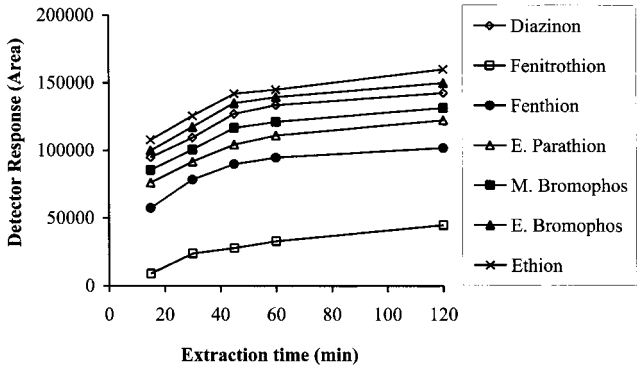


Figure 2. Plot of time of extraction versus detector response area, using a PDMS 100 μm fiber for the selected insecticides at 1 μg/L.

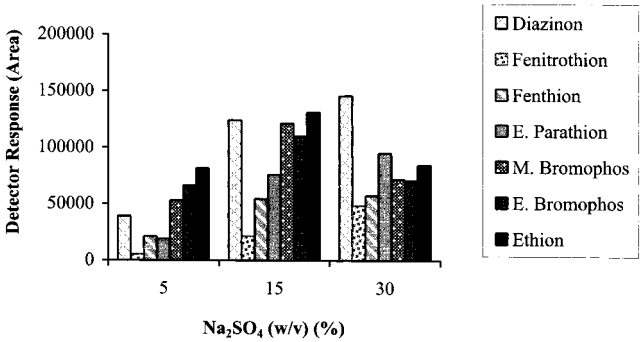


Figure 3. Plot of sodium sulfate of extraction versus detector response area, using a PDMS 100 μm fiber for the selected insecticides at 1 μg/L.

The choice of an appropriate coating is essential for the HS-SPME method. Four types of coatings were investigated: 100 μm PDMS, a 85 μm PA, a 65 μm PDMS−DVB, and a 65 μm CW−DVB. Distilled water samples were spiked with a concentration of 1 μg/L of target analytes and analyzed by HS-SPME (three replicates). The FTD response for the HS-SPME injection was calculated from the FTD calibration curve generated with injections of standards solvent (*29*) (**Figure 1**). The PDMS 100 μm coating exhibited the highest extraction efficiency for the most analytes and was selected for the subsequent experiments.

The effect of temperature on the extraction process of insecticides from the juice samples was examined over a range of 25−85 °C. HS-SPME was carried out for 45 min. The obtained results have shown that the increase of temperature influences the extraction of analytes. The temperature of 75 °C was optimized and was selected for the subsequent experiments.

Table 2. Analyzed Insecticides, Retention Times, Linearity Data, Limits of Detection (LOD), and Relative Standard Deviations (RSD) in the GC-FTD and GC-MS Systems Using SPME in Strawberry and Cherry Juices

	GC-FTD				GC-MS			
insecticide	t_R	linearity	LOD (μg/L)	RSD (%)	t_R	linearity	LOD (μg/L)	RSD (%)
Strawberry Juices								
diazinon	16.12	0.997	0.025	5	28.65	0.995	0.030	6
fenitrothion	22.64	0.998	0.030	9	32.34	0.992	0.040	13
fenthion	24.60	0.999	0.025	10	33.49	0.996	0.040	11
parathion-ethyl	24.92	0.995	0.025	10	33.72	0.992	0.040	11
bromophos-methyl	26.83.	0.997	0.025	7	34.49	0.998	0.040	10
bromophos-ethyl	31.95	0.992	0.030	12	37.43	0.994	0.050	12
ethion	37.40	0.988	0.025	11	43.04	0.993	0.050	14
Cherry Juices								
diazinon	16.12	0.997	0.030	7	28.65	0.997	0.050	7
fenitrothion	22.64	0.999	0.030	10	32.34	0.993	0.040	14
fenthion	24.60	0.997	0.030	10	33.49	0.994	0.045	11
parathion-ethyl	24.92	0.999	0.030	10	33.72	0.993	0.050	13
bromophos-methyl	26.83.	0.991	0.030	10	34.49	0.994	0.040	10
bromophos-ethyl	31.95	0.995	0.025	12	37.43	0.993	0.040	15
ethion	37.40	0.983	0.030	14	43.04	0.992	0.050	19

Table 3. Mean Relative Recoveries (Percent) and Relative Standard Deviations (RSD) ($n = 3$) of the Selected Insecticides from Strawberry Juices and Their Dilutions Using the SPME Technique with an FTD Detector

	mean relative recovery at a spiked concn of									
insecticide	0.5 μg/L	RSD (%)	1 μg/L	RSD (%)	10 μg/L	RSD (%)	25 μg/L	RSD (%)	50 μg/L	RSD (%)
Juices										
diazinon	32	8.7	28	7.6	44	5.8	51	7.7	55	8.1
fenitrothion	87	7.7	84	8.6	90	8.8	91	6.7	97	6.8
fenthion	61	9.9	68	9.2	78	8.9	72	9.6	74	9.3
parathion-ethyl	59	10.6	65	10.4	81	10.8	83	9.7	76	9.8
bromophos-methyl	58	8.9	64	9.6	69	9.9	59	10.1	60	9.6
bromophos-ethyl	57	11.1	61	11.5	63	11.8	57	12.1	62	11.9
ethion	39	18.6	45	18.9	48	18.1	56	18.4	47	17.9
Diluted Juices (30% Water)										
diazinon	78	6.7	86	6.9	84	6.3	86	5.9	91	6.9
fenitrothion	97	7.1	99	8.2	96	5.4	102	6.7	112	5.2
fenthion	81	6.6	87	6.1	90	7.5	88	8.1	90	8.0
parathion-ethyl	78	8.9	75	9.2	86	7.8	98	8.3	104	7.7
bromophos-methyl	80	7.9	85	8.1	90	8.8	93	8.9	113	7.9
bromophos-ethyl	80	10.8	74	10.5	84	10.7	94	11.4	89	11.0
ethion	80	16.9	87	16.7	91	16.5	105	17.0	107	17.0

The time required to reach equilibrium between the stationary phase and the sample headspace was also determined. The juice samples spiked with 1 μg/L of target compounds were exposed for times ranging from 15 to 120 min at 75 °C (**Figure 2**). An extraction time of 45 min was selected as a compromise between analyte response and time of analysis.

Sample agitation enhanced the extraction, especially for higher molecular mass analytes. Three replicate analyses were taken at four different stirring rates: 0 (static case), 260, 480, and 960 rpm. Faster stirring rates were avoided as they resulted in worse agitation by stirring bar vibrations. The optimum stirring rate was observed at 960 rpm and was used in all subsequent experiments.

The effect of ionic strength on extraction efficiency was evaluated by analyzing the amount of insecticides extracted in juice samples containing 5, 15, and 30% (w/v) of sodium sulfate (Na_2SO_4) (**Figure 3**). The optimum Na_2SO_4 concentration for the extraction of the tested insecticides was considered to be 15% (w/v).

Table 4. Mean Relative Recoveries (Percent) and Relative Standard Deviations (RSD) ($n = 3$) of the Selected Insecticides from Cherry Juices and Their Dilutions Using the SPME Technique with an FTD Detector

	mean relative recovery at a spiked concn of									
insecticide	0.5 μg/L	RSD (%)	1 μg/L	RSD (%)	10 μg/L	RSD (%)	25 μg/L	RSD (%)	50 μg/L	RSD (%)
Juices										
diazinon	40	9.2	47	9.9	50	10.1	55	8.9	66	8.1
fenitrothion	83	7.9	79	8.2	80	8.0	83	7.7	84	7.3
fenthion	52	9.7	60	9.9	65	10.1	66	9.8	63	9.7
parathion-ethyl	53	10.8	59	11.0	64	11.1	63	11.6	70	10.9
bromophos-methyl	63	9.3	68	10.2	72	10.4	76	9.7	81	9.6
bromophos-ethyl	66	11.9	68	12.2	73	12.5	74	11.8	84	11.7
ethion	25	18.9	36	19.0	37	18.7	40	18.9	52	18.9
Diluted Juices (50% Water)										
diazinon	74	8.6	81	8.7	80	7.6	82	7.9	92	8.3
fenitrothion	95	7.1	93	6.7	95	6.5	100	6.2	117	6.4
fenthion	78	8.6	85	8.7	80	9.0	81	8.6	90	8.8
parathion-ethyl	74	9.6	76	8.9	78	9.4	90	9.9	101	9.7
bromophos-methyl	84	8.4	93	8.8	89	8.7	107	8.9	112	8.6
bromophos-ethyl	81	9.9	76	9.7	86	9.9	96	9.7	100	9.6
ethion	101	18.1	102	17.6	99	17.8	105	18.2	117	18.4

To investigate the potential effect of the pH of the real juice (pH 3.5−4), a salted standard solution in water buffered to pH 3.5 was extracted by PDMS fiber and compared with a salted standard solution in distilled water that was not modified (pH 5.7). The pH of the juice samples was not adjusted for the next experiments because all of the analytes had an acceptable response at this value.

The method was tested on both strawberry and cherry juice samples. Triplicates of juice samples were spiked at 0.5, 1, 10, 25, and 50 μg/L. The recoveries of all analytes were found to be >60% in both strawberry and cherry juice samples, apart from diazinon and ethion, the recoveries of which were <50% (**Tables 3** and **4**). The relative recovery that is determined as the peak area ratio of juice sample and ultrapure water sample spiked with analytes at the same level (instead of absolute recovery as used in exhaustive extraction procedures) was applied because SPME is a nonexhaustive extraction procedure.

The dilution effect with water on extraction efficiency is shown in **Figure 4**. The addition of small amounts of water in the solution enhanced the recovery of the target compounds from the juice matrix. The maximum response for the more polar insecticides, such as diazinon, fenitrothion, fenthion, and parathion-ethyl, in the strawberry juice was obtained when the solution was diluted with water to give 30% (v/v) water content. In the case of less polar compounds such as bromophos-methyl, bromophos-ethyl, and ethion the peak responses decreased when the water content was >50% (v/v).

The effect of dilution was more pronounced in the cherry juice. The maximum response for all compounds was obtained when the solution was mixed with water at 1:1 [0% (v/v) water content]. After dilution, the strawberry and cherry juice samples yielded mean relative recoveries for all analytes from 82 to 102% (**Tables 3** and **4**). The amounts extracted (nanograms) by the fiber after spiking of juices and diluted juices are shown in **Table 5**.

Sampling from the headspace reduced the interaction between the sample matrix and the fiber. Thus, the coating was protected and the lifetime was increased. However, fiber destruction after extensive use could not be avoided. This parameter affects analysis reproducibility and clearly suggests that any routine use of the HS-SPME approach especially for complex matrices such as fruit juices should include frequent calibration runs after

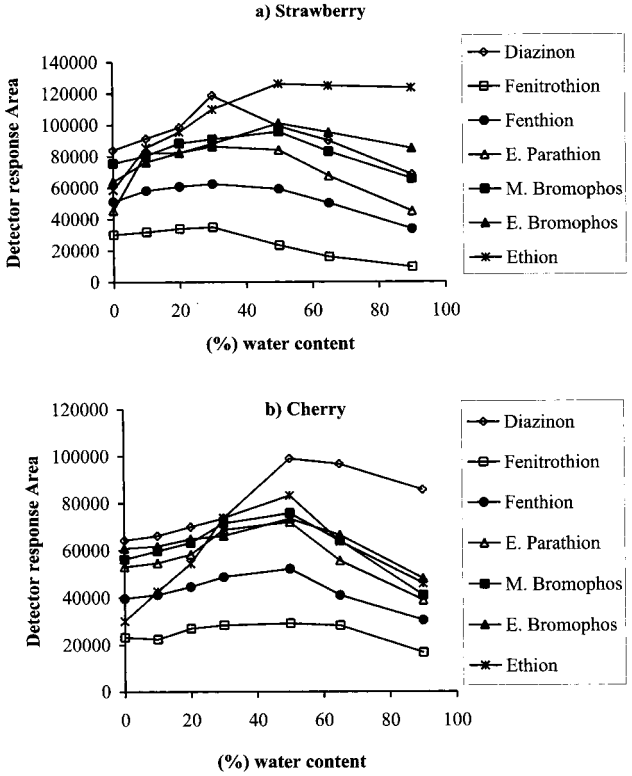


Figure 4. Plot of dilution in juices of extraction versus detector response area, using a PDMS 100 μm fiber: (a) in strawberry juice; (b) in cherry juice at a concentration level of 1 μg/L, $n = 3$.

Table 5. Amount (Nanograms) of Each Insecticide Extracted from Strawberry and Cherry Juices and Diluted Juices by Using SPME with an FTD Detector

	strawberry		cherry	
insecticide	juices[a]	juices[b] [30% (v/v) water content]	juices[a]	juices[b] [50% (v/v) water content]
diazinon	13.13	26.58	16.26	25.64
fenitrothion	2.40	2.70	2.19	2.67
fenthion	18.33	22.46	15.75	21.43
parathion-ethyl	16.02	19.31	13.61	18.43
bromophos-methyl	13.53	20.07	15.71	21.16
bromophos-ethyl	25.17	35.24	30.63	36.92
ethion	22.67	45.33	18.33	50.64

[a] Juice samples were spiked with 40 μg/L and analyzed: extraction time, 60 min; 15% (w/v) Na_2SO_4; 75 °C. [b] Juice samples were spiked with 40 μg/L, and the juice solution was diluted with 30% (v/v) and 50% (v/v) water content for strawberry and cherry samples, respectively, and analyzed: extraction time, 60 min; 15% (w/v) Na_2SO_4; 75 °C.

several uses. The PDMS 100 μm fiber was proven to be effective after almost 100 runs for the analysis of OP insecticides in strawberry and cherry juices, corresponding to the fiber life of environmental water samples using the HS-SPME mode (*15*).

Analytical Characteristics. Once the preliminary investigations were completed and extraction parameters (extraction time, temperature, salt addition, stirring rate, and effect of dilution) were optimized (in the FTD system), the feasibility of the HS-SPME method was investigated with regard to the linearity, precision, and limit of detection for strawberry and cherry juices with both GC-FTD and GC-MS instruments.

The linearity of the method was investigated by plotting the measured detector response over a series of concentration levels at 0.5, 1, 10, 25, and 50 μg/L. Each solution was run in triplicate.

Table 6. Precision of the SPME Procedure for Determination of Selected Insecticides in Spiked Strawberry Juices [30% (v/v) Water Content] ($n = 5$) with the FTD Detector

	RSD (%)		
insecticide	1 μg/L	10 μg/L	25 μg/L
diazinon	6.1	5.2	6.8
fenitrothion	7.2	6.2	5.9
fenthion	12.1	9.8	8.8
parathion-ethyl	13.4	14.2	8.9
bromophos-methyl	12.7	9.9	9.5
bromophos-ethyl	14.3	12.8	10.2
ethion	17.1	16.7	14.8

Table 7. Precision of the SPME Procedure for Determination of Selected Insecticides in Spiked Cherry Juices [50% (v/v) Water Content] ($n = 5$) with the FTD Detector

	RSD (%)		
insecticide	1 μg/L	10 μg/L	25 μg/L
diazinon	8.2	7.7	7.1
fenitrothion	8.6	8.6	7.0
fenthion	13.9	10.8	10.0
parathion-ethyl	15.1	13.3	9.7
bromophos-methyl	12.7	10.7	9.9
bromophos-ethyl	15.3	13.5	12.6
ethion	18.7	17.2	15.2

Table 8. Precision of the SPME Procedure for Determination of Selected Insecticides in Spiked Strawberry Juices [30% (v/v) Water Content] ($n = 5$) with the MSD Detector

	RSD (%)		
insecticide	1 μg/L	10 μg/L	25 μg/L
diazinon	5.5	5.1	5.9
fenitrothion	7.6	8.9	7.4
fenthion	7.6	6.8	5.6
parathion-ethyl	10.1	9.8	7.3
bromophos-methyl	10.7	9.1	8.8
bromophos-ethyl	12.5	11.7	10.1
ethion	14.2	14.7	13.7

Table 9. Precision of the SPME Procedure for Determination of Selected Insecticides in Spiked Cherry Juices [50% (v/v) Water Content] ($n = 5$) with the MSD Detector

	RSD (%)		
insecticide	1 μg/L	10 μg/L	25 μg/L
diazinon	6.8	6.6	5.8
fenitrothion	9.2	8.8	8.1
fenthion	9.2	9.4	7.6
parathion-ethyl	11.2	10.1	8.4
bromophos-methyl	10.9	10.2	10.1
bromophos-ethyl	13.2	13.1	13.7
ethion	15.2	15.7	15.1

Peak areas for each compound were plotted against the actual concentration to obtain calibration curves for each analyte. In all cases, a significant linear regression ($p < 0.05$) was observed for each analyte at the concentration range tested. The obtained results have shown linear regression with correlation coefficients between 0.986 and 0.999 with both FTD and MSD detectors (**Table 2**).

The precision of the HS-SPME method was determined by performing five consecutive extractions of spiked juice samples over the concentration range of 1, 10, and 25 μg/L, respectively.

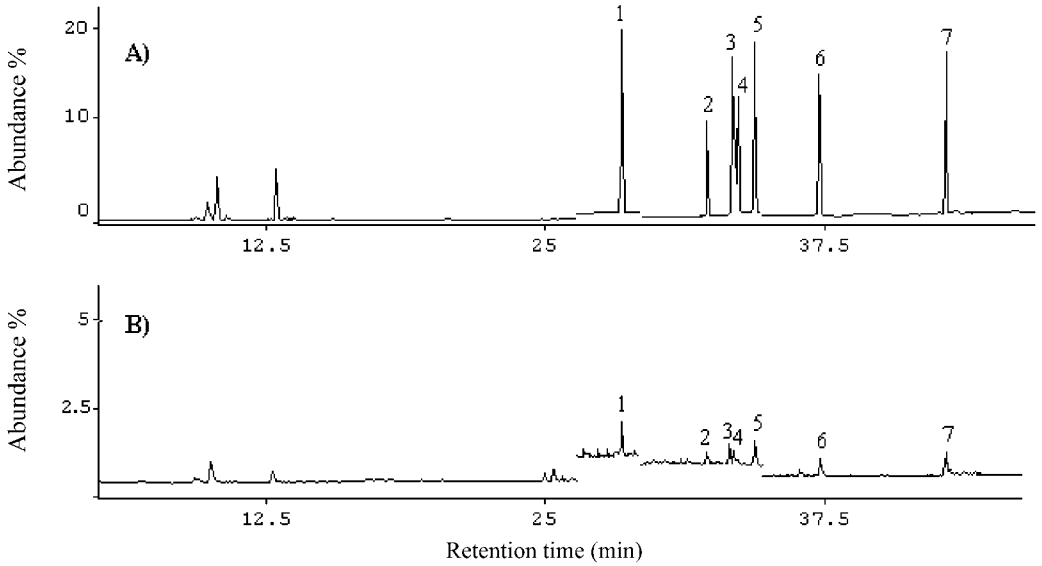


Figure 5. Typical GC-MS (SIM) chromatogram of the selected insecticides obtained from spiked strawberry diluted juice sample [30% (v/v) water content] after extraction with a PDMS 100 μm fiber: (A) 10 μg/L; (B) 0.15 μg/L. (1 = Diazinon, 2 = fenitrothion, 3 = fenthion, 4 = parathion-ethyl, 5 = bromophos-methyl, 6 = bromophos-ethyl, and 7 = ethion.)

The corresponding standard deviation was then calculated for these extractions and expressed as a percentage. The relative standard deviation (percent) (% RSD) values obtained were <20% in all cases, ranging from 5.2 to 18.7% in GC-FTD (**Tables 6** and **7**) and from 5.1 to 15.7% in GC-MS (**Tables 8** and **9**) for both strawberry and cherry juices. Higher values were obtained for the late-eluting compounds.

Limits of detection (LOD) were estimated on the basis of 3:1 signal to noise ratios obtained with standards containing the compounds of interest at low concentration levels. SPME analysis was performed as described under calibration procedures with GC-FTD and GC-MS. The SIM mode of mass spectrometry (sum of both characteristic masses of each compound) was used to estimate the detection limits. The average signal to noise ratio of three measurements was used to calculate the LOD. The method allowed detection of the insecticides in juice samples at concentrations <50 ng/L with both GC-FTD and GC-MSD instruments (**Table 2**). Thus, the limits of maximum residues (LMR) required by European and international regulations for the majority of products can be verified without difficulty (*26*).

Figures 5 and **6** shows typical GC-MS (SIM) and GC-FTD chromatograms obtained after SPME applied over a diluted [30% (v/v) water content] strawberry juice sample spiked with the selected OP insecticides: (A) 10 μg/L and (B) 0.15 μg/L. The obtained FTD chromatogram shows the presence of several nonidentified compounds in the strawberry juice sample, especially in the first part of the chromatogram, but no significant intereferences with the standard insecticides were observed. A similar profile was also observed for cherry juice samples.

Conclusions. A method for the extraction and analysis of priority organophosphorus insecticide residues from strawberry and cherry juices was developed. Optimization of the parameters affecting the method sensitivity should be carefully developed in order to enable substantial increase in the amount extracted of most analytes and to improve the limit of detection. This methodology has the advantages of simplicity, shorter duration, and not needing a cleanup step for the sample. Finally, it has been used routinely in combination with GC and GC-MS for screening analysis of complex matrices such as juice samples.

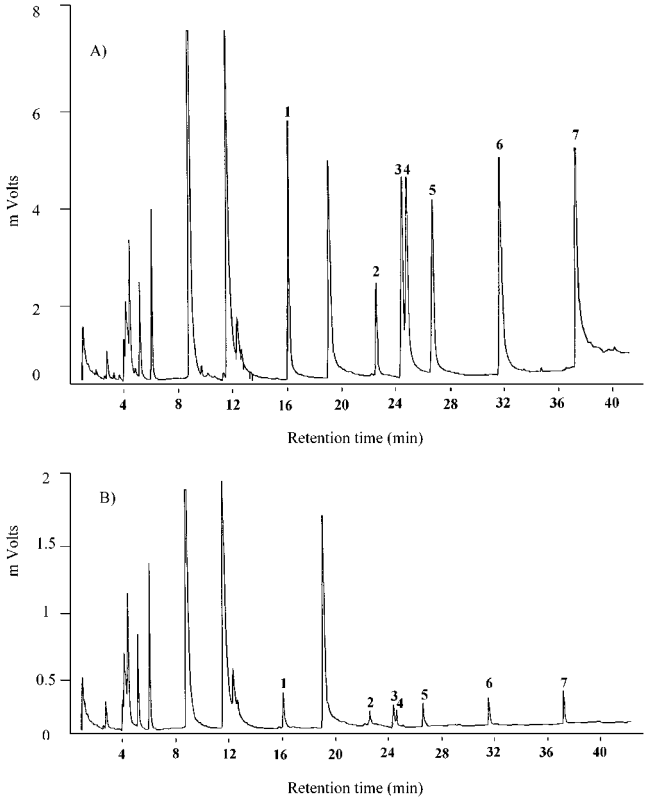


Figure 6. Typical GC-FTD chromatogram of the selected insecticides obtained from spiked strawberry diluted juice sample [30% (v/v) water content] after extraction with a PDMS 100 μm fiber: (A) 10 μg/L; (B) 0.15 μg/L. (1 = Diazinon, 2 = fenitrothion, 3 = fenthion, 4 = parathion-ethyl, 5 = bromophos-methyl, 6 = bromophos-ethyl, 7 = ethion.)

Although many aspects of the application of HS-SPME for analyzing insecticide residues in complex matrices still have to be investigated, this extraction technique should make it a valuable tool for the food analysis of insecticides or other classes of pesticides in the future.

LITERATURE CITED

(1) Community Directive 93/58 EEC. *Off. J. Eur. Communities* **1993**, L 211/6.

(2) FAO. Agriculture towards 2010. In *C 93/24 Document of 27th Session of the FAO Conference*; Rome, Italy, 1993.

(3) Pylypiw, H. M., Jr. Rapid gas chromatographic method for the multiresidue screening of fruits and vegetables for organochlorine and organophosphate pesticides. *J. AOAC Int.* **1993**, *76*, 1369−1373.

(4) Lehotay, S. Supercritical fluid extraction of pesticides in foods. *J. Chromatogr. A* **1997**, *785*, 289−312.

(5) Torres, C. M.; Pico, Y.; Manes, J. Comparison of octadecylsilica and graphitized carbon black as materials for solid phase extraction of fungicide and insecticide residues from fruit and vegetables. *J. Chromatogr. A* **1997**, *778*, 127−137

(6) Pihlström, T.; Österdahl, B. G. Analysis of pesticide residues in fruit and vegetables after cleanup with solid phase microextraction using ENV + (polystyrene−divinylbenzene) cartridges. *J. Agric. Food Chem.* **1999**, *47*, 2549−2552.

(7) Van der Hoff, R. G.; Van Zoonen, P. Trace analysis of pesticides by gas chromatography. *J. Chromatogr. A* **1999**, *843*, 301−322.

(8) Kataoka, H.; Lord, H. L.; Pawliszyn, J. Applications of solid phase microextraction in food analysis. *J. Chromatogr. A* **2000**, *880*, 35−62.

(9) Lambropoulou, D.; Sakellarides, T.; Albanis, T.; Fresenious. Determination of organophosphorus incecticides in natural waters using SPE-disks and SPME followed by GC/FTD and GC/MS. *Fresenius' J. Anal. Chem.* **2000**, *368*, 616−623.

(10) Beltran, J.; Lopez, F. J.; Cepria, O.; Hernadez, F. Solid phase microextraction for quantitative analysis of organophosphorus pesticides in environmental water samples. *J. Chromatogr. A* **1998**, *808*, 257−263.

(11) Simplicio, A. L.; Boas, L. V. Validation of a solid phase microextraction method for the determination of organophosphorus pesticides in fruits and fruit juice. *J. Chromatogr. A* **1999**, *833*, 35−42.

(12) Hu, R.; Hennion, B.; Urruty, L.; Montury, M. Solid phase microextraction of pesticide residues from strawberries. *Food Addit. Contam.* **1999**, *16*, 111−117.

(13) Beltran, J.; Lopez, F. J.; Hernandez, F. Solid phase microextraction in pesticide residue analysis. *J. Chromatogr. A* **2000**, *885*, 389−404.

(14) Hernandez, F.; Beltran, J.; Lopez, F. J.; Gaspar, J. V. Use of solid phase microextraction for the quantitative determination of herbicides in soil and water samples. *Anal. Chem.* **2000**, *72*, 2313−2322.

(15) Lambropoulou, D. A.; Albanis, T. A. Optimization of headspace solid phase microextraction conditions for the determination of organophosphorus insecticides in natural waters. *J. Chromatogr. A* **2001**, *922*, 243−255.

(16) Ng, W. F.; Teo, M. J. K.; Lakso, H. A. Determination of organophosphorus pesticides in soil by headspace solid phase microextraction. *Fresenius' J. Anal. Chem.* **1999**, *363*, 673−679.

(17) Mills, G.; Walker, V. Headspace solid phase microextraction procedures for gas chromatographic analysis of biological fluids and materials. *J. Chromatogr. A* **2000**, *902*, 267−287.

(18) Zhang, Z.; Pawliszyn, J. Headspace solid phase microextraction. *Anal. Chem.* **1993**, *65*, 1843−1852.

(19) Hulden, M.; Hansen, C. M. Water permeation in coatings. *Prog. Org. Coatings* **1985**, *13*, 171−194.

(20) Urruty, L.; Montury, M. Influence of ethanol on pesticide extraction in aqueous solutions by solid phase microextraction. *J. Agric. Food Chem.* **1996**, *44*, 3871−3877.

(21) Correia, M.; Delerue-Matos, C.; Alves, A. Multi-residue methodology for pesticide screening in wines. *J. Chromatogr. A* **2000**, *889*, 59−67.

(22) Jimenez, J. J.; Bernal, J. L.; del Nozal, M. J.; Martin, M. T.; Mayorga, A. L. Solid phase microextraction applied to the analysis of pesticide residues in honey using gas chromatography with electron capturre detection. *J. Chromatogr. A* **1998**, *829*, 269−277.

(23) Fernandez, Padron C.; Marconi, L.; Ghini, S.; Colombo, R.; Sabatini, A. G.; Girotti, S. Determination of organophosphorus pesticides in honeybees after solid phase microextraction. *J. Chromatogr. A* **2001**, *922*, 257−265.

(24) Hella, D. G.; Sakellarides, T. M.; Konstantinou, I. K.; Albanis, T. A. Influence of salinity and dissolved humic acids on pesticides extraction from water using solid phase disks. *Int. J. Environ. Anal. Chem.* **1997**, *67*, 1−14.

(25) Fielding, M.; Barceló, D.; Helweg, A.; Galassi, S.; Torstensson, L.; Van Zoonen, P.; Wolter, R.; Angeletti, G. *Pesticides in Ground and Drinking Water*; Water Pollution Research Report 27; Commission of the European Communities: Brussels, Belgium, 1992; pp 1−136.

(26) EC Council Directive listing the maximum content on pesticide residues of some fruits and vegetables, 93/58/EEC. *Off. J. Eur. Communities* **1993**.

(27) Noble, A. Partition coefficient (*n*-octanol−water) for pesticides. *J. Chromatogr. A* **1993**, *642*, 3−10.

(28) Bolles, H. G.; Dixon-White, H. E.; Peterson, R. K.; Tomerlin, J. R.; Day, E. W.; Oliver, G. R. U.S. market basket study to determine residues of the insecticide chlorpyrifos. *J. Agric. Food Chem.* **1999**, *47*, 1817−1822.

(29) DeBruin, L.; Josephy, D.; Pawliszyn, J. Solid phase microextraction of monocyclic aromatic amines from biological fluids. *Anal. Chem.* **1998**, *70*, 1986−1992.

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