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Optimization of headspace solid-phase microextraction conditions for the determination of organophosphorus insecticides in natural waters

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Abstract

Headspace solid-phase microextraction (HS-SPME) has been developed for the analysis of seven organophosphorus insecticides, i.e. diazinon, fenitrothion, fenthion, ethyl parathion, methyl bromophos, ethyl bromophos and ethion in natural waters. Their determination was carried out using gas chromatography with flame thermionic and mass spectrometric detection. To perform the HS-SPME, two types of fibre have been assayed and compared: polyacrylate (PA 85 μm), and polydimethylsiloxane (PDMS 100 μm). The main parameters affecting the HS-SPME process such as temperature, salt additives, memory effect, stirring rate and adsorption–time profile were studied. The method was developed using spiked natural waters such as ground, sea, river and lake water in a concentration range of 0.05–1 $\mu\text{g}/\text{l}$. The HS-SPME conditions were optimized in order to obtain the maximum sensitivity. Detection limits varied from 0.01 to 0.04 $\mu\text{g}/\text{l}$ and relative standard deviations (RSD <17%) were obtained showing that the precision of the method is reliable. The method showed also good linearity for the tested concentration range with regression coefficients ranging between 0.985 and 0.999. Recoveries were in relatively high levels for all the analytes and ranged from 80 to 120%. Water samples collected from different stations along the flow of Kalamas river (NW Greece) were analyzed using the optimized conditions in order to evaluate the potential of the proposed method to the trace-level screening determination of organophosphorus insecticides. The analysis with HS-SPME has less background interference and the advantage of its non-destructive nature reveal the possibility of the repetitive use of the SPME fibre. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Organophosphorus insecticides (OPPs) have been used extensively for agricultural purposes for more than 40 years. 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. OPPs have been found in groundwaters, surface waters, lagoons and drinking water in varying concentrations and therefore there is an increasing environmental concern with regard to these compounds [1].

In general, environmental waters cannot be ana-

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lyzed without sample pretreatment because they are too diluted or too complex. A sample preparation step is necessary to extract traces of pesticides from aqueous medium, bring the analytes to a suitable concentration level and remove them from interference components in the matrix (cleanup) before the chromatographic analysis. Current methods of analysis for aqueous or solid samples involve liquid–liquid extraction (LLE), supercritical fluid extraction (SFE), solid-phase extraction (SPE) and solid-phase microextraction (SPME) [2–6]. However, in both LLE and SPE toxic organic solvents are used, multiple operation steps are needed, pre-concentration of the extract prior to analysis is required, and interferences were often introduced due to the extraction procedure. SPME is a novel solvent free extraction technique, which integrated sampling, extraction, concentration and sample introduction into a single step. This technique was found applicable for the extraction of pesticides from aqueous media [7–9] or from other sample matrices [10–12].

The extraction of a sample by SPME is usually conducted directly with the coating fibre immersed in the liquid phase of the sample. Headspace (HS) SPME, where the fibre is exposed to the sample headspace to extract target analytes — which are partitioned between the gaseous and aqueous phase — is another development of the technique. In comparison with direct SPME, headspace SPME can shorten the time of extraction significantly because of the faster diffusion rate of the analytes in gaseous phase than in the aqueous phase [13]. The headspace approach is preferred when the sample matrix contains undissolved particles or non-volatile dissolved material; which may be transferred to the GC injector or non-polar non-volatile material which may contaminate the coated fibre. Furthermore, it can totally eliminate the possibility of introducing trace-level water caused by the wick effect in direct SPME [14].

HS-SPME is especially suitable when used in the analysis of volatile and semi-volatile organic compounds because these compounds can easily diffuse into the sample headspace from liquid matrix.

In this study seven insecticides — diazinon, fenitrothion, fenthion, ethyl parathion, methyl bromophos, ethyl bromophos and ethion — were selected as important insecticides that have been

detected in surface waters in Greece [15,16] and other Mediterranean countries [16] and are also included to the list of European Union [17] for control of their residues in water. Although most of experimental SPME conditions described in the literature use fibre immersion for organophosphorus pesticides analyses [18], this work intends to study the possibility using the headspace sampling, where the interferences coming from a complex matrix as natural waters are expected to be reduced.

The regulations of European Union for drinking water quality set a limit in concentration of 0.5 $\mu\text{g}/\text{l}$ for the sum of all pesticides and 0.1 $\mu\text{g}/\text{l}$ for each compound, so that detection limits below the 0.1 $\mu\text{g}/\text{l}$ are required for monitoring drinking water. In order to achieve the above mentioned requirements for the analysis of organic micro-pollutants in water, sensitive extraction and chromatographic techniques are required.

The aims of this work were (1) to develop an efficient multi-residue method on the basis of HS-SPME and gas chromatography with flame thermionic detection (FTD) and electron impact ionization mass spectrometry detection (EI-MS) for the pre-concentration and chromatographic analysis of the selected insecticides, (2) to evaluate the HS-SPME process comparing two coating materials with different polarity and thickness and (3) to demonstrate the ability of this technique to extract the selected semi-volatile organophosphorus insecticides in various natural waters (underground, river, lake and sea water) without suffering from background interferences.

2. Experimental

2.1. Reagents and standards

The tested insecticides diazinon, fenitrothion, fenthion, ethyl parathion, methyl bromophos, ethyl bromophos and ethion were purchased from Riedel-Haën (Seelze, Germany) (Table 1). Stock standard solutions of 50 $\mu\text{g}/\text{l}$ of each compound were prepared in methanol. Working standards solutions were prepared by diluting the stock solutions with methanol. The stock and working standards were

Table 1

Physicochemical properties of the selected insecticides chemical structure, molecular mass, solubility in water, vapour pressure and Henry constants

Insecticides	Chemical structure	Molecular mass	Water solubility (mg/l)	Vapour pressure (mPa)	Henry constants (m ³ Atm/mol) ^a ($K_H = P_i/S$)	Log K_{ow} ^b
Diazinon		304.3	40	8 at 20°C	0.60	3.30
Fenitrothion		277.2	30	18 at 20°C	1.64	3.40
Fenthion		278.3	55	4 at 20°C	0.20	4.09
Parathion ethyl		291.3	24	5 at 20°C	0.60	3.76
Bromophos methyl		366.1	40	17 at 20°C	1.54	4.88
Bromophos ethyl		394.0	2	6.1 at 30°C	11.87	5.68
Ethion		384.5	1	0.2 at 25°C	0.76	5.07

^a Henry constants were estimated by using the equation $K_H = P_i/S$ (Where S is the solubility in mg/l and P_i the vapour pressure).

^b Log K_{ow} : octanol–water partition coefficients [23].

stored at 4°C. Aqueous solutions were prepared by spiking the water with an appropriate amount of the working solution. HPLC-grade water and methanol

were purchased from Pestiscan (Labscan, Dublin, Ireland). Anhydrous sodium sulfate was purchased from Merck (Darmstadt, Germany).

2.2. SPME fibres

SPME holder and fibre assemblies for manual sampling were provided from Supelco (Bellefonte, PA, USA) and used without modification. The fibre coatings assayed were polyacrylate (PA 85 μm) and polydimethylsiloxane (PDMS 100 μm). Before measurements the PDMS fibre was conditioned in the injector for 3 h at 240°C, with the split vent open and PA fibre was conditioned overnight at the same conditions, to fully remove any contaminant which might have caused very high baseline noise and large ghost peaks. Then the fibre was repeatedly injected into the GC until interfering peaks disappeared. During this desorption process the GC column oven temperature was maintained at 240°C.

2.3. Headspace solid-phase microextraction procedure

Five (5) ml volume of water samples was placed in 10-ml crimp-top headspace vials, sealed with open centered aluminum caps and PTFE-gray butyl septa spiked with appropriate amount of the standard solution of insecticides and mixed with 0.75 gr of anhydrous sodium sulfate. The overall methanolic concentration during these experiments was always less than 0.1% (v/v). Sample vials were heated by supporting them with a clamp in a water bath on top of the hot plate stirrer. Sample vials in the water bath were placed on a large silicone septum to insulate the vials from the surface of the hot plate. After 10 min the needle of the SPME device pierced the septum of the vial and the fibre was exposed for 45 min to the headspace of the vial, 1 cm above the spiked water which was kept at (75°C \pm 1) and stirred by a small PTFE-coated bar. Finally the fibre was retracted into the needle, pulled out from the vial and immediately inserted into the hot injector of the GC systems for analysis. Thermal desorption of insecticides was carried out for 10 min. A fibre blank experiment was performed to ensure that there were no contaminants on the fibre.

2.4. Water samples description

Water samples for spiking procedure were collected from the River Arachthos, Lake Pamvotis and

the Ionian Sea in May 2000. Ground water was obtained from the main area of Ioannina City (Greece). All water samples were used without previous treatment or filtration. Distilled water was also used. The water samples were analyzed with SPE conventional procedure by using C₁₈ disks [5] prior to have being spiked, to ensure that they were free of interfering compounds. The samples were stored in darkness at 4°C and were analyzed within 48 h of collection. The physicochemical characteristics of the water samples are shown in Table 2.

2.5. Gas chromatographic conditions

2.5.1. GC-FTD

Chromatographic analysis was performed using a Shimadzu 14A capillary gas chromatograph equipped with FTD system at 250°C. The DB-1 column used, 30 m \times 0.32 mm I.D., contained dimethylpolysiloxane (J & W Scientific, Folsom, CA, USA). The column was programmed from 150°C (2 min) to 200°C (8 min) at 5°C/min, from 200 to 210°C (2 min) at 1°C/min and from 210 to 270°C (4 min) at 10°C/min. The injection temperature was 240°C.

Helium was used as the carrier at 25 cm/s and nitrogen was used as make-up gas at 25 ml/min. The detector gases were hydrogen and air, and their flow-rates were regulated at 150 ml/min and 3.5 ml/min, respectively. The FTD ion source was an alkali metallic salt (Rb₂SO₄) bonded to a 0.2-mm spiral of platinum wire.

2.5.2. GC-MS

A GC-MS system, QP 5000 Shimadzu equipped with a capillary column DB-5-MS, 30 \times 0.25 mm, 0.25 μm , contained 5% phenyl-methylpolysiloxane (J& W Scientific) was used at the following chromatographic conditions: injector temperature 220°C, oven temperature program 55°C (2 min) to 210°C (20 min) at 5°C/min and to 270°C at 10°C/min. Helium was used as the carrier gas at 96.5 kPa. The interface was kept at 290°C. The spectra were obtained at 70 eV. The splitless mode was used for injection with the valve opened for 30 s.

Two ions were selected from the spectrum of each compound to quantify the response under SIM mode: 137(100) and 304(26) for diazinon, 125(100) and

277(38) for fenitrothion, 278(100) and 125(76) for fenthion, 109(100) and 291(49) for ethyl parathion, 125(68) and 329(74) for methyl bromophos, 97(100) and 357(60) for ethyl bromophos and 97(100) and 231(67) for ethion. The values in parentheses are the relative abundance (%) of each peak in the spectrum. The ion traces were divided into three groups that were recorded sequentially during the injection, on the basis of the retention times of the single substances. In this way different compounds which give common fragment ions belong to a different retention time group and could be easily identified.

3. Results and discussion

3.1. Selection of SPME coating

The choice of an appropriate coating is essential for the SPME method. The sensitivity of each fibre is different depending on the molecular mass and the polarity of the analytes to be extracted. Four commercially available SPME fibres [PDMS, PA, PDMS–divinyl benzene (DVB), Carbowax (CW)–DVB] were tested for efficiency of headspace SPME extraction of organophosphorus insecticides from water samples. The PDMS and PA fibres were selected for the further experiments based on the criteria of the insecticide amount extracted onto the fibre and the reproducibility of SPME technique with the above coatings. The chance to compare these results with other reported, concerning these types of fibres is another motive. Moreover, the PDMS–DVB and CW–DVB coatings have showed higher extraction of interfering substances in natural water samples as well as a significant fouling after repeated use, which can visually be observed by accelerated darkening of the coating.

3.2. Parameters influencing the HS-SPME process

HS-SPME is an equilibrium process that involve the partitioning of analytes from aqueous phase to gas phase and eventually into the polymeric phase according to their partition coefficients K_d [19]. The optimization of parameters that influence the partition of analytes between the headspace and the solution are thus extremely important. Temperature,

appropriate time period for the extraction, memory effect, methanol content, stirring rate and ionic strength, are the main parameters that should be take into account. The pH of the sample influence occasionally the headspace SPME procedure by the percentage of a free or anionic form of organic acids or bases and thus their partition between the headspace and solution. For organophosphorus pesticides the variation of the pH over a range from 2 to 11 did not significantly affect the extraction by the fibre [20] and thus the pH of the water samples was not adjusted. The optimization of above parameters was checked with both fibres.

3.2.1. Effect of temperature

Extraction temperature should be optimized first since it plays the most important role in the extraction process by controlling the diffusion rate of analytes into the coating. The effect of temperature in the extraction yield was investigated varying the temperature between 25 and 95°C with a constant extraction time of 45 min.

The adsorption–temperature profiles obtained for the selected insecticides using PDMS 100 μm and PA 85 μm fibres, were shown in Figs. 1 and 2, respectively. Attending to the expected behavior of the pesticides, increasing the temperature improved the mobility of the insecticides through liquid and gas phase and better recoveries were obtained until 75°C. At higher temperatures the ability of the SPME fibre to adsorb the tested insecticides begins to decrease. This is because adsorption is an exothermic process and therefore, disfavored at high temperature. Thus increasing the temperature the distribution constant at equilibrium decreases [21].

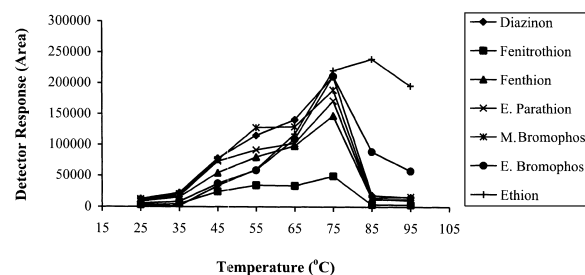


Fig. 1. Influence of temperature on detector response area, by using a PDMS 100 μm fibre for the selected insecticides at concentration level of 1 $\mu\text{g}/\text{l}$ (desorption time 10 min at 240°C).

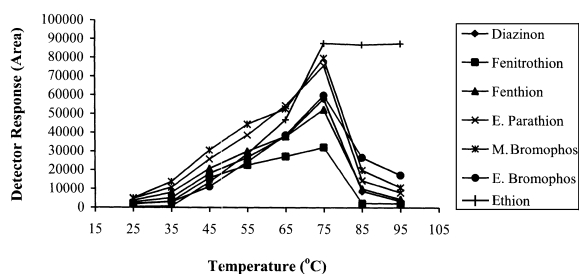


Fig. 2. Influence of temperature on detector response area, by using a PA 85 μm fibre for the selected insecticides at concentration level of 1 $\mu\text{g}/\text{l}$ (desorption time 10 min at 240°C).

Moreover, the decrease of the extraction yield could be due to the enhanced hydrolysis of organophosphorus insecticides at elevated temperature [10]. Thus, the optimum extraction efficiency was achieved at 75°C and this temperature was selected for the subsequent experiments.

3.2.2. Effect of extraction time

Since the HS-SPME technique is an equilibrium process of the analytes between the vapour phase and the fibre coating, it is important to determine the time required reaching the equilibrium. When analytes have low Henry's constant values, low concentrations at the vapour pressure are expected, thus translated on a small concentration gradient and so there is a subsequent and longer periods to reach the equilibrium needed. Also analytes with high molecular mass are expected to need longer equilibrium times, due to their lower diffusion coefficient (the equilibrium time is inversely proportional to the diffusion coefficient) [22].

Under the above studied optimum conditions, adsorption–time profiles for PDMS 100 μm and PA 85 μm fibres were generated for each insecticide and were presented in Figs. 3 and 4, respectively. Each data point is the average of three independent measurements. A unique adsorption–time curve was produced, reflecting the affinity of the insecticide for the SPME fibre coating and the FTD response. The estimated Henry constants, K_H (Table 1) were similar for most of the studied organophosphorus insecticides with the exception of ethyl bromophos. Furthermore, ethyl, methyl bromophos and ethion have relatively higher molecular mass as compared with other insecticides. Thus similar ad-

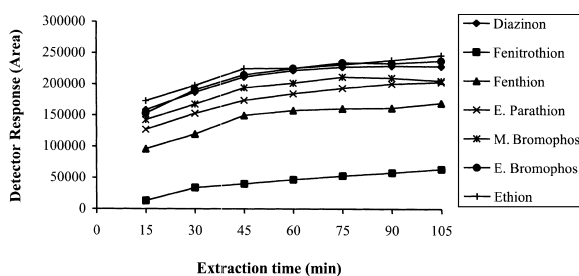


Fig. 3. Influence of adsorption time on detector response area, by using a PDMS 100 μm fibre for the selected insecticides at concentration level of 1 $\mu\text{g}/\text{l}$ (desorption time 10 min at 240°C).

sorption–time profiles were observed for all the analytes as it was expected.

The detector response for the PA fibre, is proportional to the adsorption, for the first 75 min for all the analytes, reaching a plateau for most analytes after that time which corresponds to the equilibration time (Fig. 4). For the PDMS fibre, the equilibration time of most analytes is shorter and almost reached after 45 min. The state of matter of both fibre phases is an important factor that influences the attainment of equilibrium. Since the PDMS coating is a viscous liquid polymer the diffusion of coefficient of the analyte in it will be orders of magnitude higher than its diffusion coefficient in a solid polymer. Therefore, since the dynamics of mass transport in a well-stirred solution is controlled by the diffusion coefficient of analyte in the coating, the extraction time required with a liquid polymer coating will be considerably less than that required with a solid-phase polymer [24]. Thus the longer equilibration time for PA coating can be explained. Another limitation of PA for the extraction of organophos-

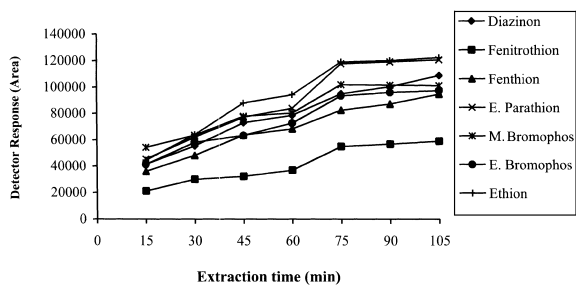


Fig. 4. Influence of adsorption time on detector response area, by using a PA 85 μm fibre for seven insecticides at concentration level of 1 $\mu\text{g}/\text{l}$ (desorption time 1 min at 240°C).

phorus insecticides is the more polar character of its coating.

It is not a required by the SPME analysis the equilibrium to be reached, as long as the extractions are carefully timed and the mixing conditions and extractions volumes remain constant [25]. Also, the use of the equilibrium time in the adsorption step is not necessary if the limits of detection (LODs) and relative standard deviation (RSD) values obtained are acceptable [7]. Since the above LOD and RSD limitations were fulfilled for the studied insecticides, an adsorption time of 45 min has been selected for the extraction for both PA and PDMS fibres.

3.2.3. Effect of stirring rate

The intensity of stirring is one important parameter that affects the time profile. For headspace, stirring should be vigorous and has to be maintained constant in all experiments. The actual stirring rate required depends on the dimensions of the vial (10 ml) and the magnetic stirring bar (2.5 mm). The optimum stirring rate was determined by analyzing samples containing 1 $\mu\text{g}/\text{l}$ of target insecticides at different stirring rates between 500 and 960 rpm. From the obtained results it can be stated that for agitation at 500 rpm a poor extraction level was achieved and the extraction efficiency increased with increasing the stirring rate. The extraction efficiency was notably enhanced by stirring for the analytes that are less volatile (lower Henry constants) such as diazinon, fenthion and ethyl parathion because the transfer of the compounds from water to headspace could conceivably be speeded up through agitation. The stirring rate at 960 rpm was considered the most adequate and was used in all subsequent experiments.

3.2.4. Effect of salt additives

In SPME procedure the salting-out effect can be employed to modify the matrix by adding salt, e.g. Na_2SO_4 and 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 fibre. Saturation with a salt can be used not only to lower the detection limits of determination, but also to normalize the influence of a random salt concentration in sample matrix. In order to investigate the

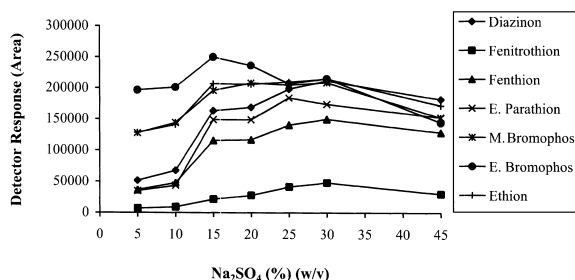


Fig. 5. Influence of sodium sulfate on detector response area, by using a PDMS 100 μm fibre for the selected insecticides at concentration level of 1 $\mu\text{g}/\text{l}$ (desorption time 10 min at 240°C).

salting-out effect, different types of salt in the extraction of analytes by the fibre were used. A salted water sample with Na_2SO_4 15% (w/v), was extracted using the PDMS 100 μm fibre, and was compared with a salted water sample with NaCl 15% (w/v). The results indicated that sodium sulfate was most effective in increasing amount of analytes extracted by the fibre. This effect of Na_2SO_4 additives was also reported in other studies with various pesticides [6,26,27].

The results on the effect of sodium sulfate concentration added to the spiked water samples as the salting out agent for the tested fibres are shown in Figs. 5 and 6. Sodium sulfate concentration added was varied between 5 and 45% (w/v) and the above optimum conditions were used.

The salting-out effect on analytes has a relationship with their solubilities in the aqueous phase [25]. The greater the solubility of analytes in water the greater the influence on adsorption will be by adding salt. Thus regarding to the PDMS fibre the com-

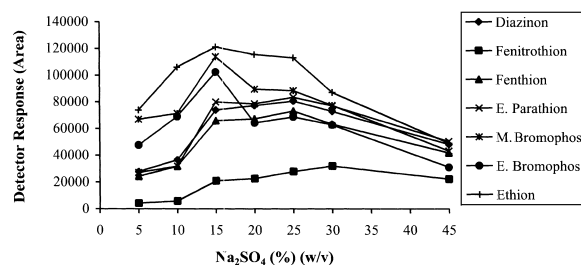


Fig. 6. Influence of sodium sulfate on detector response area, by using a PA 85 μm fibre for the selected insecticides at concentration level of 1 $\mu\text{g}/\text{l}$ (desorption time 10 min at 240°C).

pounds with higher water solubility (diazinon, methyl bromophos and fenthion) showed an increase in extraction yield with the addition of increasing Na_2SO_4 concentration until 30% (w/v). However, no effect or even a decrease in extraction yield was observed for compounds of low water solubility (ethion and ethyl bromophos) after 15% (w/v). At 45% (w/v) concentration of Na_2SO_4 that corresponds to the saturation of the solution, the extraction yield of all analytes was slightly decreased. For the PA fibre similar behavior was observed except from methyl bromophos whose the extraction efficiency decreased after 15% of Na_2SO_4 additives.

This variable effect of salt additives was also reported in other studies with various pesticides [9,25]. Thus the optimum Na_2SO_4 concentration for the extraction of the tested insecticides was considered at 15% (w/v).

3.2.5. Effect of methanol on analyte recovery

The amount of methanol in each 5 ml sample varied from 0.5 to 5 μl . This was not expected to affect the results, as previous works had shown that methanol concentrations greater than 0.5% affect the recovery [26,28]. However, this previous work was performed in a very different system and so the effect of methanol concentration on the recovery of insecticides was assessed as a possible source in the decrease caused by covering the main part of the available surface. It was found that, when in the current system, methanol volume varied above 0.5% (v/v) in the sample, a significant reduction in analyte recovery was observed. This effect was also observed by other researchers [6,7]. Therefore, a final methanol concentration level below 0.1% (v/v) was applied to all spiked and real water samples for the subsequent experiments.

3.2.6. Effect of carry over

In SPME techniques, a significant amount of the analytes often remain absorbed on the fibre after the desorption step in the GC injection system. This problem becomes more serious when low volatility compounds are analyzed [7]. This parameter was studied by exposing the fibre in the injector for lengths of time ranging from 5 to 10 min after extraction of seven studied insecticides at a 1 $\mu\text{g}/\text{l}$ concentration.

The experiments was carried out at 250°C. The results from the carryover profiles showed that most of the target analytes were efficiently desorbed off the fibre during the 10-min injector desorption for GC-FTD. Only with the PDMS coating a low carryover effect was observed for ethion. In order to eliminate this effect and avoid deviations and false signals in subsequent analysis, a blank injection (the fibre was placed in the injector without prior exposure to the sample) following the initial desorption was routinely applied between the samples as a cleaning step. With the PA coating no carryover effect was observed and all the analytes are completely desorbed from the fibre during 10 min of injector desorption for GC-FTD analysis.

3.3. Comparison of fibres

The selection of a fibre based only on the physico-chemical properties of the compounds was difficult according to the studies published to date [29]. The fibres, 85 μm PA and 100 μm PDMS, were compared by determining the recovery of the selected organophosphorus insecticides. As expected pesticides with different chemical characteristics showed different behavior when submitted to HS-SPME.

In general the PDMS fibre is preferred for the extraction of non-polar pesticides, with very low solubility in water, such as organochlorine pesticides and some of the non-polar organophosphorus insecticides, whereas the more polar polyacrylate (PA) fibre shown to be more appropriate for the more polar nitrogen-containing herbicides and for phenols [30–32]. However, the PA coating was also shown to have some affinity for non polar analytes because the structure of the PA coating consists of a hydrocarbon chain backbone with polar ester side chains [29].

From the results, it was observed that compounds with higher octanol–water partition coefficients (K_{ow}), such as ethyl bromophos and ethion ($\log K_{ow}=5.68$ and $\log K_{ow}=5.07$, respectively), were the more extensively adsorbed at equilibrium when the PDMS fibre is used due to the higher affinity to the non-polar fibre coating. Compounds with higher polarity such as fenitrothion ($\log K_{ow}<4$) were absorbed in a minor extension at the equilibrium. On the contrary, when the PA fibre is used the less polar insecticides were less effectively

extracted with a decrease at equilibrium adsorbed amount of 50–70% in relation to the PDMS fibre.

Fenitrothion which is a compound with high porality ($\log K_{ow}=3.40$) was adsorbed in a similar extension at equilibrium on both fibres. This may be explained by the fact that the adsorption of analytes on coatings is influenced not only by the affinity of analytes to the fibres but also by the film thickness of the fibre. The thicker the coating, the more analyte is absorbed by the coating, and vice versa. Thus the higher affinity of fenitrothion to PA coating is balanced by the higher equilibrium time (lower diffusion in the solid polyacrylate matrix) and the higher absorbed amount on PDMS 100 μm fibre.

3.4. Calibration curve and recoveries

Series of five levels were obtained by spiking HPLC-grade water with all the organophosphorus insecticides in a concentration range from 0.05 to 1 $\mu\text{g/l}$. Each solution was run in triplicate. In all cases, there was significant linear regression ($P<0.05$) for the analyte concentration range tested. Fig. 7a show typical chromatogram obtained after extraction of the tested organophosphorus insecticides with PDMS 100 μm fibre at 0.5 $\mu\text{g/l}$ concentration level of pesticides in water sample from the Kalamas River.

The linear regression values for all insecticides with two fibres in the range 0.05–1 $\mu\text{g/l}$ were >0.985 . Moreover, the linearity of the method has been investigated over the whole range 0.5–50 $\mu\text{g/l}$ (GC–FTD and GC–MS) and once more all insecticides had correlation coefficients greater than 0.985 with two fibres when linear regression analysis was performed. The linearity was checked also with real samples of natural waters using the same concentration levels as for HPLC-grade water. The obtained results have shown linear regression with correlation coefficients between 0.985 and 0.999 and RSD values less than 17%.

LODs were calculated by comparing the signal-to-noise ratio (S/N) of the lowest concentration to a $S/N=3$. The data of the Table 3 show that the method allows detection of the insecticides in water at concentrations lower than 40 ng/l.

The precision of the method was obtained by analyzing five replicate spiked water samples consecutively at three concentration levels (0.1, 1 and 10

$\mu\text{g/l}$). The RSD values obtained were lower than 15% for FTD and slightly more elevated values ($<17\%$) were monitored for ethion and methyl bromophos for MS.

The mean recoveries obtained for the seven selected organophosphorus insecticides spiked in four different types of water (see Table 2) are shown in Table 4. The relative recovery that is determined as the peak area ratio of real sample and ultra pure 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 non-exhaustive extraction procedure. The recovery of all analytes ranged between 80 and 120%. It should be noted that the recoveries obtained by both fibres for the most of studied organophosphorus insecticides were similar and for all samples of natural waters. This demonstrates that HS-SPME is not influenced by the undissolved particles or low volatile compounds contained in natural waters which thus act competitively for the adsorption sites of the fibre. The use of HS-SPME for the analysis of pesticides in complex matrices such as estuarine or lake waters or soil leachates where organic and particulate interferences can reduce the extraction efficiency, is a promising technique.

3.5. Comparison between HS-SPME, direct SPME and SPE disks

In order to fully validate the HS-SPME based procedure its performance was compared to that of two efficient multiresidue procedures based on solid-phase extraction and direct solid-phase microextraction (DI-SPME). The analytical data presented in other previous works for the determination of organophosphorus insecticides [7,28,33–35] were used for the comparison with the results from the present work. The procedures were applied to natural water samples and the pesticide concentration obtained as the mean value for three replicates. In comparison with other previous works [7,28,33–35], these results indicate that all procedures show good agreement for the extraction of selected insecticides in various natural waters, in terms of the concentration of analytes spiked in the samples, giving comparable recoveries. Moreover, the analytical characteristics of the HS-SPME method, linearity,

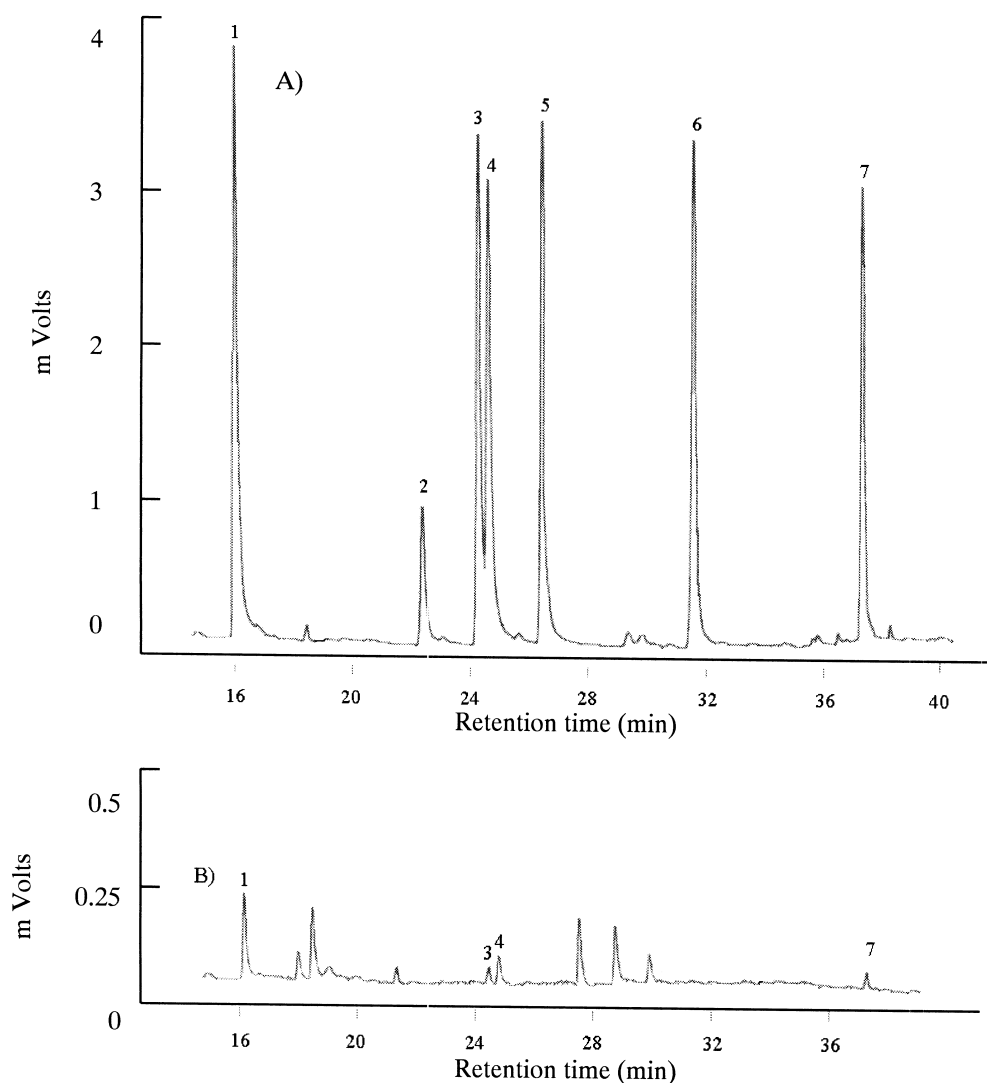


Fig. 7. GC–FTD chromatogram obtained by PDMS 100 μm fibre for (A) $\approx 0.5 \mu\text{g}/\text{l}$ of the selected insecticides in spiked river water, and (B) water sample of Kalamas River. Peak numbers: 1 = diazinon, 2 = fenitrothion, 3 = fenthion, 4 = ethyl parathion, 5 = methyl bromophos, 6 = ethyl bromophos and 7 = ethion.

precision and limits of detection were comparable with those of the SPE and DI-SPME for all analytes. Generally, SPE is a much more expensive and time-consuming method, which makes the SPME methods more favorable techniques for trace analysis work.

3.6. Reproducibility and coating lifetime

A coating lifetime is important for practical applications (changes of efficiency with number of analy-

sis). The coating is damaged mainly during the extraction due to interference between the matrix of samples and the fibre. This effect is more pronounced when the sampling performed directly from the aqueous solution (immersion SPME). In this case the presence of organic and particulate matter in the samples reduce the fibre life and the GC response after several extractions, possibly by covering the fiber surface irreversibly, resulting in a carry over effect or alteration of the fibre surface. As a final

Table 2
Characteristics of selected natural waters

Origin of water sample	pH	Conductivity ($\mu\text{mhos/cm}$)	Total suspended matter (mg/l) ^a	TOC ^b (mg/l)	Salinity (%)
Distilled water	5.89	1.1	–	b.d.l. ^c	–
Underground water	7.93	450	15	0.03	–
Arachthos River	8.72	350	115	4.16	0.36
Kalamas River	7.08	155	97	1.89	0.10
Pamvotis Lake	8.25	350	385	9.65	0.55
Ionian Sea	8.02	14 400	240	1.32	34.40

^a Total suspended matter was measured by filtration through a 0.45- μm PTFE filter (millipore).

^b TOC = total organic carbon.

^c b.d.l. = below detection limit (0.01 mg/l).

result the fibre sorptive capacity and efficiency is reduced. On the contrary, in the HS-SPME the fibre is suspending in the headspace above liquid layer of the samples and there is no interference between the matrix of samples and the coating. Thus the coating is protected and the lifetime is increased. In conventional SPME process (immersion technique) each fibre can be re-used around 30 times for surface water samples and 27 times in run-off water [29,36]. As it is observed in this study using headspace technique the fibres can be re-used almost 100 times that corresponds to the fibre lifetime with immersion SPME for distilled water.

A high temperature in the injection port of the gas chromatograph also damages the coating. Some loss of capacity and slow decrease of the adsorbed amount (~10%) of the analytes by the fibre was observed after several uses. The influence of this effect on analysis reproducibility is important and clearly suggests that any routine use of the HS-SPME approach for complex waters should include frequent calibration runs.

3.7. Environmental levels

The effectiveness of the proposed method to

Table 3
Analyzed insecticides, limits of detection and linearity data in GC-FTD, GC-MS by using SPME PDMS 100 μm and PA 85 μm fibres

Peak no./ compounds	t_R (min)	Linearity	GC-FTD		GC-MS	
			LOD ($\mu\text{g/l}$)	RSD (%)	LOD ($\mu\text{g/l}$)	RSD (%)
PDMS 100 μm						
(1) Diazinon	16.12	0.997	0.010	4	0.020	8
(2) Fenitrothion	22.64	0.990	0.025	8	0.040	10
(3) Fenthion	24.60	0.999	0.020	7	0.030	9
(4) Parathion ethyl	24.92	0.995	0.020	8	0.030	9
(5) Bromophos methyl	26.83	0.997	0.020	9	0.030	10
(6) Bromophos ethyl	31.95	0.985	0.020	9	0.030	11
(7) Ethion	37.40	0.986	0.015	13	0.025	15
PA 85 μm						
(1) Diazinon	16.12	0.992	0.020	6	0.030	8
(2) Fenitrothion	22.64	0.999	0.030	7	0.040	9
(3) Fenthion	24.60	0.997	0.025	9	0.035	10
(4) Parathion ethyl	24.92	0.999	0.025	9	0.035	10
(5) Bromophos methyl	26.83	0.991	0.025	8	0.035	10
(6) Bromophos ethyl	31.95	0.990	0.020	11	0.035	13
(7) Ethion	37.40	0.988	0.020	15	0.030	17

LOD, limit of detection; RSD, relative standard deviation.

Table 4

Mean recovery of the selected organophosphorus insecticides in natural water samples by using SPME PDMS 100 μm and PA 85 μm fibres

Peak no./ compounds	Mean recoveries (%)			
	Underground water	Arachthos River	Ionian Sea	Pamvotis Lake (Ioannina)
PDMS 100 μm				
(1) Diazinon	105.7	105.0	117.2	120.6
(2) Fenitrothion	95.4	93.0	115.2	106.1
(3) Fenthion	97.3	98.3	115.2	106.1
(4) Parathion ethyl	95.4	104.7	114.0	124.5
(5) Bromophos methyl	97.0	83.1	95.8	96.4
(6) Bromophos ethyl	83.8	87.4	96.0	85.8
(7) Ethion	96.7	117.5	118.0	109.5
PA 85 μm				
(1) Diazinon	94.0	119.2	117.6	120.2
(2) Fenitrothion	95.6	106.4	113.7	117.5
(3) Fenthion	85.2	113.1	115.2	117.7
(4) Parathion ethyl	98.6	104.9	111.3	82.6
(5) Bromophos methyl	80.5	81.5	80.7	81.8
(6) Bromophos ethyl	91.2	119.9	108.8	109.0
(7) Ethion	94.4	88.8	95.0	108.9

Spiking levels of 0.05, 0.1, 0.25, 0.5, 1 $\mu\text{g/l}$, $n=3$.

determine the selected insecticides from river water was tested by performing replicate analysis of samples from four different stations on the flow of Kalamas river from the sources in the estuaries. These sampling stations were chosen in order to have water samples with different organic matter and particulate content, which causes most of the matrix interferences.

The corresponding GC–FTD chromatogram obtained by SPME PDMS 100 μm for a Kalamas River water sample is shown in Fig. 7b. The obtained chromatogram shows the presence of several non-identified compounds in the sample as well, but no significant interferences with the studied insecticides were observed. First, full-scan GC–MS confirmation was performed on the selected samples in order to determine the presence of pollutants. Then, using a selected ion monitoring (SIM) mode prepared with some characteristic ions of the pesticide quantified carried out the quantification of the pesticides detected. The quantification of the samples was carried out according to the procedure described previously and the concentrations of the detected insecticides resulting from an average value of three measurements.

The t -test was used to compare the slopes of the

calibration curves of the insecticides based on HPLC-grade and Kalamas River water using the proposed method of PDMS fibre. The t -test is preceded by an F -test, which is required to compare the variances of those means for each analyte since unequal variances do not allow the use of the standard t -test. All variances passed the F -test at the 95% confidence level ($F < F_{\text{crit}} = 19$). The results demonstrated that the differences between the slopes of HPLC-grade and Kalamas River water as estimated with t -test were statistically insignificant at the 95% confidence level ($t < t_{\text{crit}} = 4.303$, $n=3$). These slopes prove that HS-SPME method was almost independent of the matrix of the river water sample. The results from analysis showed that fenthion, ethyl parathion and ethion had been detected in trace levels, nearly at the limit of detection only in the first and second station of the Kalamas River. Only diazinon had been detected in all stations at a concentration level between 0.08 and 0.16 $\mu\text{g/l}$.

4. Conclusions

Headspace solid-phase microextraction with polydimethylsiloxane and polyacrylate coatings is a

precise, reproducible technique for both qualitative and quantitative determination of priority organophosphorus residues in natural water (surface and underground) samples. 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.

The combination of HS-SPME with GC-MS and GC-FTD can be achieved very low limits of detection (10–40 ng/l), as the total amount of extracted analytes is used for the determination. Thus the maximum level set by the European Union for organophosphorus insecticides in drinking water can be verified without difficulty.

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