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Determination of fungicides in natural waters using solid-phase microextraction and gas chromatography coupled with electron-capture and mass spectrometric detection

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Abstract

This study develops a method for the analysis of seven fungicides in environmental waters, using solid-phase microextraction (SPME). The analyzed compounds — dicloran, chlorothalonil, vinclozolin, dichlofluanid, captan, folpet and captafol — belong to different classes of chemical compound (chloroanilines, sulphamides, phthalimides and oxazolidines) and are used mainly in agriculture and as antifouling paints. Their determination was carried out by gas chromatography with electron-capture and mass spectrometric detection. To perform SPME, four types of fibre have been assayed and compared: polyacrylate (85 μm), polydimethylsiloxane (100 and 30 μm), carbowax–divinylbenzene (CW–DVB 65 μm) and polydimethylsiloxane–divinylbenzene (65 μm). The main parameters affecting the SPME process such as pH, salt additives, methanol content, memory effect, stirring rate and adsorption–time profile were studied. The method was developed using spiked natural waters such as ground water, sea water, river water and lake water in a concentration range of 0.1–10 μg/l. Limits of detection of studied compounds were determined in the range of 1–60 ng/l, by using electron-capture and mass spectrometric detectors. The recoveries of all fungicides were in relatively high levels (70.0–124.4%) and the average R^2 values of the calibration curves were above 0.990 for all the analytes. The SPME conditions were finally optimized in order to obtain the maximum sensitivity. The potential of the proposed method was realized by applying it to the trace-level screening determination of fungicides and antifouling compounds in sea water samples originating from various Greek marinas.

Keywords: Water analysis; Environmental analysis; Solid-phase microextraction; Pesticides

1. Introduction

Fungicides are a group of chemicals which are used primarily to control spoilage of crops through fungal attack. Crops which are especially susceptible to attack include soft fruits such as grapes grown for wine production, strawberries, and vegetables. Grain crops, rice and ornamental plants are also frequently treated with fungicides to minimize possible spoilage [1]. As a result of their widespread use, fungicide residues were found to contaminate crops, wells, rivers and estuaries due to spills, spraying or run off [1,2].

In the Mediterranean region, it can be estimated that the total amount of fungicides used is approximately the same as the insecticides, but probably slightly less than that of herbicides (IAEA/FAO/

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UNEP, 1990) [3]. For example, quantities of fungicides (active ingredients) used in Greece (1987) were 3 600 000 kg respectively compare with 3 000 000 kg for herbicides [1].

Additionally, in the last few years fungicides have been widely used as alternative additives in paints in order to reduce primary colonization by algae and growth of seaweeds in boats [4]. Fungicides such as dichlofluanid, chlorothalonil, folpet, ziram, maneb and zineb were reported to be used in antifouling paints [5]. In accordance with this paper, important coastal concentrations of these compounds have been found in various marinas and ports [6,7].

Current methods of analysis for aqueous or solid samples involve liquid–liquid extraction (LLE), supercritical fluid extraction (SFE) and solid-phase extraction (SPE) [6,8]. LLE is efficient but has some disadvantages such as the large amount of solvent consumed, the formation of emulsions and extensive time-consuming cleanup procedures. Although SPE methods eliminates the above disadvantages of LLE, the presence of particulate matter in the samples can cause plugging of the cartridges or the disks, and the large sample volume required still poses certain problems to SPE applications.

Solid-phase microextraction (SPME), which allows simultaneous extraction and pre-concentration of analytes from a sample matrix, has recently become commercially available. In addition it is significantly more rapid and simple than LLE and SPE; the requirement of solvents has been eliminated and only a small volume of sample is required [9–12]. Investigation of different stationary phases, concentrating on polydimethylsiloxane and polyacrylate, has provided evidence that a variety of different groups of analytes can selectively be extracted [11,13,14].

In this study seven fungicides — dicloran, chlorothalonil, vinclozolin, dichlofluanid, captan, folpet and captafol — were selected as important fungicides and antifouling paints detected in waters from the Mediterranean region [2] and are listed by the European Union [1] for control of their residues in water. The regulations of the European Union for drinking water quality set a limit on the concentration of 0.5 μg/l for the sum of all pesticides and 0.1 μg/l for each compound, so that detection limits below 0.1 μg/l are required for monitoring drinking water. In order to achieve the above-mentioned requirements for the analysis of organic micropollutants in water, sensitive chromatographic techniques are required.

The aims of this work were: (1) to develop an efficient multi-residue method based on SPME and gas chromatography with electron-capture detection (ECD) and electron-impact ionization mass spectrometry (EI–MS) detection for the pre-concentration and chromatographic analysis of the selected fungicides belonging to the following classes classes of compound: chloroanilines, sulphamides, phthalimides and oxazolodines and (2) to apply the analytical method for the monitoring of the selected fungicides in various environmental waters (underground, river, lake and marine water). Finally, the method was evaluated for the monitoring of fungicides used in antifouling paints in sea water samples originated from various Greek marinas.

2. Experimental

2.1. Reagents and standards

The tested fungicides — dicloran (chloroaniline), vinclozolin (oxazolodine), dichlofluanid (sulphamide), chlorothalonil, captan, folpet and captafol (phthalimides) — were purchased from Promochem (Wesel, Germany). Their solubilities in water range from 0.6 to 7.0 mg/l and the corresponding octanol–water coefficients (K_{ow}) range from 3.42 to 3.71. Stock standard solutions of 10 mg/l of each compound were prepared in methanol. Working standard solutions were prepared by diluting the stock solutions with methanol. The stock and working standard solutions were 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).

Sodium chloride, potassium dihydrogenphosphate, hydrochloric acid and potassium hydroxide were purchased from Merck (Darmstadt, Germany).

2.2. SPME fibres

The SPME holder and fibre assemblies for manual sampling were provided by Supelco (Bellefonte, PA, USA) and used without modification. The fibre

coatings assayed were as follows: polyacrylate (PA, 85 μm), polydimethylsiloxane (PDMS, 100 and 30 μm), carbowax–divinylbenzene (CW–DVB, 65 μm) and polydimethylsiloxane divinylbenzene (PDMS–DVB, 65 μm). Before measurements the fibres were conditioned in the injector for 3 h at 220°C, with the split vent open, to fully remove any contaminant which might have cause very high baseline noise and large ghost peaks. Then the fibre was repeatedly injected into the GC system until interfering peaks disappeared. During this desorption process the GC column oven temperature was maintained at 240°C.

2.3. SPME analytical procedure

A 3-ml volume of water was placed in 4-ml vials, sealed with hole-caps and PTFE-lined septa. The samples were stirred before and during extraction. The fibre was then exposed to the aqueous phase for an appropriate time period of 30 min, with stirring at room temperature (25±2°C). After extraction, the fibre was directly exposed to the hot injector of the GC systems for analysis. Thermal desorption of fungicides was carried out for 5 min. After this period no significant blank values were observed. The overall methanolic concentration during these experiments was always less than 0.5% (v/v).

2.4. Water samples description

Water samples for spiking procedure were collected from the River Arachthos, Lake Pamvotis and the Ionian Sea in September 1999. Ground water was obtained from the main area of Ioannina (Greece). All water samples were used without previous treatment or filtration. Distilled water was also used. The water samples were analyzed prior to have being spiked, to ensure that they were free of interfering compounds. Their characteristics are shown in Table 1. Sea water samples from the marinas of Igoumenitsa, Preveza and Patras, (N.W. and W. Greece) were collected in November 1999 and January 2000. The samples were stored in darkness at 4°C and were analyzed within 48 h of collection.

2.5. Optimization procedure of the SPME process

Aqueous fungicide-containing solutions were extracted under varying sodium chloride and sodium sulfate (NaCl, Na_2SO_4) concentrations, pHs, stirring rates and methanol concentrations to establish optimum extraction parameters. The effect of salt addition was determined with NaCl and Na_2SO_4 standard solutions in a range of 0–100% of saturated level, for initial concentration of fungicides at 10 μg/l. Before introducing the needle of the SPME device to the injection port, the fibre was rinsed with distilled water (a few ml) in order to remove small amounts of adhering sodium chloride. Analysis of the rinsing solutions indicated that no loss of analytes, due to rinsing of the fibre, was demonstrable. The above step is important for the protection of the fibre and the GC injection port. The optimum stirring rate was also determined by analyzing samples containing 10 μg/l of target fungicides at different stirring rates.

The effect of pH was investigated for the values 2, 4, 6, 8 and 10 by using appropriate concentrations of phosphate buffer. The phosphate buffer employed for pH adjustment of the samples was prepared from a 100-ml solution of 0.1 *M* dipotassium hydrogenphosphate, adding the appropriate amounts of 0.1 *M*

Table 1
Characteristics properties of selected natural waters

Origin of water sample	pH	Conductivity (μS/cm)	Total suspended matter (mg/l)[a]	TOC[b] (mg/l)
Distilled water	6.15	2	–	b.d.l.[c]
Underground water	7.43	554	15	0.05
Arachthos River	7.65	286	127	3.10
Pamvotis Lake	7.86	283	326	10.95
Ionian Sea	7.45	14,400	240	1.32

[a] TSM (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).

KOH and/or 0.1 *M* HCl solutions. The effect of methanol content on extraction was studied on aqueous solutions containing 0.5, 1, 5, 10, 15 and 20% methanol (v/v). An extraction time of 30 min was used for all experiments except for the adsorption–time profiles, noted below. Extractions at ambient temperature of 10 μg/l of aqueous fungicide solutions, saturated with sodium chloride at pH 4.0, were performed for 0–120 min.

Quantification of samples was made using calibration curves of aqueous standards (between 0.1–10 μg/l, using HPLC-grade water) extracted in the same way as the samples and using peak area measurements.

2.6. Gas chromatographic conditions

2.6.1. GC–ECD

Chromatographic analysis was performed using a Shimadzu 14A capillary gas chromatograph equipped with a ^{63}Ni electron-capture detector at 300°C. Analytes were separated with a DB-1 column (J & W Scientific, Folsom, CA, USA), 30 m×0.32 mm I.D., containing dimethylpolysiloxane with a phase thickness of 0.25 μm. The temperature program used for the analysis was: from 80°C (2 min) to 290°C (10 min) at 21°C/min. The injection temperature was 250°C. The splitless mode was used for injection with the valve opened for 60 s. The linear purge was closed during desorption of analytes from the SPME fibre in the split/splitless injector (5-min delay time).

Helium was used as the carrier at 25 cm/s and nitrogen was used as make-up gas at 25 ml/min according to the optimization results of the instrument given by the manufacturer.

2.6.2. GC–MS

A QP 5000 Shimadzu instrument, equipped with a capillary column DB-5-MS, 30×0.25 mm, 0.25 μm, containing 5% phenyl–methylpolysiloxane (J & W Scientific) was used at the following chromatographic conditions: injector temperature 220°C, oven temperature programme 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 ion source and transfer were kept at 200°C and 240°C, respectively. 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 in the selected-ion mode (SIM) mode: 124 (100) and 206 (87) for dicloran, 266 (100) and 264 (73) for chlorothalonil, 212 (100) and 285 (99) for vinclozolin, 123 (100) and 167 (37) for dichlofluanid, 79 (100) and 264 (10) for captan, 260 (100) and 262 (70) for folpet and 79 (100) and 107 (12) for caprafol. The values in parentheses give 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. Parameters influencing the SPME process

SPME is an equilibrium process that involves the partitioning of analytes from a liquid sample into the polymeric phase according to their partition coefficients, K_d [9]. The mass extracted and the linear range are depended on the partition coefficient and the volume of the stationary phase. The optimization of parameters that influence the partition coefficient and the choice of an appropriate stationary phase are thus extremely important. Stirring rate, pH, ionic strength, solvent content and the appropriate time period for the extraction are also the main parameters that should be into account.

The optimization of parameters was checked for all fibres. The 100 μm PDMS fibre was selected for the next experiments of optimization, as the extraction level for the less extractable fungicides — captan, folpet and captafol — was higher by using this fibre.

3.1.1. Effect of salt additives

Addition of a salt (sodium chloride or sodium sulfate) often improves the recovery when conventional extraction methods are used [15]. The influence of the salt concentration on the ECD re-

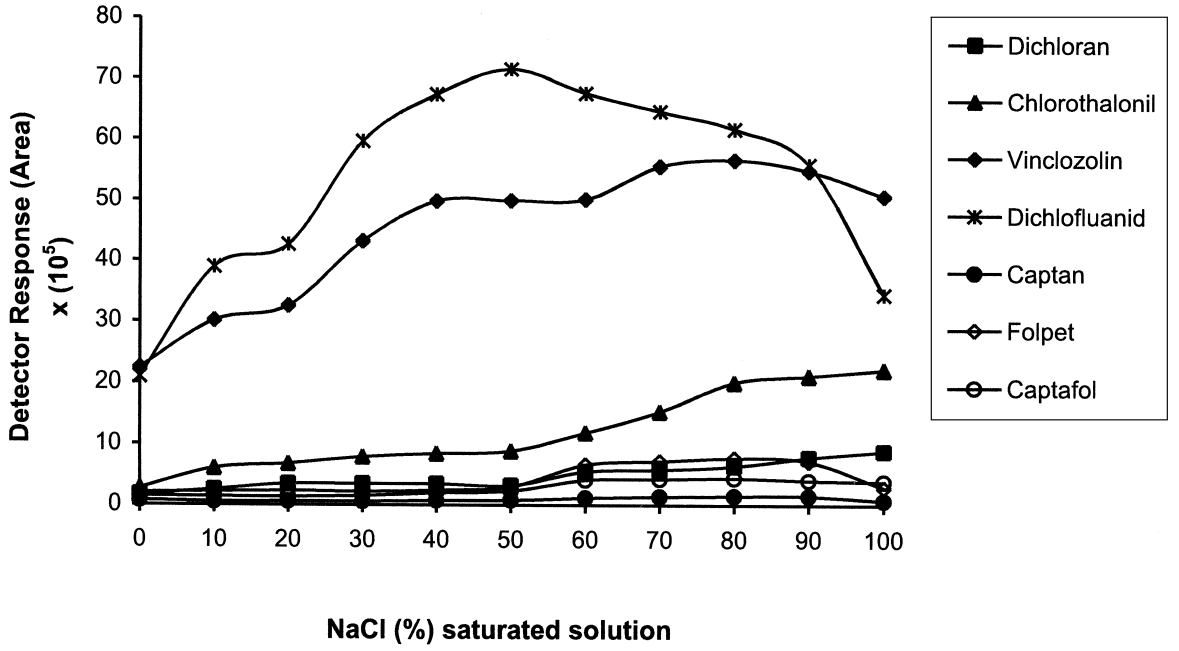

Fig. 1. Influence of sodium chloride on detector response area, by using a 100 μm PDMS fibre for seven fungicides at concentration level of 10 μg/l (desorption time 5 min at 240°C).

sponse is shown in Fig. 1, for the studied fungicides at concentration level of 10 μg/l. The salting-out effect was indeed observed as reported in previous results for SPME use for various pesticides [16–20]. It is well known that aqueous solubility of many organic compounds decreases in the presence of salt excess. On the other hand, the addition of salt may lead to a decreased extraction when the compounds' solubility does not change. This is related to the dependence of partition coefficients on the activities of rather than the concentrations of analytes in a solution. In addition, high salt content results in high ionic strength in the solution, which may cause a significant decrease of the activity coefficients of some analytes [9]. This salting-out effect was higher for vinclozolin, dichlofluanid and chlorothalonil but rather limited for captan, folpet, captafol and dicloran. The optimum extraction level for captan, folpet and captafol was observed at 80% of saturated solution salt (25%, w/v). The optimum extraction for dichlofluanid and vinclozolin was observed for 50% and 70% of saturated solution respectively. Finally, the extraction of dicloran and chlorothalonil increase as the salt content reaches 100% of saturated solution. Because the extraction level of captan, folpet and captafol was less pronounced for all fibres used, the selected extraction salt content was 80% of saturated solution, which was used in all further studies.

Similar extraction profiles were observed for Na_2SO_4 addition with the exception of dichlofluanid, in which the extraction decrease for salt content above 60% of saturated solution is more pronounced compared to NaCl addition (Fig. 2).

The addition of Na_2SO_4 was not preferred due to its hydroscopic properties which can cause problems in the determination of the concentration of fungicides [21].

3.1.2. Effect of methanol content on peak response

The recovery of fungicides studied decreases with small added amount of methanol, greater than 2% (v/v). Methanol addition decreases the absorptivity of SPME fibre by covering the main part of the available surface. Although methanol cannot be considered as a representative compound for all organics, the extraction results demonstrate that the effect of polar organics is less pronounced than

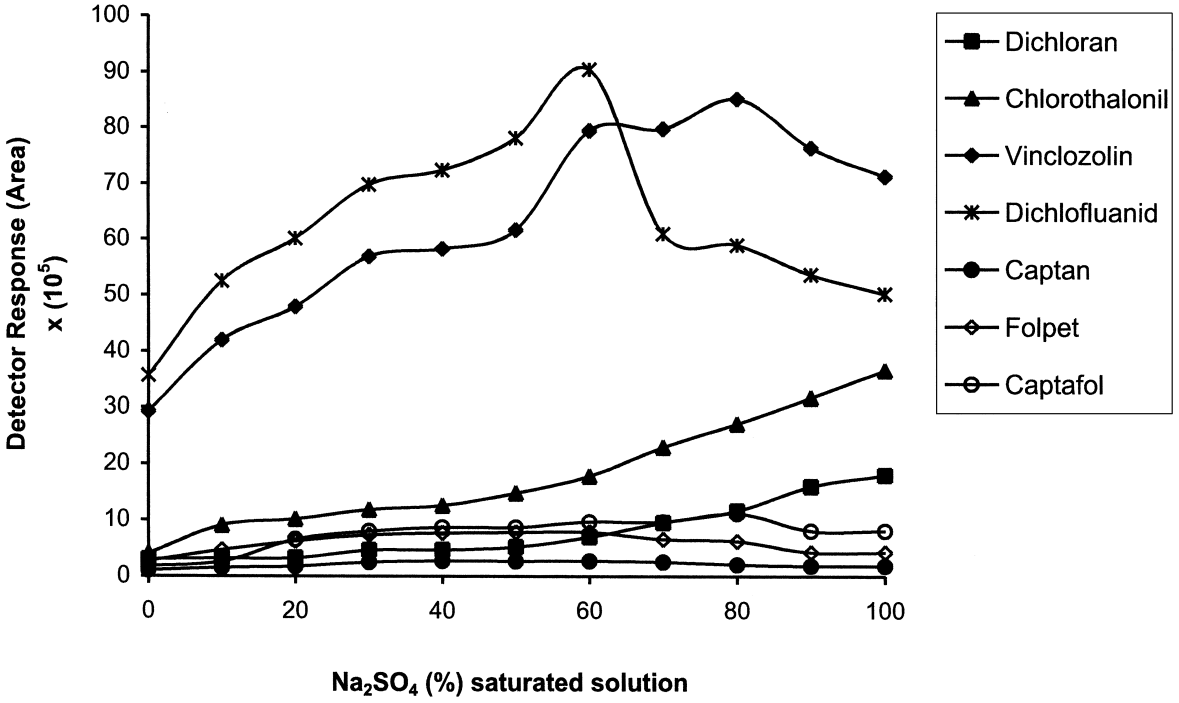

Fig. 2. Influence of sodium sulphate on detector response area, by using a 100 μm PDMS fibre for seven fungicides at concentration level of 10 μg/l (desorption time 5 min at 240°C).

expected. Similar conclusions, based on the properties of various pesticides, have been also reported by other workers [20,22].

3.1.3. Effect of carry over

In SPME techniques, a significant amount of the analytes often remain adsorbed on the fibre after the desorption step in the GC injection system. This problem becomes more serious when low-volatility compounds are analyzed [23]. In order to study this effect, a blank desorption experiment was run after extraction of seven studied fungicides at a 10 μg/l concentration. For the tested fungicides, no carryover from previous run was observed, indicating that these compounds are readily desorbed from the fibre during the 5 min of injector desorption for GC–ECD analysis.

3.1.4. Effect of stirring rate

The rate at which the extraction process reaches an equilibrium state primarily depends upon the rate of mass transfer in the aqueous phase [24,25] which is improved by stirring. The optimum stirring rate was determined by analyzing samples containing 10 μg/l of target fungicides at different stirring rates. From the obtained results it can be stated that without agitation, a very poor extraction level was achieved and the extraction efficiency increased with the stirring rate. In other studies it was reported that high stirring rates or sonication of the solution enhance or do not significantly alter SPME binding [17,20]. However, the amount of extracted analytes decreases with agitation over 1250 rpm due to stirring bar vibrations at higher speeds resulting in worse agitation in the sample. Thus, the selected optimum stirring rate was 960 rpm.

3.1.5. Influence of pH on response area

The investigation of the effect of pH on fungicide extraction by SPME fibres was undertaken in order to find a pH value at which the extraction of the fungicides was enhanced in general or was not significantly decreased for some of the tested compounds. In varying the pH value from 2 to 8, no

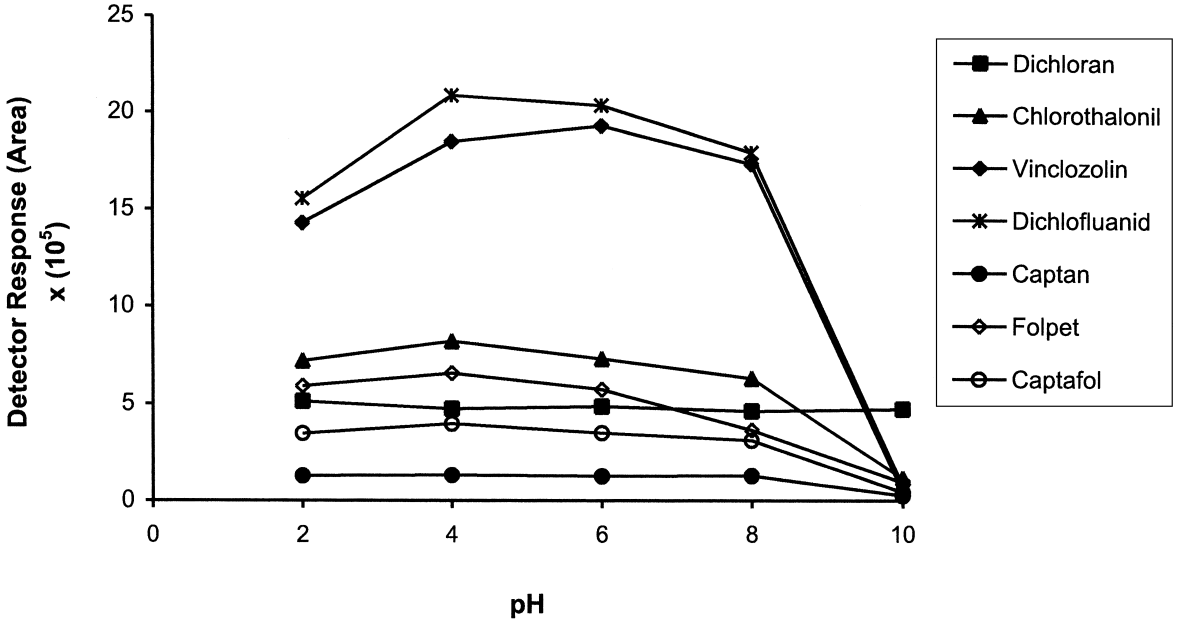

Fig. 3. Influence of pH values on detector response area, by using a 100 μm PDMS fibre for seven fungicides at concentration level of 10 μg/l (desorption time 5 min at 240°C).

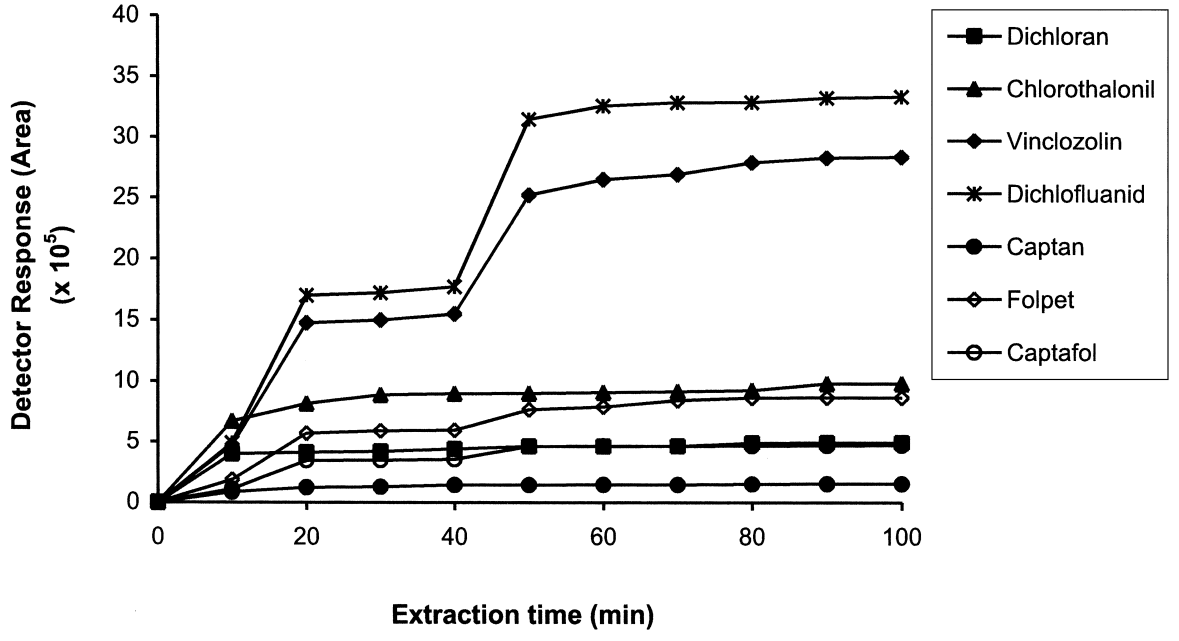

Fig. 4. Influence of adsorption–time on detector response area, by using a 100 μm PDMS fibre for seven fungicides at concentration level of 10 μg/l (desorption time 5 min at 240°C).

significant effect was observed on the extraction of dicloran, chlorothalonil, captan, folpet and captafol. On the contrary, at pH value 10, the extraction levels for all fungicides, except for dicloran, were decreased. The decrease was more significant for dichlofluanid and vinchlozolin (Fig. 3). This fact could be attributed to the higher hydrolysis rate of dicarboximide and sulphanilide fungicides in alkaline media [26–28]. The optimum value of pH was 4 for all fungicides and all subsequent SPME extractions were performed at pH 4.

3.1.6. The adsorption process: Comparison of fibres

The selection of a fibre, based only on the physicochemical properties of the compounds, was difficult according to the studies published to date [12].

Two factors are to be taken into account when the proper fibre is to be selected: the equilibration time and the amount of analyte extracted by the fibre. Four fibres, 85 μm PA, 100 and 30 μm PDMS, 65 μm PDMS–DVB and 65 μm CW–DVB were compared by determining the recovery of the selected fungicides. The PDMS–DVB is a non-polar fibre and CW–DVB is a weakly polar fibre. 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 PA fibre was shown to be more appropriate for the more polar nitrogen-containing herbicides and for

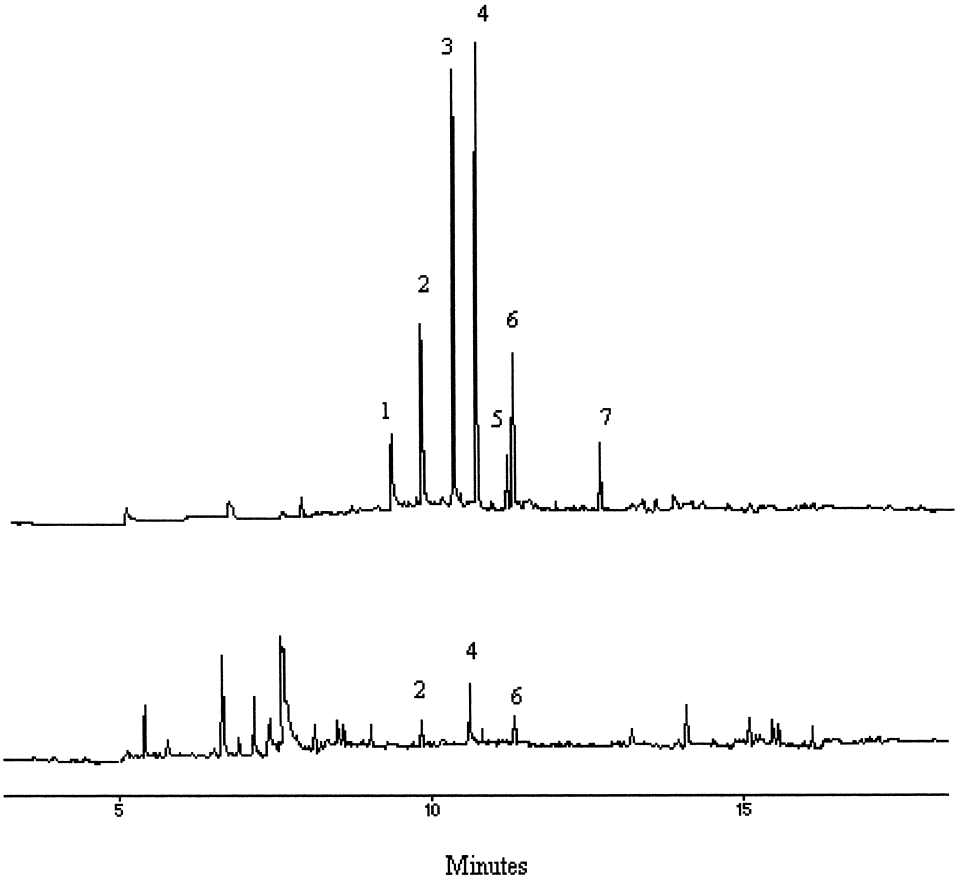

Fig. 5. GC–ECD chromatogram obtained by using a 100 μm PDMS fibre and (A) ca. 0.5 μg/l of seven selected fungicides in spiked sea water, and (B) water sample of Preveza marine. DB-1 column, 30-m long, containing dimethylpolysiloxane was programmed from 80°C (2 min) to 290°C (10 min) at 21°C. Peaks: 1=dicloran, 2=chlorothalonil, 3=vinclozolin, 4=dichlofluanid, 5=captan, 6=folpet and 7=captafol.

phenols [12,17,24,29]. 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 [12].

Mixed phase coatings such as PDMS–DVB and CW–DVB have complementary properties to PDMS and PA. In the mixed phase, the DVB porous microspheres are immobilized onto the fibre by using either carbowax or PDMS, as a glue to hold them together. In addition, the pores of the template DVB are uniform resulting in less absorption discrimination as a function of the molecular weights of the analytes.

Generally, the affinity of fungicides for the fibre coating cannot be explained by solubility or hydrophobicity of fungicides when different functionalities are considered. Only in the case of dicloran, which is the compound with the higher solubility and lower K_{ow} value, is the adsorption is more pronounced in the PA and CW–DVB fibres, which are more appropriate for polar compounds. The carboximide fungicides captan, folpet and captafol have similar solubilities and hydrophobicities shown similar affinities for the fibre coatings increasing in order PDMS>PA>PDMS–DVB>CW–DVB.

The adsorption of fungicides on PDMS coatings is influenced by the film thickness of the fibre. The extracted amount of all analytes was increased using 100 μm PDMS compared to 30 μm PDMS. The above observation is also reported elsewhere for other groups of pesticides [12]. It was also established that the film thickness has an effect on sorption kinetics. Although that the equilibrium is reached faster with 30 μm fibre, the extracted amount is higher with the 100 μm fibre. The mixed phase coatings PDMS–DVB and CW–DVB show similar extraction capacity for all the analytes.

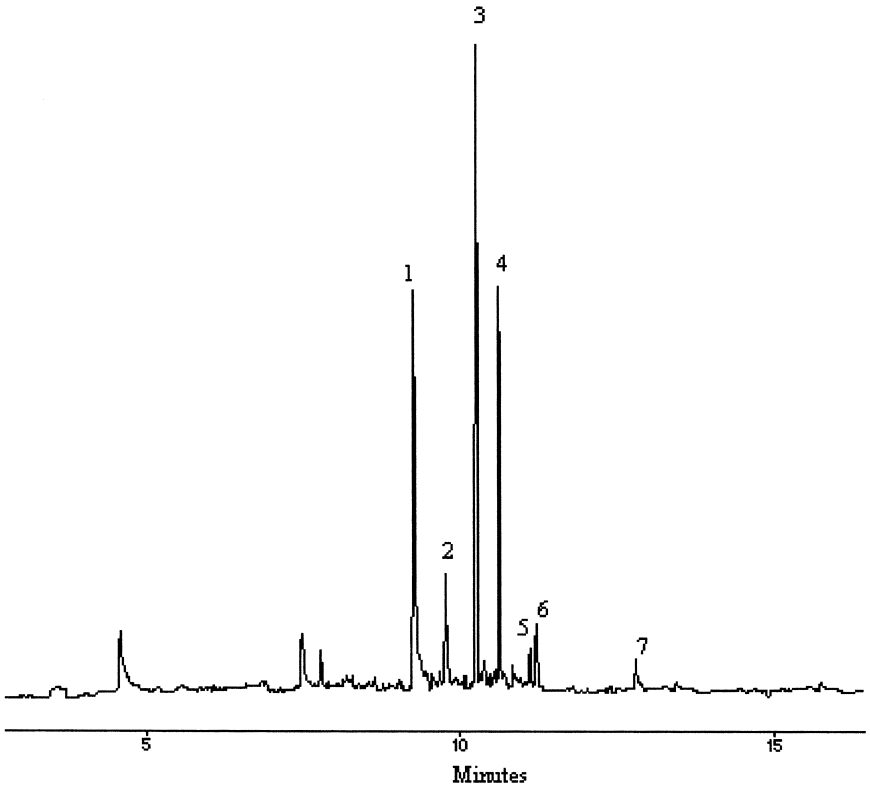

Fig. 6. GC–ECD chromatogram obtained by using a 85 μm PA fibre and (A) ca. 0.5 μg/l of seven selected fungicides in spiked sea. Peaks: 1=dicloran, 2=chlorothalonil, 3=vinclozolin, 4=dichlofluanid, 5=captan, 6=folpet and 7=captafol).

Under the above-mentioned optimum conditions, adsorption–time profiles for the 100 μm PDMS fibre were generated for each fungicide and were presented in Fig. 4. Each data point is the average of three independent measurements. A unique adsorption–time curve was produced reflecting the affinity of the fungicide for the SPME fibre coating and the ECD response to that fungicide. As can be seen from the graph, the desorption rate is high and the detector response is proportional to the adsorption, for the first 50 min, reaching a plateau after that time, which corresponds to the equilibration time (Fig. 4). Although the advantages of using the equilibration time as the adsorption period are interesting (higher extractions and smaller deviations), practical considerations — namely, the need to speed up the analysis — prohibit this. It is not a requirement for analysis that equilibrium be reached to utilize SPME, as long as the extractions are carefully timed and the mixing conditions and extractions volumes remain constant [17]. The use of the equilibrium time in the adsorption step is not necessary if the limits of detection (LODs) and relative standard deviations (RSDs) obtained are acceptable [23]. Since the above LOD and RSD requirements were fulfilled for the studied fungicides under the optimal extraction conditions previously discussed, an adsorption time of 30 min was selected for the extraction.

3.2. Calibration curve and recoveries

A series of seven levels were obtained by spiking HPLC-grade water with all the fungicides in a concentration range from 0.1 to 10 μg/l. Each solution was run in triplicate. In all cases, there was significant linear regression ($P<0.05$) for the analyte concentration range tested. Figs. 5 and 6 show typical chromatograms obtained after extraction of the tested fungicides with the 100 μm PDMS and 85 μm PA fibres at a 0.5 μg/l concentration of pesticides in water samples from the Ionian Sea. Due to the selectivity of the detector, no interferences were noticed in the GC–ECD retention time data of these compounds.

LODs were calculated by comparing the signal-to-noise ratio (S/N) of the lowest concentration to a $S/N=3$. The data of Tables 2 and 3 show that the method allows detection of the fungicides in water at

Table 2
Mean recoveries of the selected fungicides in natural water samples by using solid-phase microextraction with 100 and 30 μm PDMS fibres[a,b]

Peak No./Compound	Mean recovery (%)			
	Underground water	Arachthos River	Pamvotis Lake	Ionian Sea
PDMS 100 μm				
1. Dicloran	117.6	123.2	109.3	100.7
2. Chlorothalonil	120.5	124.4	118.6	103.1
3. Vinclozolin	110.7	113.0	114.3	109.0
4. Dichlofluanid	111.6	117.9	112.9	103.2
5. Captan	106.5	119.2	121.1	97.6
6. Folpet	123.2	110.8	106.4	98.6
7. Captafol	99.2	112.5	90.5	103.0
PDMS 30 μm				
1. Dicloran	94.4	85.2	108.9	87.0
2. Chlorothalonil	104.3	100.4	116.7	94.0
3. Vinclozolin	108.2	88.2	85.6	78.7
4. Dichlofluanid	104.6	96.0	76.2	78.7
5. Captan	94.4	90.3	112.1	84.6
6. Folpet	116.0	110.8	87.6	95.5
7. Captafol	96.8	97.6	75.4	102.3

[a] Spiking levels of 0.1, 0.25, 0.5, 1, 2.5, 5, 10 μg/l, $n=3$.
[b] Mean of three replicate experiments, average RSD values of 5–15%.

concentrations lower than 60 ng/l. The precision obtained, expressed as RSD, was lower than 10% for ECD and slightly more elevated values (<14%) were observed for captan, folpet and captafol for MS. The precision of the method could be improved by automating the whole process due to the fact that the extraction efficiency is based on an equilibrium directly affected by the time.

The mean recoveries obtained for the seven selected fungicides spiked in four different types of water (see Table 1) are shown in Tables 2 and 3. It should be noted that the recoveries obtained with the 100 μm PDMS fibre were higher than that with the other fibres for the most of studied fungicides and for all samples of natural waters. The main difference between the studied surface water are the high salinity and conductivity in the Ionian Sea water and the higher concentration of the total organic carbon in the Pamvotis Lake water samples. The recovery of all fungicides ranged between 70.0% and 124.4%. Relative recovery was employed because SPME is a non-exhaustive extraction procedure. Note that relative recovery is defined as the ratio of peak areas of real and ultra-pure water samples, spiked with analytes at the same level (instead of absolute recovery, as used in exhaustive extraction procedures).

All coatings are suitable to extract the selected fungicides from water samples but the 100 μm PDMS fibre showed lower LODs and better linearity for the most of the analytes (Tables 4 and 5).

The linearity was checked, also with real samples of natural waters using the same concentration levels

Table 3
Mean recovery of the selected fungicides in natural water samples by using SPME fibres PA (85 μm), PDMS–DVB (65 μm) and CW–DVB (65 μm)[a,b]

Peak No./Compound	Mean recovery (%)			
	Underground water	Arachtos River	Pamvotis Lake	Ionian Sea
PA 85 μm				
1. Dicloran	120.0	91.7	95.5	114.9
2. Chlorothalonil	89.3	110.7	115.2	97.6
3. Vinclozolin	100.6	81.1	92.3	89.3
4. Dichlofluanid	89.6	92.9	108.2	71.3
5. Captan	103.8	70.9	70.0	91.8
6. Folpet	114.2	72.6	74.2	77.9
7. Captafol	101.8	71.6	70.5	72.3
PDMS–DVB 65 μm				
1. Dicloran	110.8	93.8	90.5	114.7
2. Chlorothalonil	118.0	83.4	71.5	101.2
3. Vinclozolin	122.3	101.5	71.4	108.4
4. Dichlofluanid	123.3	103.8	70.6	109.7
5. Captan	99.2	101.1	101.4	114.5
6. Folpet	95.3	105.5	85.6	117.2
7. Captafol	77.0	109.5	72.0	101.4
CW–DVB 65 μm				
1. Dicloran	99.1	111.9	88.6	102.9
2. Chlorothalonil	91.3	109.8	89.9	85.2
3. Vinclozolin	100.9	99.6	75.9	102.5
4. Dichlofluanid	86.6	102.3	95.0	72.5
5. Captan	79.2	90.1	101.9	106.8
6. Folpet	84.2	81.7	85.2	96.1
7. Captafol	70.4	95.9	90.5	76.1

[a] Spiking levels of 0.1, 0.25, 0.5, 1, 2.5, 5, 10 μg/l, $n=3$.
[b] Mean of three replicate experiments, average RSD values of 5–15%.

as that for HPLC-grade water. The obtained results have shown linear regression with correlation coefficients between 0.988 and 0.999 and RSD values less than 15%.

3.3. Environmental levels

Natural water samples, collected from the marinas Igoumenitsa, Preveza, Patras (Greece), were analyzed by the proposed method and by a conventional SPE-C_{18} method [30]. The determined concentrations (μg/l) are given in Table 6. The corresponding GC–ECD chromatogram, obtained by the SPME PDMS 100 μm fibre for the Preveza marine water sample, is shown in Fig. 5.

The concentrations of fungicides detected are similar to those reported for surface waters in the Mediterranean region [1,31]. The reported differences at the determined levels between methods could be explained by considering that the SPME technique shows higher recoveries in comparison with SPE for most of the analytes in all the types of water [32]. Particularly in the case of dichlofluanid, the higher recoveries obtained by SPME (103%) compared with those obtained by SPE (40%) made the determination possible at low environmental levels. This observation is also reported elsewhere [4,32]. The obtained chromatogram shows the presence of several non-identified compounds in the sample, as well. However, such eluted compounds do not interfere with the determination of the analytes of interest. The identity of these fungicides was also confirmed in the SPE-C_{18} extract by GC–MS according to the procedure described previously.

The effect of organic and particulate matter on the SPME fibre is unknown, but they appear to reduce the fibre life and the GC response after several extractions, possibly by covering the fibre surface irreversibly, resulting in a carry over effect or alteration of the fibre surface. As a final result, the fibre sorptive capacity and efficiency is reduced. Each fibre can be re-used many times with natural waters, e.g. 15–20 times depending on the water content of organic and particulate matter. Fibres were used more than 30 times for distilled and drinking water. Without salt addition in the water samples, the fibre life is increased and could be used for about 100 times in distilled water. Fibres have

Table 4
Analysed fungicides, retention times and limits of detection (LODs) in the GC–ECD and GC–MS systems with PDMS fibres (100 and 30 μm) and optimum value of parameters

Peak No./Compound	t_R (min)	Linearity (R^2)	GC–ECD		GC–MS	
			LOD[a] (μg/l)	RSD[b] (%)	LOD[a] (μg/l)	RSD[b] (%)
PDMS 100 μm						
1. Dicloran	9.56	0.997	0.010	5	0.020	9
2. Chlorothalonil	9.85	0.997	0.005	5	0.015	8
3. Vinclozolin	10.43	0.998	0.001	3	0.010	6
4. Dichlofluanid	10.78	0.998	0.002	4	0.010	7
5. Captan	11.23	0.997	0.015	9	0.040	10
6. Folpet	11.35	0.998	0.010	9	0.020	10
7. Captafol	12.75	0.999	0.015	9	0.040	11
PDMS 30 μm						
1. Dicloran	9.56	0.997	0.030	9	0.040	10
2. Chlorothalonil	9.85	0.997	0.015	7	0.030	6
3. Vinclozolin	10.43	0.998	0.010	5	0.020	5
4. Dichlofluanid	10.78	0.997	0.010	9	0.020	9
5. Captan	11.23	0.996	0.050	10	0.060	13
6. Folpet	11.35	0.998	0.030	10	0.040	9
7. Captafol	12.75	0.998	0.050	10	0.060	14

[a] LOD=Limit of detection.
[b] RSD=Relative standard deviation (mean of three replicate experiments, $n=3$).

Table 5
Analysed fungicides, retention times and limits of detection (LODs) in the GC–ECD and GC–MS systems with fibres PA (85 μm), PDMS–DVB (65 μm) and CW–DVB (65 μm) and optimum value of parameters

Peak No./Compound	Linearity (R^2)	GC–ECD		GC–MS	
		LOD[a] (μg/l)	RSD[b] (%)	LOD[a] (μg/l)	RSD[b] (%)
PA 85 μm					
1. Dicloran	0.998	0.010	7	0.030	10
2. Chlorothalonil	0.995	0.015	6	0.010	8
3. Vinclozolin	0.999	0.001	5	0.015	5
4. Dichlofluanid	0.999	0.010	8	0.015	10
5. Captan	0.998	0.030	10	0.040	12
6. Folpet	0.999	0.020	9	0.030	9
7. Captafol	0.998	0.030	9	0.040	11
PDMS–DVB 65 μm					
1. Dicloran	0.998	0.020	10	0.040	10
2. Chlorothalonil	0.997	0.010	10	0.030	10
3. Vinclozolin	0.999	0.005	9	0.010	10
4. Dichlofluanid	0.997	0.010	9	0.020	10
5. Captan	0.995	0.040	10	0.060	13
6. Folpet	0.998	0.030	10	0.050	11
7. Captafol	0.994	0.050	10	0.060	13
CW–DVB 65 μm					
1. Dicloran	0.996	0.015	8	0.030	10
2. Chlorothalonil	0.997	0.005	5	0.010	8
3. Vinclozolin	0.998	0.001	5	0.010	8
4. Dichlofluanid	0.998	0.010	9	0.020	10
5. Captan	0.987	0.050	10	0.060	12
6. Folpet	0.987	0.030	9	0.050	11
7. Captafol	0.997	0.050	10	0.060	11

[a] LOD=Limit of detection.
[b] RSD=Relative standard deviation (mean of three replicate experiments, $n=3$).

Table 6
Concentrations (μg/l) of fungicides detected in water samples from Greek marinas using SPE (C_{18} disks) and SPME (PDMS 100 μm)

Compound (peak number)	Igoumenitsa		Preveza		Patras	
	SPE	SPME	SPE	SPME	SPE	SPME
November 1999						
Chlorothalonil (2)	0.029	0.037	0.025	0.032	–	–
Dichlofluanid (4)	0.020	0.055	0.027	0.053	0.104	0.205
Folpet (6)	0.009	0.011	–	–	–	–
January 2000						
Chlorothalonil (2)	0.027	0.034	0.055	0.070	0.017	0.022
Dichlofluanid (4)	0.006	0.012	0.056	0.109	0.012	0.023
Folpet (6)	–	–	–	–	0.012	0.014

been used more than 100 times with distilled water and 27 times in run-off water as reported elsewhere [12,25,26].

4. Conclusions

In conclusion, SPME with PDMS, PA, PDMS–DVB and CW–DVB coatings is a precise and reproducible technique for both qualitative and quantitative determination of priority fungicide residues in environmental water (surface and underground) samples. Solubilities and/or hydrophobicities are not sufficient to explain the observed affinities and to be used as criteria for the fibre selection. 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.

Moreover, the fibre can be used repeatedly (without any matrix effects) (in contrast to SPE where the disks are used only once). Finally, the small sample volume needed (2–5 ml) may be attractive for many applications where the sample volume is limited. If combined with GC–MS and GC–ECD, very low limits of detection (1–60 ng/l) can be achieved, as the total amount of extracted analytes is used for the determination. Thus, the maximum level set by the European Union for fungicides in drinking water can be verified without difficulty.

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