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Analysis of antifouling biocides Irgarol 1051 and Sea Nine 211 in environmental water samples using solid-phase microextraction and gas chromatography

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Abstract

This study develops a method for the analysis of biocides Irgarol 1051 and Sea Nine 211 in environmental water samples, using solid-phase microextraction (SPME). Their determination was carried out using gas chromatography with flame thermionic (FTD), electron-capture (ECD) and mass spectrometric detection. The main parameters affecting the SPME process such as adsorption–time profile, salt additives and memory effect were studied for five polymeric coatings commercially available for solid-phase microextraction: poly(dimethylsiloxane) (100 and 30 μm), polyacrylate, poly(dimethylsiloxane)–divinylbenzene (PDMS–DVB 65 μm) and Carbowax–divinylbenzene (65 μm). The method was developed using spiked natural waters such as tap, river, sea and lake water in a concentration range of 0.5–50 μg/l. All the tested fiber coatings have been evaluated with regard to sensitivity, linear range, precision and limits of detection. Typical RSD values (triplicate analysis) in the range of 3–10% were obtained depending on the fiber coating and the compound investigated. The recoveries of biocides were in relatively high levels 60–118% and the calibration curves were reproducible and linear (R^2>0.990) for both analytes. The SPME partition coefficients (K_f) of both compounds were also calculated experimentally in the proposed conditions for all fibers using direct sampling. Finally the influence of organic matter such as humic acids on extraction efficiency was studied, affecting mostly Sea Nine 211 uptake by the fiber. Optimum analytical SPME performance was achieved using the PDMS–DVB 65 μm fiber coating in ECD and FTD systems for Sea Nine 211 and Irgarol 1051, respectively.

Keywords: Solid-phase microextraction; Environmental analysis; Water analysis; Irgarol; Sea Nine; Antifouling compounds

1. Introduction

In the late 1980s, the use of organotin compounds as active ingredients in antifouling paints for small boats was restricted after the regulations introduced by the European Union (EU) [1], due to their severe impact on nontarget organisms, e.g. bivalves and gastropods, at very low concentrations [2]. Therefore, there has been a return to the use of copper-based antifouling paint formulations [3].

Among them Irgarol 1051 (2-methylthio-4-*tert*.-butylamin-6-cyclopropylamin-*s*-triazine) and Sea Nine 211 (4,5-dichloro-2-*n*-octyl-4-isothiazolin-3-one), that are used as additives in antifouling paints in order to inhibit the primary growth of copper-resistant fouling organisms such as algal slimes and the growth of seaweed. To date, the majority of the

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studies concerning these compounds have been mostly interested in their environmental occurrence and fate [4–10] as well as their toxicity behavior [11–14]. The presence of these compounds (especially Irgarol 1051) has been reported in the aquatic environment in several European areas at concentration levels ranged between 0.0025 and 0.64 μg/l for Irgarol 1051 [4,7,8] and 0.049–3.3 μg/l in the case of Sea Nine 211 [9,15].

Due to their occurrence at low concentration levels, a pre-concentration technique is usually required for their determination in environmental water samples. In most cases, determination of pesticide residues relies on the use of liquid–liquid extraction (LLE) [4,15,16], solid-phase extraction (SPE) [16–18] or supercritical fluid extraction [19] as described in many papers and as referenced in several US Environmental Protection Agency (EPA) methods.

These procedures usually require an extensive and time-consuming step of sample preparation, prior to final concentration because in most cases typical environmental samples cannot be directly analyzed by the usual chromatographic method applied. This step usually includes an extraction step and a clean-up procedure in order to obtain a final extract fully compatible with the chromatographic determination. In the last few years, several authors have indicated the need for a major simplification in the sample preparation accounting for a miniaturization in scale, which will also result in a reduction of time and solvent consumption [20,21].

As a result of the effort devoted in this research field of sample treatment reduction, Pawliszyn and co-workers developed the solid-phase microextraction (SPME) technique in the early 1990s which provides a simple solvent-free approach for organic pollutant determination [22]. Since its introduction, SPME has had an increasing interest in the field of pesticide residue analysis as shown in the literature. It has been applied for the determination of several groups of pesticides such as organophosphorus [23,24], organochlorine or triazine compounds [25–27]. In most cases, SPME is carried out by direct dipping of the fiber into the aqueous sample [21,28], but it can also be carried out by sampling the headspace of the sample contained in a hot vial [29]. To date few data are available in the literature concerning the determination of Irgarol 1051 using SPME [28]. To our knowledge, this represents the first survey of Sea Nine 211 using SPME in environmental water samples while a more extended survey compared to previous findings has been conducted in the case of Irgarol 1051 using five commercially available fibers.

In this paper, an in depth study on the applicability of direct sampling of SPME, for the quantitative determination of Irgarol 1051 and Sea Nine 211 in environmental water samples (tap, river, sea and lake water) has been carried out. Optimization of parameters that affect the SPME procedure has been conducted by a well-structured step-by-step approach using coating materials with different polarity and thickness. Partition coefficients (K_f) were determined by applying direct SPME sampling based on the characteristics of the fibers and of the analytes, while the relation of K_f with water solubility and biocides hydrophobicity was examined. The effect of humic acids on the extraction efficiency was also examined. Finally, the method was applied for the screening of the target analytes in the Greek marine environment.

2. Experimental

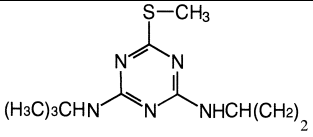
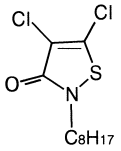
2.1. Reagents and standards

Irgarol 1051 was purchased from Ciba-Geigy (UK) and Sea Nine 211 was a kind offer by Rohm-Haas (Table 1). Stock standard solutions of 50 μg/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 stored at 4 °C. Aqueous solutions were prepared by spiking the water with an appropriate amount of the working solution. Methanol and sodium chloride were supplied by Pestiscan (Labscan, Dublin, Ireland) and Merck (Darmstadt, Germany), respectively. Humic acids were purchased from Fluka (Steinheim, Germany).

2.2. SPME fibers

SPME holder and fiber assemblies for manual sampling were provided by Supelco (Bellefonte, PA, USA) and used without modification. The fiber coatings assayed were as follows: polyacrylate (PA,

Table 1
Physicochemical properties of Irgarol 1051 and Sea Nine 211, chemical structure, molecular mass, solubility in water, octanol–water partition coefficients (K_{ow}) and sorption coefficient normalized to organic carbon content (K_{oc})

Biocide	Chemical structure	Molecular mass	Water solubility (mg/l)	Log K_{ow} [30–32]	Log K_{oc} [30]
Irgarol 1051		253.36	7.0	3.95	3.0–3.3
Sea Nine 211		282.23	6.5	2.80	–

85 μm), poly(dimethylsiloxane) (PDMS, 100 and 30 μm), Carbowax–divinylbenzene (CW–DVB, 65 μm) and poly(dimethylsiloxane)–divinylbenzene (PDMS–DVB, 65 μm). Before measurements the fibres were conditioned in the injector for 3 h at 240 °C, with the split vent open, to fully remove any contaminant which might have caused very high baseline noise and large ghost peaks. Then the fiber 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. Solid phase microextraction analysis

Five-milliliter water samples were placed in 8-ml vials, sealed with hole-caps and PTFE-lined septa. The samples were stirred before and during extraction. The fiber was exposed to the aqueous phase for an appropriate time period of 30 min, with a stirring rate of 960 rpm at room temperature (25±2 °C). After extraction, the fiber was directly exposed to the hot injector of the GC systems for analysis. Thermal desorption of biocides was carried out for 5 min. After this period no significant blank values were observed. The overall methanol concentration during these experiments was less than 0.1% (v/v) in all cases.

2.4. Water sample description

Water samples for spiking procedure were collected from Louros River, Pamvotis Lake and the Ionian Sea. Tap 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. A portion of the collected water samples was analyzed with a conventional SPE procedure using C_{18} disks [33] prior to have being spiked, to ensure that they were free of contaminating-interfering compounds (including antifouling biocides in the case of marine water). Their characteristics are shown in Table 2. Water samples from the most contaminated Greek marinas (Piraeus and Elefsina), according to our previous survey [9], were collected during the period of June–August 2001. The samples were stored in darkness at 4 °C and were analyzed within 48 h of collection.

2.5. Gas chromatographic conditions

2.5.1. GC–flame thermionic detection (FTD)

Chromatographic analysis was carried out using a Shimadzu 14A capillary gas chromatograph equipped with a FTD system at 250 °C. The DB-5 column, 30 m×0.32 mm I.D., used contained 5% phenyl–methylpolysiloxane (J&W Scientific, Fol-

Table 2
Characteristic properties of selected natural waters

Origin of water sample	pH	Conductivity (μS/cm)	Total suspended matter (mg/l)[a]	TOC (mg/l)
Distilled water	5.89	2	–	b.d.l.
Tap water	7.43	554	115	0.05
Louros River	7.67	309	119	6.01
Pamvotis Lake	8.12	321	350	12.84
Ionian Sea	7.45	52 800	240	1.32

TOC, total organic carbon; b.d.l., below detection limit (0.01 mg/l).
[a] TSM (total suspended matter) was measured by filtration through a 0.45-μm PTFE filter (Millipore).

som, 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 1.5 ml/min and make-up gas (40 ml/min). The detector gases were hydrogen and air, and their flow-rates were regulated at 120 and 4.0 ml/min, respectively. The ion source of the FTD system was an alkali metallic salt (Rb_2SO_4) bonded to a 0.2 mm spiral of platinum wire.

2.5.2. GC–electron-capture detection (ECD)

Chromatographic analysis was carried out using a Shimadzu 14B capillary gas chromatograph equipped with a ^{63}Ni ECD system working at 300 °C. Analytes were separated with a DB-1 column (J&W Scientific), 30 m×0.25 mm I.D., containing dimethylpolysiloxane with a phase thickness of 0.25 μm (splitless mode). 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 240 °C. Helium was used as the carrier at 1.5 ml/min and nitrogen was used as make-up gas at 35 ml/min according to the optimization results of the instrument given by the manufacturer.

2.5.3. GC–MS

A Shimadzu QP 5000 GC–MS system equipped with a DB-5-MS 30 m×0.25 mm×0.25 μm capillary column, containing 5% phenyl–methylpolysiloxane (J&W Scientific) was used with the following chromatographic conditions: injector temperature 240 °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 1.0 ml/min. The interface was kept at 290 °C and the spectra were obtained at 70 eV. Three ions were selected from the spectrum of each compound to quantify the response in the selected ion monitoring (SIM) mode: 253(58), 238(61) and 182 (100) for Irgarol 1051, 169(33), 182(18) and 246(11) for Sea Nine 211. The values in parentheses give the relative abundance (%) of each peak in the spectrum.

3. Results and discussion

3.1. SPME optimization

SPME is an equilibrium process that involves the partitioning of analytes from a liquid phase into the polymeric phase according to their partition coefficients, K_f [22]. Therefore, the choice of the optimum parameters that affect the SPME process was of outstanding importance in order to achieve higher extraction efficiency for both compounds. The fibers used, PDMS, PDMS–DVB, PA and CW–DVB, cover a wide range of polarities.

Methanol content was always less than 0.1% (v/v) in spiked and real water samples, since as described in previous studies, the presence of high concentrations of organic solvents in aqueous samples leads to an important decrease in extraction efficiency [34,35].

Although ECD is much more sensitive than FTD for the analysis of Sea Nine 211, the FTD system was chosen for both recovery and optimization parameter experiments as well as for the sensitivity, linear range, precision and limits of detection, in order to monitor both biocides simultaneously. However, the ECD system was also used in order to

check the above analytical characteristics in the case of Sea Nine 211 under optimum extraction conditions.

3.1.1. Extraction time profiles

The first step in the development of an SPME method is the determination of the time needed for the analyte to reach equilibrium between the sample and the fiber. Thus, a number of spiked water sample aliquots were extracted using five different coated fibers for times ranging from 15 to 180 min.

The equilibration time is reached when a further increase in the extraction time does not result in a significant increase in the detector response. Fig. 1a–e shows the extraction time profiles for PDMS 100 μm, PDMS 30 μm, PA 85 μm, PDMS–DVB 65 μm, and CW–DVB 65 μm fibers. Each data point is the average of three independent measurements.

It can be deduced from the curves that Irgarol 1051 reaches equilibration conditions after 90 min for the CW–DVB 65 μm fiber and 120 min for PA 85 μm and PDMS 100–30 μm, respectively, while in the case of PDMS–DVB 65 μm equilibrium is reached after 180 min. For Sea Nine 211, the equilibration time is shorter and almost reached after 60 and 75 min, for CW–DVB and PA fibers, respectively. For the other fiber coatings, a behaviour similar to Irgarol 1051 has been observed almost reaching equilibrium after 120 min.

Higher uptake was observed for PDMS–DVB coating for both compounds. The partition coefficients of Irgarol 1051 and Sea Nine 211 for PDMS–DVB fiber were four and two orders of magnitude higher than the other fibers, respectively. The equilibrium time with this fiber was long because of a thin, static layer of water surrounding the fiber coating. This static layer is extremely difficult to eliminate, even when the aqueous solution is stirred rapidly [36]. The large partition coefficients of the analytes between the coating and the aqueous phase mean that more analyte molecules have to pass through this static water layer, with a very low diffusion coefficient, to reach the coating. Therefore, as K_f increases, so will the equilibrium time since a greater mass must diffuse across the static layer [36]. Thus the diffusion of analyte was a more significant factor in the equilibration of PDMS–DVB rather than PDMS, PA and CW–DVB, at which the mass transferred onto the fiber was much lower.

For quantitative analysis, it is not necessary for the analytes to reach equilibrium as long as the extractions are carefully timed and the mixing condition volumes remain constant [37,38]. Therefore, a 30-min extraction time was selected due to the sufficient analytical sensitivity despite the fact that the analytes had not reached equilibrium at this time point. In addition, this sampling time was similar to the chromatography run time, thus allowing us to achieve a maximum sample throughput.

3.1.2. Enhancement with salt

Addition of salt to the sample may have several effects on the extraction. More commonly, the presence of salt in the solutions improves extraction efficiencies by altering the solvation environment of the target analytes [34–38]. Extraction is usually enhanced with increasing salt concentration and increased polarity of the compound (salting-out effect). The effect of the salt on the extraction efficiency was investigated by analyzing samples which contained different amounts of sodium chloride (NaCl) in the range from 0 to 30% (w/v). The influence of NaCl concentration, as the salting out agent, on the FTD response for the tested fibers is shown in Fig. 2a–d. As can be seen from the figure the addition of salt does not have the same effect for both biocides. For the more hydrophobic compound, Irgarol 1051 (log K_{ow}=3.97, where K_{ow} is the octanol–water partition coefficient), optimum extraction was reached at about 20% (w/v) content of NaCl in the sample for all fibers. The above observation is also in agreement with the study by Penalver et al. [28] concerning the optimum salt addition using the PA 85 μm. However, in the case of Sea Nine 211, no effect or even a decrease in extraction yield was observed after 5% (w/v) NaCl with the exception of PDMS 100 μm at which optimum extraction was observed at 10% (w/v) NaCl. This was somewhat unexpected since Sea Nine 211 is more polar (log K_{ow}=2.86) than Irgarol 1051 and an increase in extraction yield with the addition of increasing NaCl concentration would be more reasonable.

A possible explanation for this observation may be apart from the salting out effect, the change in the

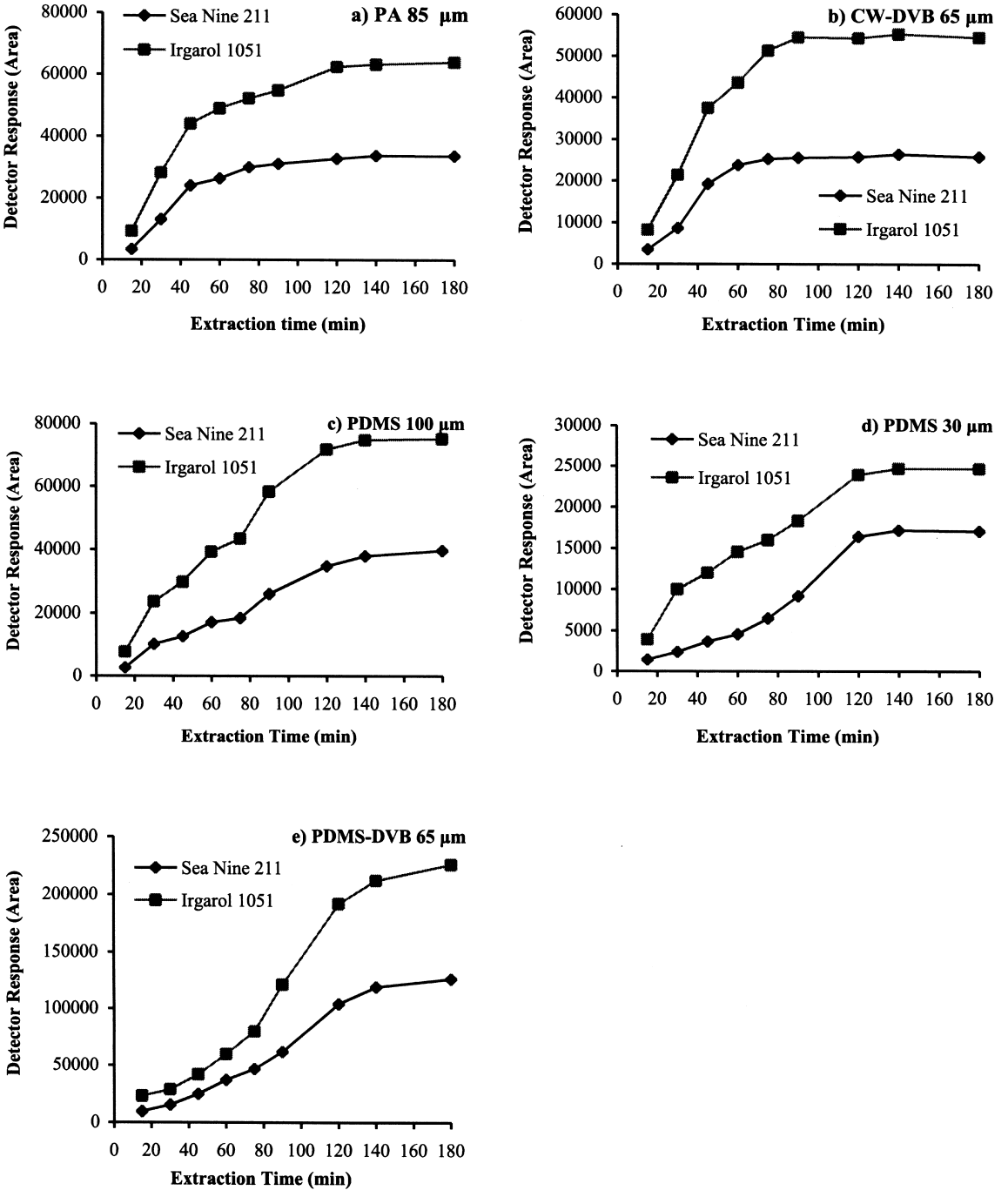


Fig. 1. Influence of extraction time on detector response area for Irgarol 1051 and Sea Nine 211 at a concentration level of 10 µg/l with (a) PA, (b) CW–DVB, (c,d) PDMS and (e) PDMS–DVB fibers.

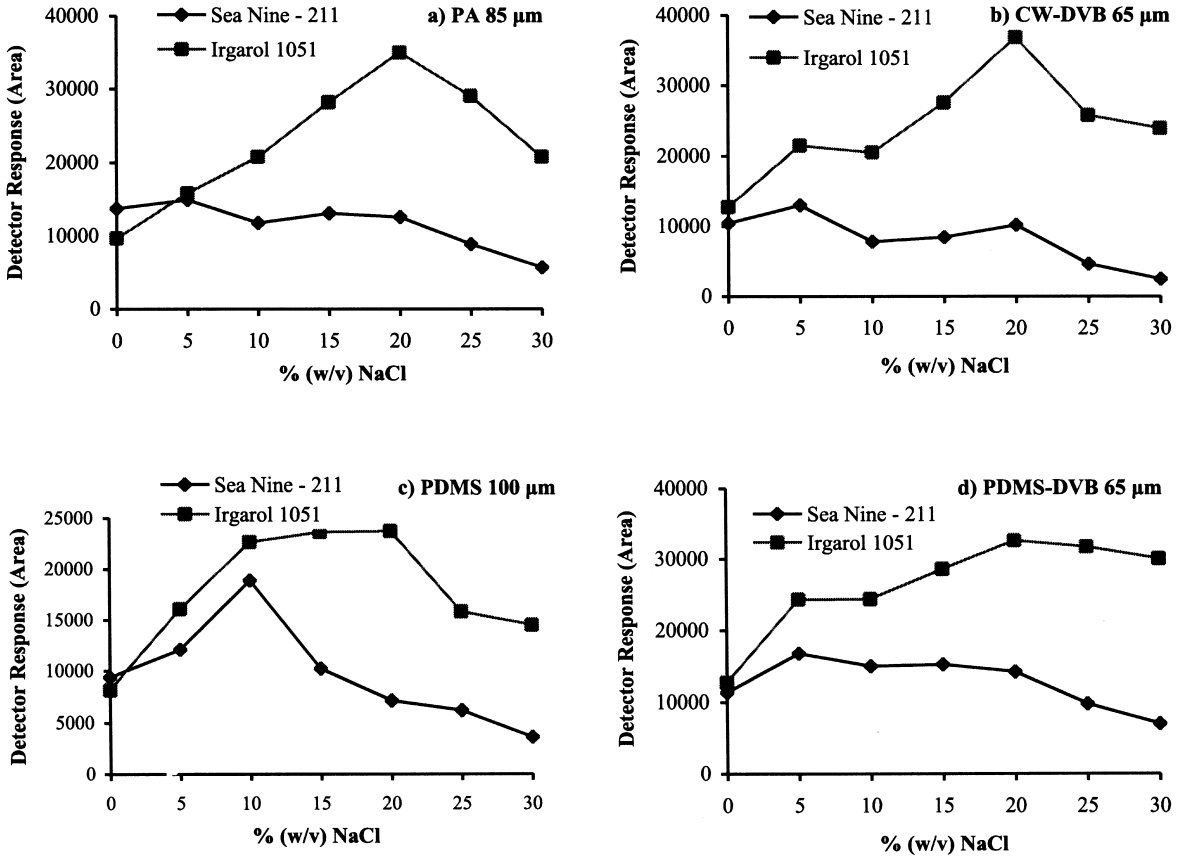


Fig. 2. Influence of sodium chloride on detector response area for Irgarol 1051 and Sea Nine 211 at a concentration level of 10 μg/l with (a) PA, (b) CW–DVB, (c) PDMS and (d) PDMS–DVB fibers.

physical properties of the static aqueous layer to the fiber due to the presence of dissolved NaCl [39–41], leading to the reduction of the diffusion rate of the target analyte. This means that when salt concentration increases, the diffusion of analytes towards the fiber becomes more and more difficult, resulting in limited extraction. These effects compensate each other and it is likely that this competition was more pronounced in the case of Sea Nine 211. Thus, the extraction efficiency of Sea Nine 211 is little enhanced by salt addition, or even decreases when moderate or high NaCl concentrations are used. A similar effect of salt has also been reported concerning other polar compounds [41,42].

The salt content of 5% (w/v) NaCl with the CW–DVB fiber was chosen for subsequent experiments taking into consideration that Sea Nine 211, which was the compound with the lower response in the FTD system, had the greater uptake in this salt concentration. Furthermore, fast degradation of the CW–DVB fiber occurred under high salt content in agreement with other studies [43] although Irgarol 1051 uptake was increased with increasing salinity (Fig. 2b). The salt content of 5% (w/v) enabled us to use a single fiber extraction of over 50 samples without significant degradation of the fiber coating.

In the case of PDMS 100 μm, the salt content of 10% (w/v) was chosen due to the optimum extraction efficiency for Sea Nine 211, although the response of Irgarol 1051 at this value was slightly lower than those at 20% (w/v) NaCl.

When PA 85 μm and PDMS–DVB 65 μm fibers were applied 5% (w/v) salt content has been proven to be the optimum value for Sea Nine 211. Under

these conditions the detector response was similar to that obtained at 20% (w/v) salt content which has been demonstrated to be the optimum in the case of Irgarol 1051. Thus, 20% (w/v) salt content was chosen for the quantitative determination of both biocides with the above fibers.

3.1.3. Carryover

Another step was to ensure that exposure time of the fiber in the GC injector was long enough to completely desorb the compounds from the stationary phase. This parameter was studied by leaving the fibers in the injector for lengths of time ranging from 2 to 5 min. The experiments were carried out at 240 °C. Carryover was determined by analyzing the fiber blank directly after the initial injection and expressing as a percentage of the initial peak area. A carryover effect was observed for both analytes with all fiber coatings after 2 min desorption time in the injector except for the PDMS 30 μm fiber at which both analytes were completely desorbed. The corresponding values for Irgarol 1051 were 1.1% for PDMS–DVB 65 μm, 0.6% for CW–DVB 65 μm, 0.7% for PA 85 μm and 0.9% for PDMS 100 μm. Sea Nine 211 demonstrated lower carryover values of 0.8, 0.3, 0.4 and 0.8%, respectively. In order to avoid this carryover effect, a desorption time of 5 min was chosen for the subsequent experiments because after this period of time both biocides are completely desorbed for all fibers. A higher background signal was observed for PA 85 μm and CW–DVB 65 μm fibers throughout the chromatogram compared to PDMS 100 μm and PDMS–DVB μm fibers, also reported by other studies [44,45].

3.2. Analytical characteristics

Considering the quantitative requirements of the procedure and the need for validation of analytical methods, some experiments were carried out to obtain the analytical characteristics of the method such as linear range, precision, reproducibility, and limits of detection.

The linearity of the method was investigated over a range between 0.5 and 50 μg/l for all detectors. A series of seven concentration levels was obtained by spiking distilled water with both biocides to generate the calibration curves. Each solution was run in triplicate in both ECD and FTD systems for Sea Nine 211 and FTD in the case of Irgarol 1051. The line of best fit for the relationship between the mean peak area and the concentration of analyte in the sample was determined by linear regression. Squared regression coefficients (R^2) were higher than 0.990 for both compounds using FTD, ECD and MSD. The linearity was also checked with real samples of natural waters using the same concentration levels as for distilled water. The results obtained using the FTD system have shown linear regression with correlation coefficients between 0.988 and 0.999 and RSD values less than 15%.

The limits of detection (LODs) were determined according to published guidelines by comparing the signal-to-noise ratio (S/N) of the lowest concentration to a $S/N=3$. The data indicated that, as expected, the coatings that exhibited higher K_f values gave lower detection limits for both analytes. Thus, the detection of Irgarol 1051 and Sea Nine 211 using the coated fibers with the lower K_f values (PDMS and PA) and GC–FTD, yielded detection limits from 10 to 60 ng/l. However, the use of PDMS–DVB and CW–DVB fiber coatings greatly improve the sensitivity and detection limits were found in the range of 5–30 ng/l for both compounds in the same system. It should be noted that the detection limits of Sea Nine 211 are approximately 10 times lower with ECD compared to FTD. This was expected since the electron-capture detector is much more sensitive than the flame thermionic detector for the analysis of Sea Nine 211. The data in Table 3 show that the method allows detection of the biocides in water at concentrations lower than 60 ng/l for all detectors with all types of fibers. Thus, the procedure developed is fully applicable for the determination of these biocides in environmental waters, meeting the restrictive requirements of EU directives for public supply water.

The precision of the method was obtained by analyzing five replicate spiked water samples consecutively at three concentration levels (1, 5 and 10 μg/l). The RSD values obtained were lower than 7% for FTD and ECD, but slightly higher values (<10%) were observed for MS. In any case, the values obtained are comparable or even lower than those reported in the literature for SPME determination of pesticides [21,23–26] or SPE [24,33,47] procedures combined with GC determination.

To our knowledge, few published SPME data are

Table 3
Analysed biocides, linearity and limits of detection (LODs) in the GC–ECD, GC–FTD and GC–MS systems with SPME fibers in distilled water

Compound	Linearity (R^2)	GC–ECD		GC–FTD		GC–MS-SIM	
		LOD (μg/l)	RSD (%; $n=3$)	LOD (μg/l)	RSD (%; $n=3$)	LOD (μg/l)	RSD (%; $n=3$)
PDMS 100 μm							
Sea Nine 211	0.997	0.005	3	0.050	5	0.030	9
Irgarol 1051	0.997			0.010	4	0.020	8
PDMS 30 μm							
Sea Nine 211	0.997	0.010	5	0.060	6	0.040	10
Irgarol 1051	0.997			0.020	5	0.030	10
PA 85 μm							
Sea Nine 211	0.997	0.005	4	0.050	7	0.030	9
Irgarol 1051	0.997			0.010	6	0.020	9
PDMS–DVB 65 μm							
Sea Nine 211	0.997	0.002	3	0.020	5	0.020	7
Irgarol 1051	0.997			0.005	5	0.020	9
CW–DVB 65 μm							
Sea Nine 211	0.997	0.005	4	0.030	5	0.030	8
Irgarol 1051	0.997			0.010	4	0.030	9

available in the literature and only in the case of Irgarol 1051 [28]. Penalver et al. [28] have applied SPME to the determination of Irgarol 1051 using only the PA 85 μm fiber coating. Although no extensive comparison could be made, as far as the analytical characteristics are concerned, linearity and LODs obtained in our study are quite comparable while the RSD values are slightly better in relation to the values reported for the target analyte [28]. In the present study, a more extensive survey has been conducted using five commercially available fiber coatings for Irgarol 1051 while this is the first attempt for the determination of Sea Nine 211 using the SPME procedure. Among the fibers studied, the PDMS–DVB 65 μm fiber has been demonstrated to be the most efficient coating for the extraction of both analytes in natural water samples exhibiting lower LODs.

3.3. Determination of biocide fiber partition coefficients

The determination of SPME partition coefficients (K_f) and the establishment of the relationship between K_f and the characteristics of analytes is an important parameter for understanding the sorption mechanism of SPME as well as for quantitatively determining the extracted amounts of analytes [22].

Two sets of experiments, according to Doong and Chang [46] were carried out for the determination of K_f values for both analytes using the five available fibers. The first set of experiments was conducted by delivering a constant initial concentration (10 μg/l) into the vials using 5, 10 and 15 ml of sample, respectively (constant concentration system). These sample volumes were selected according to the volumes usually used for SPME analysis. In the second set of experiments different concentrations of biocides were added in a constant volume water system (5 ml) [46]. The experiment was conducted by averaging three independent determinations of different concentration levels at 5, 10 and 15 μg/l. These concentrations are well within the SPME linear range for both compounds. The experimental K_f values were calculating using the following equation:

$$K_f = \frac{nV_{vial}}{C_0 V_f V_{vial} - nV_f} = \frac{C_{fiber}}{C_{sample}} \quad (1)$$

with V_{vial} and V_f, the volumes of the vial and the fiber

coating, respectively (ml); C_0, the initial analyte concentration (ppb); n, analyte mass uptake (ng); C_{fiber}, the analyte concentration in the fiber coating at equilibrium; and C_{sample}, the analyte concentration in the sample under equilibrium conditions. K_f is defined here in terms of concentrations.

Syringe injections of the analytes in methanol were made in order to determine the actual mass desorbed from the fiber onto the column. An extraction time of 180 min was selected because both biocides had reached equilibrium in this time with all fibers and the analytical sensitivity was sufficient. All experiments were performed in triplicate.

Table 4 gives the extracted amounts and the respective K_f values for both compounds using all fibers. The results obtained showed that the partition coefficients determined in this study were similar in both experimental sets being independent from the tested concentration and volume sample. The RSD values are satisfactory ranging between 3 and 16%, depending on fiber coating. These values include variability due to the preparation of the samples, GC peak integration, and differences among individual fibers as well as variability intrinsic to the SPME technique.

For Irgarol 1051, the K_f value was lower with the PDMS 100 μm coating compared to 30 μm indicating that diffusion is either lower or incomplete in the thicker coating for this compound. Although PDMS 100 μm is expected to extract a greater amount of analytes due to its thickness, the n_s and K_f values obtained were similar to those obtained for PA and CW–DVB fibers. The highest n_s and K_f values were obtained for PDMS–DVB fiber for both biocides.

However, a correlation between K_f values and the solubility or octanol–water constant, log K_{ow} values, cannot be considered for the five coatings of interest. Irgarol 1051 and Sea Nine 211 have similar solubility but different log K_{ow} values. The more polar compound, Sea Nine 211, had higher K_f values than Irgarol 1051 for all fibers. This is an expected result when polar fibers are applied but it is less expected with the non-polar fiber coatings such as PDMS and PDMS–DVB. K_f values can vary with coating nature and thickness, depending on the analyte and this fact should be taken into account and contribute to the difficulty of comparing results obtained with fibers of various types and different thickness [26].

3.4. Recoveries

Four different types of water samples were used for recovery studies, since such water samples contain different levels of dissolved and suspended natural organic material, that may affect sample extraction. Because SPME is a non-exhaustive extraction procedure the relative recovery which is determined as the peak area ratio of real sample and Ultrapure water sample spiked with analytes at the same level (instead of absolute recovery as used in exhaustive extraction procedures) was employed. As shown in Table 5, acceptable relative recoveries and RSD values were obtained for all types of water samples. The main differences between the studied surface waters are the high salinity and conductivity in Ionian sea water and the higher concentration of the total suspended solids in Pamvotis lake water samples. The relative recoveries of both biocides were lower in the lake water samples ranging between 70 and 60% for Irgarol 1051 and Sea Nine

Table 4
Extracted amount, ns, at equilibrium and partition coefficient K_f, obtained from time profile curves performed with various fibres

Biocide	PDMS 30 μm, volume coating 1.32×10^4 ml			PDMS 100 μm, volume coating 6.6×10^4 ml			PDMS–DVB 65 μm, volume coating 3.6×10^4 ml			CW–DVB 65 μm, volume coating 3.6×10^4 ml			PA 85 μm, volume coating 5.21×10^4 ml		
	n_s[a,b] (ng)	K_f[c]	RSD (%)	n_s[a,b] (ng)	K_f[c]	RSD (%)	n_s[a,b] (ng)	K_f[c]	RSD (%)	n_s[a,b] (ng)	K_f[c]	RSD (%)	n_s[a,b] (ng)	K_f[c]	RSD (%)
Irgarol 1051	1.57	2048	10	4.25	1434	8	8.1	4073	10	2.9	1476	8	3.32	1113	16
Sea Nine 211	3.35	5090	8	17.6	5960	3	31.8	16 963	10	15.7	8832	6	19.5	6774	9

[a] Distilled water spiked with 5 μg/l, stirring, extraction time, 180 min, sample volume, 5 ml.
[b] Mean of three replicate experiments, average RSD values of 2–10%.
[c] K_f was determined by the average values for spiked water with 5, 10, 15 μg/l, extraction time, 180 min and sample volume 5 ml.

Table 5
Mean relative recoveries of the selected biocides in natural water samples using solid-phase microextraction fibers

Compound	Mean recovery (%)			
	Tap water	Arachthos River	Pamvotis Lake	Ionian Sea
PDMS 100 μm				
Sea Nine 211	98	73	65	66
Irgarol 1051	110	64	67	84
PDMS 30 μm				
Sea Nine 211	96	90	60	80
Irgarol 1051	102	95	77	111
PA 85 μm				
Sea Nine 211	93	99	60	93
Irgarol 1051	97	91	74	105
PDMS–DVB 65 μm				
Sea Nine 211	99	94	65	96
Irgarol 1051	95	89	79	90
CW–DVB 65 μm				
Sea Nine 211	83	93	62	87
Irgarol 1051	118	81	74	80

Spiking levels of 0.5, 1, 2.5, 5, 10, 25 and 50 μg/l, $n=3$. Mean of three replicate experiments, average RSD values of 5–15%; GC–FTD system.

211, respectively. The extraction efficiency reduction of analytes is attributed to the higher total suspended solids present in Pamvotis lake water samples.

3.5. Effect of humic acid on extraction biocides

In general, humic acids in water inhibit the extraction of organic compounds in an aqueous solution. The effect varies according to the amount and origin of humic acids [48]. In this study, an attempt was made to trace the effect of humic acid addition in the extraction efficiency of the biocides using SPME method and flame thermionic detector. Ten micrograms per liter aliquots of mixed biocide solutions were spiked in a range from 5 to 100 mg/l humic acids. As can be seen from Fig. 3, the presence of humic acids in water sample can primarily affect the extraction efficiency of Sea Nine 211 possibly, as in the case of salting out effect, by limiting the rate of diffusion in the static layer. It is noteworthy that the concentration of 15–20 mg/l humic acid (concentration of most natural waters) in the sample can reduce the recovery of Sea Nine 211 by about 20%. In the case of Irgarol 1051 the above effect was insignificant for the tested concentration levels of humic acids and a slight decrease in its extraction efficiency was observed after 50 mg/l concentration of humic acid present in the sample perhaps due to diffusion effects of complex matrix.

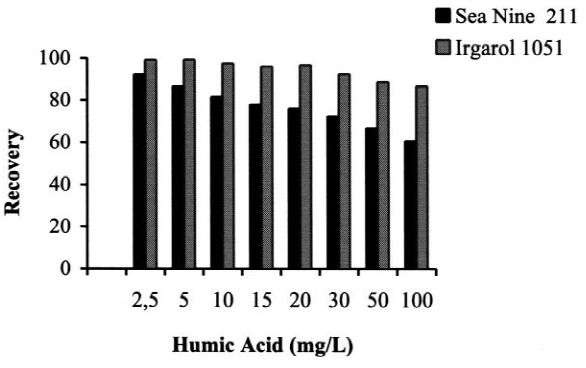


Fig. 3. Effect of humic acids on extraction efficiency of Irgarol 1051 and Sea Nine 211 at a concentration level of 10 μg/l with PDMS–DVB 65 μm fiber.

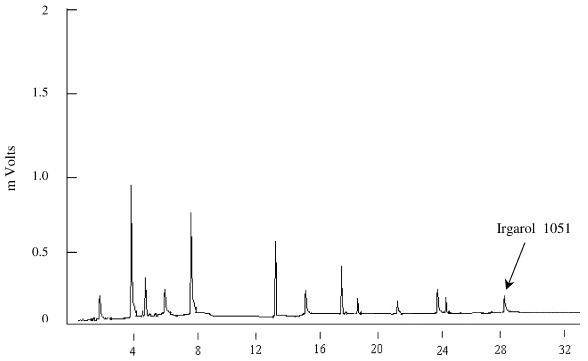


Fig. 4. GC–FTD chromatogram of Piraeus marine water sample with PDMS–DVB 65 μm fiber (August 2001).

3.6. Analysis of real water samples

In order to study the applicability of the recommended SPME procedure to water samples, the direct SPME method with PDMS–DVB 65 μm fiber was applied to several sea water samples obtained from two Greek marinas (Piraeus and Elefsina) during June–August 2001. In addition, the SPE method [9] was also applied as a reference technique. All samples were initially analyzed using GC–MS in order to confirm the identity of the compounds. The quantification of the samples was carried out according to the procedure described previously using the GC–FTD and GC–ECD systems for Irgarol 1051 and Sea Nine 211, respectively, while the concentrations of the detected biocides resulted from an average value of three measurements. Because the presence of organic solvents in the aqueous samples influences the extraction process, the same methanol content (0.1%) was added to the real samples in order to normalize the influence of methanol concentration between spiked and real water samples. The analysis performed confirmed only the presence of Irgarol 1051 in both sampling stations. The corresponding chromatogram of Piraeus sample obtained by SPME and using FTD is shown in Fig. 4. The results obtained (Table 6) were in good agreement with those obtained with the SPE method indicating that the SPME technique can be successfully applied to biocide residue determination in real water samples.

4. Conclusions

This study demonstrates that antifouling agents such as Irgarol 1051 and Sea Nine 211 can be simultaneously determined in environmental water samples following SPME and GC–FTD, –ECD and –MS. Optimization of the parameters affecting the sensitivity of SPME extraction mode should be carefully developed in order to enable a substantial increase in the amount extracted of most analytes and to improve the limits of detection. According to these results, the fiber coated with poly(dimethylsiloxane)–divinylbenzene yields higher extraction efficiency than other fibers. Detection limits of 2–60 ng/l are achieved when using SPME coupled to GC (MS, FTD, ECD) with all fibers. In addition humic substances dissolved in water over 20 mg/l can markedly reduce the extraction efficiency of Sea Nine 211 either by saturation of the sorbent or by interfering with the biocide.

Table 6
Mean detected biocide concentrations (ng/l) in real water samples using SPME and SPE analytical techniques

Biocide	Mean detected concentration (ng/l)					
	June		July		August	
	Piraeus	Elefsina	Piraeus	Elefsina	Piraeus	Elefsina
SPME						
Irgarol 1051	nd	nd	38	25	65	38
Sea Nine 211	nd	nd	nd	nd	nd	nd
SPE						
Irgarol 1051	nd	nd	41	27	70	36
Sea Nine 211	nd	nd	nd	nd	nd	nd

nd, not detected.

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