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Determination of antifouling compounds in marine sediments by solid-phase microextraction coupled to gas chromatography–mass spectrometry

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Abstract

Solid-phase microextraction (SPME) coupled to gas chromatography–mass spectrometry was applied to determine the antifouling biocides chlorothalonil, dichlofluanid, sea nine 211 and irgarol 1051 in marine sediments. Two experimental approaches were selected before the submission of the aqueous extracts to SPME prior to GC determination. The extraction of the biocides from the sediment samples was conducted using (a) water (containing 5%, v/v, acetone) and (b) acetone which was then diluted with water to give a 5% (v/v) content. The recommended procedures were found to be applicable for quantitative determination of the selected antifouling compounds in sediments with R.S.D.s below 17% and limits of detection ranging from 0.5 to 25 ng/g. The acetone/SPME procedure showed lower detection limits (0.5 to 6 ng/g) and R.S.D. values (<11%) as well as better recoveries (73 to 92%), proving that it could be successfully performed for the determination of antifouling compounds in sediment analysis, even in samples with high organic matter content. Both optimized water/SPME and acetone/SPME procedures were applied to the analysis of antifouling compounds in marine sediments and compared with the conventional liquid–liquid extraction with subsequent clean up by solid-phase extraction.

Keywords: Sediments; Environmental analysis; Solid-phase microextraction; Water–acetone extraction; Antifouling compounds

1. Introduction

The importance of the determination of antifouling compounds such as booster biocides, is now widely recognized as a result of their toxic properties and potential risk to marine biota [1–5]. Therefore, the control of their presence in the marine environment has been a topic of increasing importance [6,7].

To determine the distribution, sources, pathways and fate of these compounds in the environment, it is necessary to detect even the smallest amounts of such compounds in different compartments of an ecological system. For instance, concentrations of these antifouling compounds in marine sediments from European countries are usually in the range of a

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few nanograms or picograms per gram of sediments [8–11]. Due to their occurrence at low concentration levels, the scientific community has forced to develop analytical procedures that can be used to determine not only the presence of booster biocides in marine samples but also their concentrations with good accuracy. These methods have to be robust, precise and sensitive to be used in regulatory situations.

At present, most of the studies have been focused on the analysis of biocides in sediment samples by using liquid–liquid extraction (LLE) [10,11] and solid-phase extraction (SPE) [8,12], while more selective methodologies have also been presented [13,14]. However, the desire to reduce the time required and the quantities of organic solvents needed for the extraction of organic pollutants from solid samples, has led to the recent development of a variety of new extraction approaches including microwave assisted extraction [15,16] supercritical fluid extraction (SFE) [17], accelerated solvent extraction (ASE) [18] and subcritical water extraction [19,20]. Similar goals have led to the development of solid-phase microextraction (SPME), a truly solvent-free method. Each of these techniques dramatically reduces or eliminates the need for organic solvents in the sample extraction step and reduces the time required from several hours to <1 h.

Recent studies in solid samples have shown that pesticides can be sampled by SPME fibers and analyzed by gas chromatography (GC) [21–23]. In these studies the sampling was carried out by direct or headspace mode after addition of appropriate amount of water and organic solvent in the solid sample. To the best of our knowledge, no previous study has ever investigated the determination of booster biocide residuals in a matrix such as marine sediments, since most of the studies concerning SPME extraction of the new antifouling agents have focused on water samples [24–27].

The main objective of the present study was to demonstrate the feasibility of using SPME for the determination of four widely used antifouling compounds at the μg/kg levels in marine sediments. This was facilitated by applied both water/SPME and acetone/SPME coupled to GC–mass spectrometry (MS) analysis. The obtained results are compared to those achieved by LLE coupling to SPE as clean up step [8].

2. Experimental

2.1. Reagents and materials

Antifouling biocide standards chlorothalonil, dichlofluanid, were purchased from Riedel-de Häen (Germany) and irgarol 1051 was obtained from Giba Geigy (Germany). Sea nine 211 was a kind offer by Rohm & Haas (Philadelphia, PA, USA). Acetone, acetonitrile and methanol were supplied from Pestiscan (Labscan, Dublin, Ireland) and sodium chloride from Merck (Darmstadt, Germany). Stock solutions of 1 mg/ml for irgarol 1051, dichlofluanid, sea nine 211 and 0.5 mg/ml for chlorothalonil were prepared in methanol. They were used to prepare the corresponding solutions for the calibration graphs and to spike the sediments. The SPME holder and fiber assemblies for manual sampling were provided by Supelco (Bellefonte, PA, USA). The 100 μm (poly)-dimethylsiloxane (PDMS) fibers, used in the present study were conditioned with the supplier's recommended procedures before analysis.

2.2. Samples

Sediment samples used for the development of the method were collected from N.W. Greece (Preveza). The samples were first sieved through a screen (pore size 2 mm I.D.) to remove rocks, coarse particles and other large debris and finally were air dried to constant mass at room temperature. A portion of the sediments were analyzed—prior to have being spiked—by LLE as described by Martinez and Barceló [8], to ensure that they were free of interesting compounds. The pH value and organic content of the sediments were 7.8 and 4.5%, respectively. Sediment samples were prepared by spiking appropriate amounts of the diluted working standards solutions to get final concentrations of 25–1000 ng/g sediment. The sediments were first homogenized by hand mixing for ~2 min and afterwards in a mechanical shaker while they were left for at least 3 h at room temperature to fully evaporate the solvent.

2.3. Sediment analysis

2.3.1. Water extraction and SPME analysis

The general extraction procedure consisted of two separate steps: (a) liquid extraction of biocides from

the sediment and (b) direct SPME sorption over 10 ml of the aqueous sediment extract. Liquid extraction of the biocides from the sediment samples (5 g) was carried out firstly by the addition of 30 ml water (containing 5%, v/v, acetone) acidified with 12 *M* HCl to pH 4, to avoid hydrolysis, coupled to ultrasonic sonication for 30 min. Sediment suspensions were separated by centrifugation at 4000 rpm for 5 min, and the liquid phase was removed by using a Pasteur pipette. Then a 10-ml aliquot was subjected to SPME using the procedure described for water samples [24].

2.3.2. Acetone extraction and SPME analysis

The second extraction approach was consisted by the addition of 5 ml of acetone (1:1 ratio solvent/sediment) to sediment samples. After 30 min of ultrasonic sonication the extracts were centrifuged at 4000 rpm for 5 min and the supernatant liquid was concentrated to 0.5 ml by a gentle stream of N_2. The final concentrate was then diluted with distilled water (acidified to pH 4.0) in order to give a 5% (v/v) acetone content, and subjected to SPME.

2.4. Gas chromatographic conditions

GC was carried out with a QP 5000 Shimadzu GC–MS gas chromatograph. A DB-5-MS (5% phenyl–methylpolysiloxane) (30 m×0.25 I.D.) (J&W Scientific, Folsom, CA, USA) fused-silica capillary column with 0.25 μm film thickness was used with helium as the carrier gas at a flow-rate of 1.0 ml/min. The GC oven was operated with the following temperature program: initial temperature 150 °C held for 2 min, ramped at 5 °C/min to 200 °C, held for 5 min, followed by another ramp of 1 °C/min to 210°C, held 2 min, and finally ramped to 270 °C at 10 °C/min. The interface was kept at 290 °C and the spectra were obtained at 70 eV. Three ions were selected from the spectrum for each compound to quantify the response under selected ion monitoring (SIM) mode: 264, 266 and 268 for chlorothalonil, 123, 167 and 224 for dichlofluanid, 182, 238 and 253 for irgarol 1051, 169, 182 and 246 for sea nine 211.

2.5. Quantitation

The SPME determinations of the concentrations of the target analytes on the sediments were performed by using conventional SPME analysis of the extractant water, i.e., with quantitative calibrations being performed on the basis of external standard solutions made in pure water [19].

3. Results and discussion

3.1. Sediment extraction

3.1.1. Water extraction and SPME partitioning

The first experimental approach selected was the extraction of booster biocides from the sediments using water and then the submission of this aqueous extract to SPME prior to GC determination. The PDMS 100 μm fiber was chosen for all determinations taking into consideration the acceptable extraction efficiency of all selected analytes [24] as well as its higher resistance in complex matrices such as sediment sample extracts.

The first step for the development of the procedure was the extraction of spiked sediment samples (5 g) by the addition of different amounts of water. The SPME analysis in sediment water extract was performed following the procedure previously described [24].

The results obtained (Fig. 1) demonstrated that an increase in the responses for all the analytes were observed with the addition of 10–30 ml of water. However, a decrease was observed for volumes higher than 30 ml. Since the analytes were analyzed by SPME, it was evident that the addition of higher amounts of water would dilute the concentration of the analytes decreasing the sensitivity of the method.

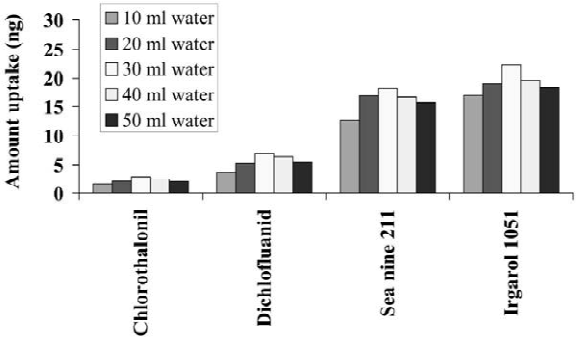

Fig. 1. Effect of water amount on the extraction of sediment samples (5 g sample, 10, 20, 30, 40 and 50 ml water). Extraction time 30 min, 10% (w/v) NaCl, stirring rate 960 rpm.

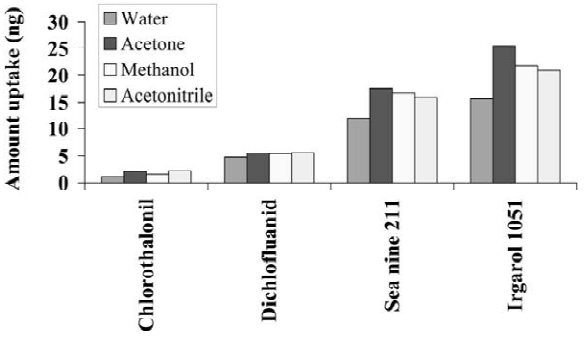

Fig. 2. Influence of the organic solvents on the extraction efficiency of analytes [5 g sample, 30 ml water containing 1% (v/v) of each solvent]. Extraction time 30 min, NaCl 10% (w/v) stirring rate 960 rpm.

Thus, 30 ml was chosen as the optimum amount added for quantitative analysis.

The next consideration was directed toward the extraction of biocides from the sediment samples with water containing small amounts of organic solvents. In this study solvents of different polarity, such as methanol, acetone and acetonitrile, in which analytes are soluble, were mixed with water prior to liquid extraction in order to enhance the release of analytes from the solid sample. The extraction efficiency after the addition of each solvent (in order to give a total of 1%, v/v, into 30 ml of aqueous extracts) is depicted in Fig. 2. The results obtained demonstrated that the liberation of analyte molecules from sediment samples was more pronounced when acetone was added showing higher sensitivity for all analytes. Thus, this solvent was chosen for the next experiments.

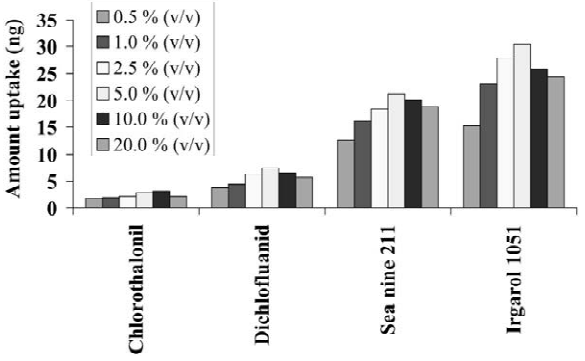

Fig. 3. Effect of acetone amount [0.5, 1, 2.5, 5, 10 and 20% (v/v)] on the extraction of analytes from spiked sediment samples. Extraction time 30 min, 10% (w/v) NaCl, stirring rate 960 rpm.

Once the choice of solvent for the extraction of analytes was established, the next step was to study the maximum operative concentration of acetone that should be present in aqueous sediment extracts (Fig. 3). The addition of small amounts of acetone progressively improved the extraction performance of the biocides from the sediment samples up to a 5% (v/v) acetone content. Above this value a decrease in the response was observed showing that acetone present is amounts higher than 5% (v/v) affects the sorption of analytes on the fiber, thus limiting their extraction efficiency. In addition, it is not feasible (nor recommendable) to directly dip the fiber into samples with high content of organic solvents due to the fast degradation and declining stability of the fiber. Taking into consideration the above aspects a compromise between stability/efficiency of the fiber and sensitivity of the overall procedure was reached.

The optimized water/SPME procedure was applied to the determination of antifouling biocides in spiked marine sediment samples at a concentration level of 300 ng/g (Table 1). The obtained results indicated that the described procedure could be considered useful as a rapid screening method for selected biocides in marine sediment samples giving acceptable detection limits (below 25 ng/g).

3.1.2. Acetone extraction and SPME partitioning

The moderate recoveries obtained with the above described procedure described especially in the case of chlorothalonil (always below 37%) force us to study an alternative liquid extraction from sediment samples using acetone as the solvent for the first extraction step of antifouling biocides. In this case, 5 g of spiked sediment sample was extracted according the procedure described in Section 2.3.2.

The obtained results were very favorable giving lower limits of detection (LODs) and RSD values as well as higher recoveries compared to water/SPME method (Tables 1 and 2).

3.2. Linear range, reproducibility and limits of detection

Both water/SPME and acetone/SPME methods shows a linear dynamic range (correlation coefficients between 0.988 and 0.998) between 50 and

Table 1
Recoveries of antifouling biocides in spiked sediment samples (300 ng/g) using water extraction/SPME, acetone extraction/SPME and acetone extraction/SPE

Biocide	Mean recoveries, (R.S.D., %)		
	Water extraction/SPME	Acetone extraction/SPME	Acetone extraction/SPE
Chlorothalonil	36 (18)	75 (12)	76 (9)
Dichlofluanid	56 (12)	82 (9)	80 (11)
Sea nine 211	58 (11)	87 (11)	85 (9)
Irgarol 1051	67 (8)	90 (4)	93 (5)

1000 ng/g and between 25 and 1000 ng/g, respectively. At concentrations higher than 1000 ng/g, the SPME fiber tends to be saturated, resulting in a lower absorbed amount of analytes.

LODs were defined as the concentration of the analytes in the sample that produces a peak with a signal-to-noise ratio (S/N) of 3 (Table 2). The LODs for all antifouling compounds ranged between 0.5 and 25 ng/g with both methods. Compared to water/SPME method, acetone/SPME extraction of all analytes showed higher sensitivity, with LODs ranging between 0.5 and 6 ng/g. These values are in the same order of magnitude as those obtained via LLE coupling to SPE as clean up step [8] and classical LLE [9]. The latter method, however, requires larger sample amounts and volume of organic solvents for the extraction, while the additional clean-up step before the introduction of the final extracts in the chromatograph can not be overlooked.

Repeatability was examined by five replicates of a sediment sample with the lowest concentration (25 and 50 ng/g for water/SPME and acetone/SPME procedures, respectively) used within the standard series. Generally, RSDs lower than 17% (Table 2), for all analytes were obtained. Acetone/SPME showed lower RSD values than the water/SPME procedure, which are in the same order of magnitude as that of classical LLE [9].

With analytical characteristics such as linearity, repeatability and sensitivity comparable to those of LLE, acetone/SPME could be characterized as a powerful tool for the determination of antifouling biocides in marine sediments.

3.3. Influence of organic matter on the acetone extraction/SPME procedure

The possibility to extent the SPME method also to

Table 2
Analytical characteristics of proposed water/SPME and acetone/SPME methods under GC/MS-SIM mode

Biocide	Water extraction/SPME					Acetone extraction/SPME				
	Linear range, R^2	Recovery (%)[a]		LOD (ng/g)	RSD (%)	Linear range, R^2	Recovery (%)[a]		LOD (ng/g)	RSD (%)
		200 ng/g	400 ng/g				200 ng/g	400 ng/g		
Chlorothalonil	0.985	37	34	25[c]	17	0.990	74	73	6.0[d]	11
Dichlofluanid	0.990	54	56	11[c]	12	0.993	84	82	1.0[e]	7
Sea nine 211	0.994	58	59	13[c]	10	0.995	88	90	1.5[e]	6
Irgarol 1051	0.993	66	69	8[c]	7	0.997	91	92	0.5[f]	4

[a] Linear curves were constructed using five samples between 50 and 1000 ng/g (50, 100, 250, 500 and 1000 ng/g) and between 25 and 1000 (25, 100, 250, 500, 1000 ng/g) for water/SPME and acetone/SPME methods, respectively.

[b] Since the SPME is an equilibrium rather than an exhaustive extraction method, ''% recovery'' refers to the antifouling compound concentrations determined rather than the actual percent of antifouling compound extracted by the SPME analysis.

[c] Calculated from the chromatograph of the sample spiked at the 50 ng/g concentration level.

[d] Calculated from the chromatograph of the sample spiked at the 25 ng/g concentration level.

[e] Calculated from the chromatograph of the sample spiked at the 10 ng/g concentration level.

[f] Calculated from the chromatograph of the sample spiked at the 5 ng/g concentration level.

marine sediments with increasing organic matter content was also investigated. For this reason sediment samples were collected from the areas of Thessaloniki and Piraeus. Their pH value and organic matter content were 7.8 and 9.2% for Thessaloniki and 7.6 and 13.9% for Piraeus, respectively.

The mean recoveries of antifouling biocides in spiked sediments with increasing organic matter content subjected to acetone/SPME procedure are shown in Fig. 4. The obtained results showed that the recoveries decrease as the organic content of the samples increases, due to the higher sorption of analytes to the organic matter. However, the values are still acceptable at the higher content of organic matter, even if larger RSD values (<25%) were observed.

3.4. Comparison between water extraction/SPME procedure, acetone extraction/SPME procedure and acetone extraction coupled to solid-phase extraction as clean up step (acetone/SPE)

The performance of both water extraction/SPME and acetone extraction/SPME approach was compared to that of conventional LLE with subsequent SPE clean up step [8] by determining antifouling compounds in a spiked sediment samples from Preveza (Table 1). Quantitation of the antifouling compounds was based on triplicate analysis of sediment samples at the 300 μg/g concentration level. The extraction results demonstrated that acetone extraction/SPME efficiency is comparable to that of the acetone extraction/SPE method, whereas the water extraction/SPME method showed lower sensitivity and higher RSD values. In general acetone/SPME and acetone/SPE procedures yielded reasonably similar concentrations for all antifouling compounds. In addition both methods showed similar RSD values (Table 1).

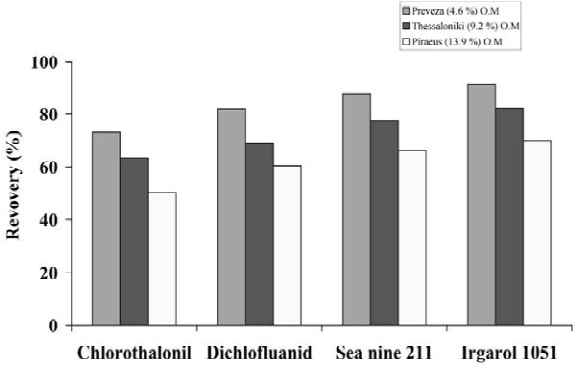

Fig. 4. Recoveries of antifouling biocides using acetone/SPME method on spiked sediment samples (500 ng/g) with increasing organic matter content.

Although, water/SPME method demonstrated lower but acceptable recoveries, precisions and LODs values, considering its simplicity and inexpensive equipment it could be a viable alternative method for rapid screening of antifouling compounds in marine sediments.

3.5. Levels of antifouling biocide in marine sediments

The acetone/SPME method was used for the determination of the antifouling biocides in sediments collected from Igoumenitsa marina of N.W. Greece. Sampling was performed during the high boating activity season (June–August 2002). The analysis performed by GC–MS confirmed only the presence of Irgarol 1051 at an average concentration of 43 ng/g. The results obtained were comparable with levels reported in 2000 in sediments of Igoumenitsa marina where 20–74 ng/g of Irgarol 1051 was detected [9]. GC–MS-SIM chromatograms obtained by acetone/SPME procedure in (A) spiked (500 ng/g) Preveza marine sediment and (B) Igoumenitsa marine sediment (July 2002) are shown in Fig. 5.

4. Conclusions

The main objective of the present work was to investigate the efficiency of the SPME procedure for antifouling biocide analysis in marine sediment samples. In order to achieve this goal water and/or acetone were used as solvents for extraction of analytes from sediment samples and the extractant was subjected to SPME analysis after optimization.

It was proven that the selected compounds could be efficiently determined under the optimum experimental conditions, while a case specific application can further enhance the analytical utility of the proposed approaches.

The acetone extraction/SPME method was by far the highest method in terms of recovery, precision

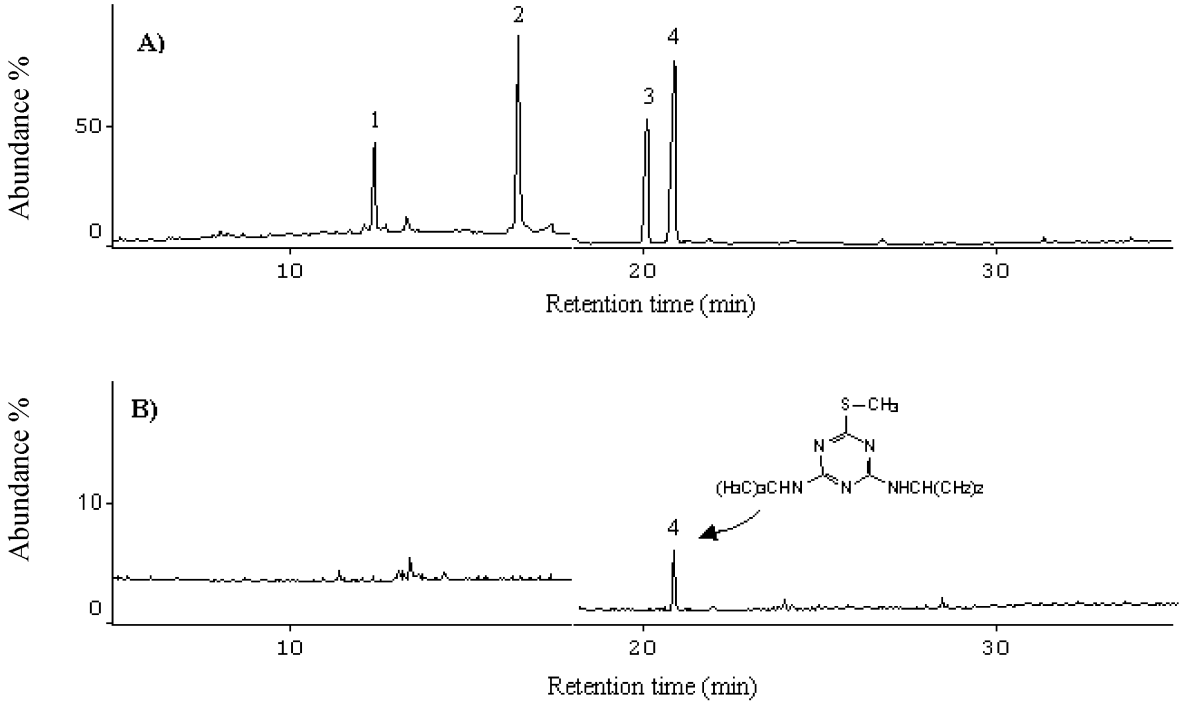

Fig. 5. GC–MS-SIM chromatograms obtained by acetone/SPME procedure in (A) spiked (500 ng/g) Preveza marine sediment and (B) real sample (Igoumenitsa marina—July 2002).

(RSD between 4 and 11%) and LOD values (0.5 to 6 ng/g) and was successfully applied for the monitoring of these antifouling compounds in marine sediment.

In light of all the obtained results, it seems that the sensitivity, simplicity and the low operating cost of the SPME method make this procedure a powerful tool for the detection of the selected antifouling compounds at accurate final determinations in marine sediment samples.

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