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Journal of Chromatography A, 1049 (2004) 17–23
JOURNAL OF CHROMATOGRAPHY A
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Application of solvent microextraction in a single drop for the determination of new antifouling agents in waters☆

Dimitra A. Lambropoulou*, Triantafyllos A. Albanis

Laboratory of Environmental Technology, Department of Chemistry, University of Ioannina, Ioannina 45110, Greece

Received 8 March 2004; received in revised form 14 July 2004; accepted 11 August 2004

Abstract

A new, rapid microextraction technique termed solvent microextraction (SME) has been developed for the simultaneous determination of new generation antifouling agents, in water samples. Chlorothalonil, dichlofluanid and Sea nine 211 were employed as model compounds to asses the extraction procedure and were determined by gas chromatography with electron capture detection. Experimental parameters which control the performance of SME, such as selection of solvent, exposure time, agitation, organic drop volume, and salt concentration were optimized. The new method provided good average enrichment factors of >10.7 for all analytes, good precision (RSD < 8.5%) and good linearity (r^2 > 0.9880). The limits of detection (LODs) were in the range of 0.00025–0.003 μg/L (S/N = 3). The SME was performed in different type of natural water samples and acceptable recoveries were obtained for the tested analytes. The results demonstrated that SME is a rapid, accurate and effective preparation method and could be successfully performed for the determination of antifouling agents in water samples.

Keywords: Water analysis; Solvent microextraction; Antifouling booster biocides; Chlorothalonil; Dichlofluanid; Sea nine 211

1. Introduction

Antifouling paints have long been the most effective method to avoid the accumulation of marine organisms. They prevent biofouling by releasing biocides such as tributyltin oxide (TBTO) and triphenyltin fluoride (TPTF) at a constant rate. Because these biocides have toxic effects on various marine species and contaminate the environment, their use has been restricted in many countries [1–4] and considerable effort is being devoted to finding new antifouling alternatives. Voulvoulis et al. [5] reviewed 11 alternative antifouling compounds named "booster biocides" that they make their appearance into the markets of antifouling paints just a few years ago.

The leaching of some of the most commonly used ones such as chlorothalonil, dichlofluanid, diuron, irgarol 1051, sea nine 211, TCMTB, zinc pyrithione, and Zineb, have been detected in waters in certain areas of the marine environment, together with the resistance to degradation observed in some of them [6–8] has been revealed as a risk for the marine biota [9] and extensive monitoring programs have been applied in different coastal zones in Europe [9–18].

Together with the toxicity in marine organisms, an additional problem arises from the low concentration levels at which these compounds have to be detected because of the dilution capacity of the environment. In this sense, the challenge for the scientists lies in the development of new analytical methods able to determine such compounds in the low picogram levels with enough guarantees about their identities.

For the enrichment of antifouling biocides from waters, either liquid–liquid extraction (LLE) or solid-phase extraction (SPE) is traditionally applied. Furthermore, solid-phase microextraction (SPME), has been recently applied [19–22]

☆ Presented at the 3rd Meeting of the Spanish Association of Chromatography and Related Techniques and the European Workshop: 3rd Waste Water Cluster, Aguadulce (Almeria), 19–21 November 2003.

* Corresponding author. Tel.: +30 326510 98363; fax: +30 26510 98795.
E-mail address: dlambro@cc.uoi.gr (D.A. Lambropoulou).

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doi:10.1016/j.chroma.2004.08.024

as a preconcentration method of antifouling biocides in natural waters showing good precision and high sensitivity.

In recent years, solvent microextraction (SME), or liquid-phase microextraction (LPME), has attracted increasing attention for water analysis of organic micropollutants [23–26]. Solvent microextraction is a type of liquid–liquid extraction in which the analyte partitions between the bulk aqueous-phase and a very small volume of organic solvent. This relatively new technique has been described in several papers and was found to be a powerful tool for the analysis of different groups of analytes, such as alcohols [27], nitroaromatic explosives [28,29], chlorobenzenes [30], drugs [31,32], volatile organic compounds (VOCs) [33] and pesticides [34–36]. SME is very inexpensive as it requires only common laboratory equipment and 1–2 μL of organic solvent and it does not suffer from carryover between extractions that may be experienced when using SPME. Other advantages of SME include simplicity, speed and potential for easy automation. To date the potential of microextraction techniques has yet been fully exploited, in both methodology and application [22,24,26].

Although SME was found to be effective for several groups of organic pollutants, no information is currently available on SME of new generation antifouling agents such as booster biocides. Therefore, the present work attention was focused on SME of antifouling biocides in water samples. The fundamental extractability by SME, SME from tap, sea and river water samples and optimization studies for all antifouling biocides were the major objectives of the present work.

2. Experimental

2.1. Reagents and materials

Chlorothalonil, dichlofluanid and vinclozolin as internal standard were purchased from Riedel-de Häen (Germany). Sea-nine 211 was a kind offer by Rohm & Haas (Pliladelphia, PA, USA). All solvents (pesticide-grade) were supplied from Pestiscan (Labscan Ltd., Dublin, Ireland) and sodium chloride from Merck (Darmstadt, Germany). Humic acids were purchased from Fluka (Steinheim, Germany). A methanolic solution of vinclozolin was prepared and used as the internal standard (IS). Stock standard solutions were prepared in methanol with concentration levels of 1000 μg/L for each compound and were stored in a freezer at about −20 °C. Working solutions were prepared by dilution of stock standards with deionised water. Water from the GFL (2108) water purification system (GFL, Germany) was used.

A 10-μl Hamilton gastight syringe (Hamilton, Bonaduz, Bonaduz, Switzerland) model 1701, with a bevel needle tip (length: 5.1 cm, i.d.: 0.013 cm, bevel 22°) was used for extraction target analytes and injecting the solvent solution to the GC for further analysis.

2.2. Water samples

Tap and river waters was obtained from the main area of Ioannina (Greece). Sea water samples used for the development of the method were collected from Ionian Sea. The water samples were collected in glass bottles and used without previous treatment or filtration. All water samples were free of the selected pesticides as found by previous analysis using the conventional SPE technique [22].

2.3. Extraction process

A 1.5 mL drop of toluene was used as the extraction solvent and immersed in the stirred sample solution for a 15 min extraction time. The sample solution was stirred at a rate of approximately 600 rpm using an 8 mm × 2.5 mm PTFE stir bar. For all quantification experiments, the same amount of internal standard solution (Vinclozolin) was added in the aqueous samples prior extraction. A Hamilton 10 mL 1701SN syringe (Hamilton, Bonaduz, Switzerland) fitted with an adapter to assist reproducibility was used in all extractions and injections. Using the adapter, the maximum syringe volume was set to 1.7 μL and the delivery volume was set to 1.5 μL. For the extraction, 1.7 μL of hexane was drawn into the syringe and the plunger was depressed with the stop button engaged, causing 0.2 μL to be expelled. The microsyringe was then positioned in the extraction stand in such a way that the tip of the extraction needle protruded to a depth of about 8 mm below the surface of the aqueous solution. The syringe plunger was then completely depressed causing a 1.5 μL drop to form on the needle tip. The drop was suspended from the needle for 15 min at which time the plunger was withdrawn to the maximum value of 1.7 μL with the needle tip still submerged in the sample solution. The contents of the syringe were then injected into the GC for analysis. In all cases, the analytical signal measured was peak area and ratios of the analyte area with the internal standard area were calculated.

2.4. GC–ECD analysis

Chromatographic analysis was performed using a Shimadzu 14B capillary gas chromatograph equipped with a ^{63}Ni electron-capture detection (ECD) system working at 300 °C. Analytes were separated with a DB-1 column (J&W Scientific, Folsom, CA, USA), 30 m × 0.25 mm i.d., contained 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 250 °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.

Table 1
Efficiency of different organic solvents evaluated for extraction of antifouling booster biocides by SME (concentration level at 1 μg/L, stirring rate 400 rpm, and drop volume 1.0 μL)

Biocides	Peak area ($\times 10^6$)			
	n-Hexane	Isooctane	Toluene	Xylene
Chlorothalonil	1.50	0.89	1.55	1.54
Dichlofluanid	0.77	0.58	0.69	1.02
Sea nine 211	2.05	1.53	1.67	3.37

2.5. Quantitation

Quantification of real water samples was performed by GC–ECD using vinclozolin as the internal standard. All the determinations were performed in triplicate except the evaluation of precision, which was performed in five replicates. The linearity of the method was investigated over the 0.010–50 μg/L range expressed as the initial concentration in water. Detection limits were calculated from a procedural blank as the concentration corresponding to three times the signal-to-noise ratio. Confirmation of analytes in real samples was performed by a GC–MS 17 A instrument (Shimatzu) in the electron impact ionization (EI) mode.

3. Results and discussion

The optimization experiments were conducted with antifouling biocides in pure water samples to study the fundamental extractability by SME. During optimization experiments, organic drop was set at 1 μL.

3.1. Selection of organic solvent

In a first experiment, attention was focused on selection of the organic solvent. The solvents had three important characteristics that made them suitable candidates for impregnation; they were immiscible with water and were of low volatility. Four solvents selected for this study, according to increasing order of polarity, were *n*-hexane (polarity index, 0.1), isooctane (0.1), toluene (2.4) and xylene (2.5). The efficiency of SME is evidenced by the changes in peak areas of antifouling biocides. As illustrate in Table 1 SME of antifouling biocides was accomplished with all the three solvents. Nevertheless, among solvents considered, xylene and toluene provided higher responses. Hexane and isooctane were not as effective as the other two solvents in the extraction of all analytes. This may be attributed to the relatively lower polarities of isooctane and hexane as compared with those of toluene and xylene.

Despite the fact that superior results were obtained with xylene, this solvent contained a minor amount of impurities, which interfered with ECD; this is a consequence of the need for a solvent with a high purity so as to minimize the surcharge of the detector by the solvent drop impurities during the analysis. Toluene can provide high extraction efficiencies as xylene and furthermore, it has low solubility in water (170 mg/L) resulting in low losses drop; therefore, it was selected as most suitable solvent for extraction.

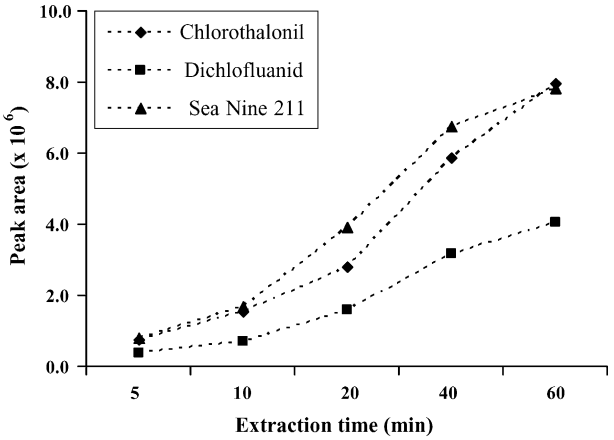

Fig. 1. Effect of extraction time on the extraction efficiency of antifouling booster biocides obtained from SME (concentration level at 1 μg/L, stirring rate 400 rpm, and drop volume 1.0 μL).

3.2. Extraction time

In addition to the organic solvent, the extraction time was established for the SME of antifouling biocides. Extractions were carried out at 5, 10, 20, 40 and 60 min (Fig. 1). The amount of antifouling biocides extracted by SME increased with increasing exposure time from 5 to 60 min. Although equilibrium could not be attained within this interval, 15 min was chosen as the sampling time because it should be comparable to the duration of the chromatographic run. Longer extraction times were not evaluated.

3.3. Ionic strength

It was of interest to examine the influence of salt addition on the efficiency of SME. For this purpose, the ionic strength of solutions was modified by addition of sodium chloride. In order to investigate the effect of ionic strength, a series of spiked samples with various concentrations of NaCl (0–10%) prepared by adding of calculated weight of NaCl into a 5 ml volume of sample solution. Plots of relative peak area versus ionic strength have been shown in Fig. 2. According to the curves, it is clear that the addition of ionic strength embarrasses the transport of the analytes to the extracting drop, especially for chlorothalonil and Sea nine 211. This means that with increased salt concentration the diffusion of analytes towards the organic drop becomes more and more difficult limiting thus the extraction. Similar observations concerning the effect of salt on the SME analysis was also made by other researchers [19,20]. Based on the above consideration all remaining extraction experiments were performed without salt addition on the water samples.

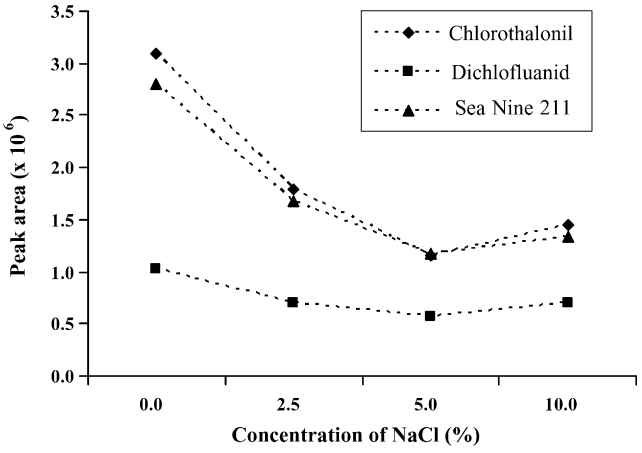

Fig. 2. Effect of sodium chloride concentration on SME for antifouling booster biocides (concentration level at 1 μg/L, stirring rate 400 rpm, and drop volume 1.0 μL).

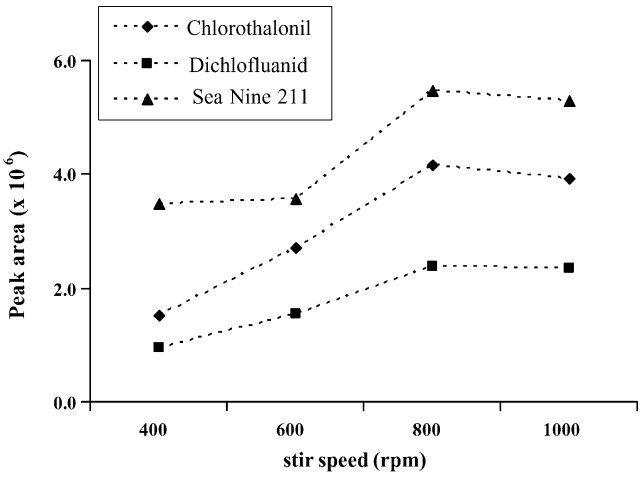

Fig. 3. Effect of stir speed on SME for antifouling booster biocides (concentration level at 1 μg/L, and drop volume 1.0 μL).

3.4. Effect of stir speed

The effect of stirring on the extraction of antifouling biocides was studied next. Extractions were performed from spiked water solutions containing 1 μg/L of the analytes of interest using different stirring rates with a 0.8 cm magnet on a stirrer plate. As can be seen in Fig. 3, the total signal increases with increase in stirring speed up to 800 rpm. A high rate of agitation increases the diffusion rate and reduces the time required to reach analyte equilibrium between the sample solution and extraction solvent. Although an agitation rate of 800 rpm resulted in the greatest efficiency of the target analyte, air bubbles formed in the solution due to mechanical forced generated which, in turn, led to occasional difficulties in the quantification on the analyte. Therefore, an optimum stir speed of 600 rpm was selected for sample analysis.

3.5. Solvent drop volume

To increase the sensitivity of the SME method, the solvent drop volume was optimized. For this purpose extractions were performed from spiked water solutions containing 1 μg/L of the analytes by increasing the drop volume from 1.0 to 2.0 μL. As can be expected, peak areas of booster biocides increased with drop volume (data not shown). However, using high drop volumes of organic solvent can result in the loss of the organic drop. Thus, 1.5 μL drop volume was used for further experiments in order to avoid these losses.

3.6. Recoveries from natural water samples and effect of humic acids on SME analysis

The feasibility of using this method for antifouling biocide screening in tap, sea and river water samples was then tested at spiked concentration levels of 1 and 10 μg/L. The optimized extraction protocol was applied to these samples and the recoveries were calculated as the ratio of the concentrations found in natural and deionized water samples, spiked with the same amount of analytes. For each sample, at each concentration, the extraction was repeated three times. Relative recoveries and precision were calculated and are listed in Table 2. As can been seen, acceptable recoveries (78–104%) and RSD values (2.9–11.7%) was obtained for all analytes in the tested water samples. The lower recoveries were observed for Sea nine 211 in river water samples may be due to the higher content of organic matter and the presence of suspended solids in these types of water samples. A chromatogram of analytes after solvent microextraction in spiked river water sample (1 μg/L) with a 1.5 μL drop of toluene is shown in Fig. 4.

Table 2
Relative recoveries and precision of SME in natural waters spiked with antifouling biocides

Biocides	Relative recoveries and RSD values (%)[a,b]											
	Tap				Sea				River			
	1 μg/		10 μg/L		1 μg/		10 μg/L		1 μg/		10 μg/L	
Chlorothalonil	102	2.9	98	3.4	94	3.4	95	3.5	86	3.7	88	4.1
Dichlofluanid	103	3.1	101	3.7	88	4.6	91	4.2	82	4.9	86	5.3
Sea nine 211	104	8.3	99	9.1	91	9.1	89	8.9	78	9.7	80	11.7

[a] Spiking levels of 1 and 10 μg/L.
[b] Mean of three replicate experiments.

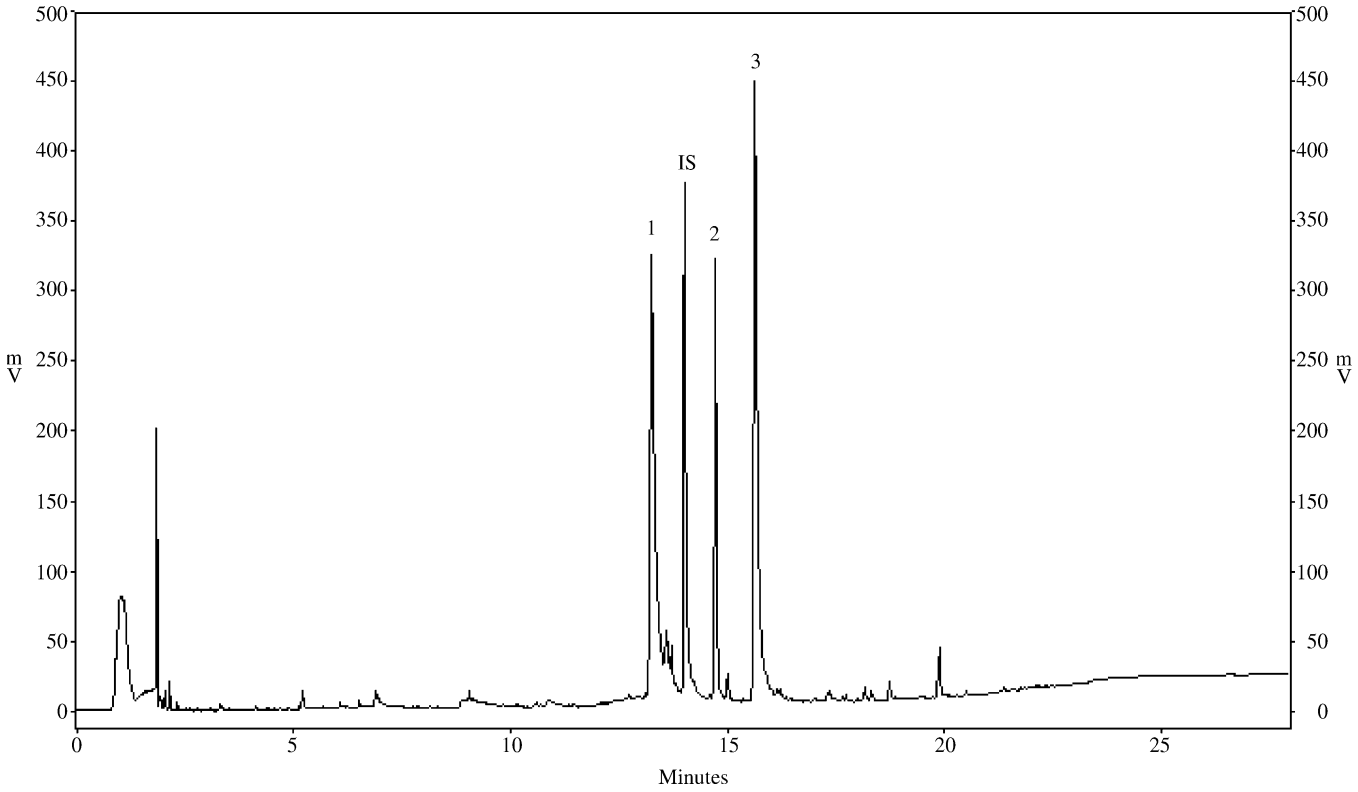

Fig. 4. GC–ECD chromatogram of antifouling booster biocides obtained from SME analysis in spiked seawater sample at concentration level of 1 μg/L. (1) Chlorothalonil, (2) dichlofluanid, (3) Sea nine 211, and (IS) vinclozolin.

The effect of matrix interferences due to humic acids (HAs) in water samples on the extraction efficiency of antifouling biocides by SME, was studied by analyzing water samples containing 1 μg/L of each of target antifouling biocide, spiked with HAs at concentrations ranging from 5 to 50 μg/L (Fig. 5). As can been seen from figure, the presence of HA is primarily affect the extraction efficiency of Sea nine 211. Its extraction is decreased, probably, as in the case of salting out effect, by limiting the diffusion towards the drop and this negative effect was more pronounced at a HA concentration value ranging between 0 and 10 μg/L. The inhibition on the extraction efficiency of Sea nine 211 by the presence of HAs was also observed in previous study [19] during the SPME analysis. In the case of chlorothalonil and dichlofluanid, the SME efficiency was less affected by the presence of HAs indicating that could be successfully performed in natural waters with low or medium organic content.

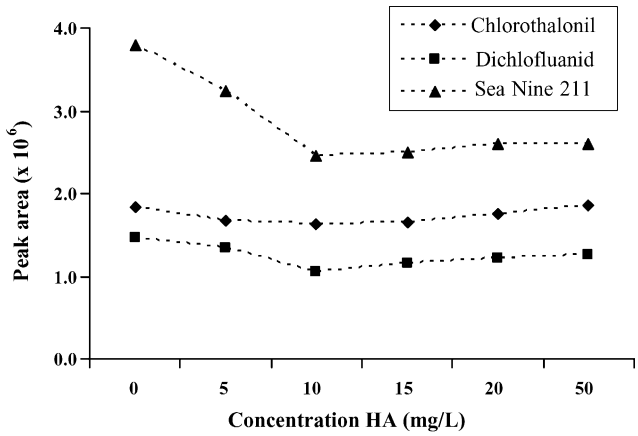

Fig. 5. Effect of humic acid on extraction efficiency of SME for antifouling booster biocides (concentration level at 1 μg/L, stirring rate 400 rpm, and drop volume 1.0 μL).

3.7. Enrichment factors (EFs)

In order to investigate the enrichment factors of each antifouling biocide, three replicate extractions were performed at optimal conditions from aqueous samples. The enrichment factor was calculated as the ratio of the peak area obtained after SME extraction to the peak area obtained after a syringe injection of a toluene solution containing 25 μg/L of each target analyte. The enrichment factors of SME were 10.7 for Sea nine 211, 17.4 for dichlofluanid and 32.6 for chlorothalonil.

3.8. Evaluation of the method performance

In order to proceed with the current evaluation of SME, repeatability, linearity and limit of detection were determined for the target analytes in water samples. Calibration curves constructed for all analytes using five spiking

Table 3
Linearity data, precision data, limits of detection (LOD) (ng/L), enrichment factors (EF) and relative standard deviation values (RSD %) of SME in GC–ECD system

Biocides	Linear range	Correlation coefficient (r^2)	LOD[a] (μg/L)	EF	Precision (RSD, %)	
					Repeatability[b]	Reproducibility[c]
Chlorothalonil	0.01–50	0.9925	0.00250	32.6	2.8	4.2
Dichlofluanid	0.01–50	0.9945	0.00300	17.7	2.3	3.9
Sea nine 211	0.01–25	0.9880	0.00025[d]	10.7	7.8	8.5

[a] LOD: calculated from 0.010 μg/L spiked level, S/N = 3.
[b] Repeatability was calculated by analyzing six water samples spiked at 1 μg/L with 1 day.
[c] Reproducibility was calculated by analyzing three water samples spiked at 1 μg/L per day for 3 days.
[d] LOD: calculated from 0.050 μg/L spiked level, S/N = 3.

levels of antifouling biocides in the concentration range of 0.010–50 μg/L. For each level three replicate extraction were performed at optimal conditions (extraction time: 15 min, drop volume: 1.5 μL, stirring rate: 600 rpm and sample volume: 5 mL). Regression analysis was used to approximate the linearity of the calibration curves. All analytes exhibited good linearity with squared regression coefficients (r^2) > 0.9880 (Table 3).

To evaluate the precision of the measurement, reproducibility and repeatability were investigated. The intra-day precision (repeatability) was performed by consecutively extracting six aqueous samples spiked at 1 μg/L within the same working day. As shown in Table 3 the repeatability of the method expressed in terms of relative standard deviation (RSD) varied between 2.3 and 7.8%. The inter-day precision (reproducibility) of the method was determined by analysing each day three water samples spiked at 1 μg/L over a period of three working days. The reproducibility of the method expressed in terms of RSD ranged from 3.9 to 8.5%. The method presented a good precision, with RSD values similar to values described in other publications [11,19–22].

The calculated LODs (see Section 2) were contrasted with the responses obtained for a standard containing 0.010 μg/L of each biocide. In case of Sea nine 211, LODs were determined by taking into account the response factors given by standards at 0.050 μg/L because, at very low concentrations, areas were higher that those expected, resulting in an increase of the LODs for this compound. LODs ranged from 0.00025 to 0.003 μg/L. The result of this work was comparable with that of GC–ECD using SPME technique [19–22], but better than that of GC–ECD followed with SPE technique [9,22].

4. Conclusions

In the present work SME was combined with GC–ECD for the determination of new antifouling agents in water samples for the first time. Determination of the model compounds (chlorothalonil, dichlofluanid and Sea nine 211) by the proposed method was possible at trace amounts (ng/L level) with good accuracy and reproducibility. A comparison of the proposed procedure for antifouling analysis with other extraction techniques, such as SPME and SPE, in terms of recovery, limits of detection and precision indicates that SME exhibits a favorable performance.

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