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Trace determination of antifouling compounds by on-line solid-phase extraction–gas chromatography–mass spectrometry

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Abstract

A new method based on solid-phase extraction (SPE) on-line coupled to gas chromatography–mass spectrometry through an on-column interface has been applied to determine three antifouling compounds in water samples. Parameters affecting the SPE process and transfer step have been optimised and the method developed has been applied to the analysis of marinas, fishing ports and Ebro river water. The method allows the analytes to be detected at 0.01 $\mu g\ l^{-1}$ in SIM acquisition mode by preconcentrating only 10 ml of water sample. Different marina and fishing port water samples have been analysed and Irgarol 1051 has been found in some of them at a concentration level equal or lower than 0.05 $\mu g\ l^{-1}$.

Keywords: Antifouling compounds; 4-Chloro-3-methylphenol; Dichlofluanid; Irgarol 1051

1. Introduction

Pesticides are one of the organic compounds which are currently added to boat paints to prevent fouling [1]. These compounds, such as 4-chloro-3-methylphenol, dichlofluanid and Irgarol 1051, can diffuse into the surrounding waters and contaminate coastal environments [2–4].

Because of their low concentration levels, a preconcentration technique is usually required to determine these compounds in water samples. However, Irgarol 1051 and dichlofluanid have not been widely studied in environmental samples to date. Liquid–liquid extraction (LLE) [5] and solid-phase extraction (SPE) [6–9] followed by gas chromatography (GC) [5,8] or high-performance liquid chromatography (HPLC) [6,8,10] are the most common preconcentration techniques applied to determine 4-chloro-3-methylphenol and Irgarol 1051 in water samples. Irgarol 1051 has also been determined by applying immunochemical techniques (ELISA) [6,11,12]. Dichlofluanid has mostly been determined in air samples by using a trapping sorbent combined with GC [13]. Recently, we have applied solid-phase microextraction (SPME) coupled to GC to determine these contaminants in water samples [14]. On-line SPE–LC–MS with an atmospheric pressure chemical ionisation (APCI) interface has also been applied by Ferrer and Barceló [15].

In this paper, an on-line SPE–GC–MS system has been selected. SPE is generally preferred to LLE because it minimises most of the drawbacks of LLE and the potential of GC–MS for the analysis of environmental samples is very well known. Likewise, on-line systems are widely used nowadays because they are easily automated and more difficult to contaminate from external sources. An on-column interface has been selected because of its wide application range.

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The aim of this work is to develop a method for determining the three pesticides mentioned in water samples by using an on-line SPE–GC–MS method with an on-column interface. To our knowledge, this on-line combination has not been tested for determining Irgarol 1051 and dichlofluanid. The presence of these pesticides along the coast from Tarragona to Cambrils, a popular area for tourists with various fishing ports and marinas, was tested for the period between March–June 1999. Most of the boats are repainted during this period, which is also high season for tourists.

2. Experimental

2.1. Reagents and standards

The compounds studied were 4-chloro-3-methylphenol, dichlofluanid and Irgarol 1051. Their structures are shown in Fig. 1. They were all purchased from Riedel-de Häen (Seelze-Hannover, Germany) with a purity higher than 98%, except Irgarol 1051 which was supplied by Ciba-Geigy (Barcelona, Spain) with a purity of 100%. A standard solution of 2000 mg l^{-1} of each pesticide was prepared in ethyl acetate and stored in the refrigerator. A solution of 100 mg l^{-1} in ethyl acetate was prepared weekly from the standard solutions and used to prepare dilute solutions and to spike water samples to the required concentrations.

Ethyl acetate and methanol, both trace analysis grade, were from Merck (Darmstadt, Germany). Acetic acid was from Probus (Barcelona, Spain) and water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Helium and nitrogen were supplied by Carburos Metálicos (Tarragona, Spain) with a quality of 99.995%.

2.2. Equipment

A Hewlett-Packard (Waldbronn, Germany) HP 6890 Series gas chromatograph equipped with an on-column injector and an HP 5973 mass selective detector was used. An HP G2399A SVE kit was installed so that larger volumes could be injected and the chromatographic separation carried out. The SVE kit consisted of a 5-m×530-μm I.D. retention gap, a

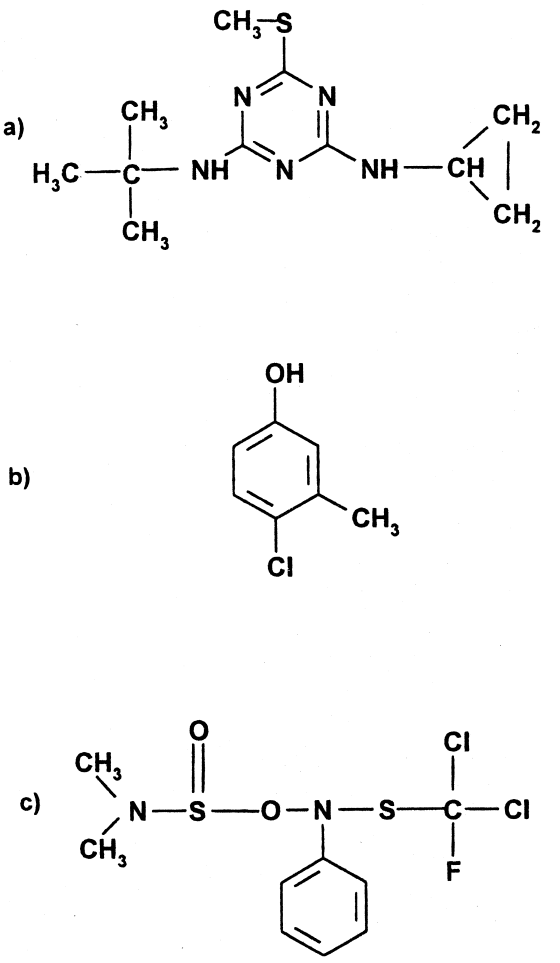

Fig. 1. Structures of (a) Irgarol 1051, (b) 4-chloro-3-methylphenol, (c) dichlofluanid.

0.25-μm retaining precolumn of 2 m×250 μm I.D. and a 0.25-μm analytical column of 30 m×250 μm I.D. and a solvent vent valve. Both columns were HP-5 MS. All connections were made with conventional glass press-frits. Chromatographic data were recorded using an HP G1701AA Chemstation which was controlled by Windows 95 (Microsoft) and equipped with the Hppest and Wiley 138 mass spectral libraries.

Three six-port Valco valves (Houston, TX, USA), controlled by the GC software, were used for the SPE process. The precolumn (10×2 mm I.D.) was hand-packed with a styrenedivinylbenzene copolymer (PLRP-S, 100 Å, 20-μm particle size) (Polymer Laboratories, Shropshire, UK). An HP 1100 pump

Table 1
Selected ions and analytical data for full-scan and SIM acquisition modes for sea water

Compounds	Selected ions[a] (relative abundance)		Full-scan			SIM		
			Linearity range ($\mu g\ l^{-1}$)	r^2	LODs ($\mu g\ l^{-1}$)	Linearity range ($\mu g\ l^{-1}$)	r^2	LODs ($\mu g\ l^{-1}$)
4-Chloro-3-methylphenol	*142* (100)	107 (80)	0.5–20	0.996	0.2	0.1–5	0.998	0.01
Dichlofluanid	*123* (100)	167 (37)	0.5–20	0.998	0.1	0.05–5	0.996	0.02
Irgarol 1051	*182* (100)	253 (61)	0.5–30	0.998	0.1	0.05–5	0.997	0.01

[a] The ions in the quantification for full-scan acquisition are shown in italics.

was used to deliver the sample and the solvents needed to clean and activate the sorbent. The ethyl acetate used in the elution step was delivered with a syringe pump (Cole-Parmer, IL, USA). A fused-silica capillary (30 cm×0.10 mm I.D.) permanently mounted in the on-column transferred the analytes from the precolumn to the GC system. A 100-μl loop made of polyether ether ketone (PEEK) tubing (HP) was used instead of the precolumn when direct injection was carried out. The scheme of the equipment required for the on-line SPE–GC–MS method has been shown in a previous paper [16].

2.3. Chromatographic conditions

The oven was set initially at 60°C and remained constant until the analytes were preconcentrated and transferred to the GC. The temperature was then increased to 250°C at 30°C min^{-1} and kept constant for 7 min. The on-column injector worked in the track oven mode and the carrier gas (helium) was maintained at a flow-rate of 1.2 ml min^{-1}.

The MS transfer line was kept at 280°C to prevent the analytes from recondensing. The ion energy used for the electron impact (EI) ionization was 70 eV and the MS was tuned to m/z 69, 219 and 502 for the EI corresponding to perfluorobutylamine (PFTBA). The mass range was 50–350 in the full scan mode with a rate of 4.72 scans s^{-1}. Selected ion monitoring (SIM) acquisition was carried out by acquiring data from the two most abundant ions, as can be seen in Table 1.

2.4. On-line trace enrichment

As described in a previous paper [16], three six-port valves connected in series were used for the on-line sample enrichment. The precolumn was conditioned by flushing 3 ml of methanol and 3 ml of water. Then, 10 ml of sample, previously acidified with 1% acetic acid, were preconcentrated and 10% of methanol was added. The tubes were purged with the corresponding solution before it was passed through the precolumn. The flow-rate throughout the process was 2 ml min^{-1}. Before the elution, the precolumn was dried with 3 bar nitrogen for 30 min. Finally, the analytes were desorbed in the backflush mode with 100 μl ethyl acetate pumped at 47 μl min^{-1} with a syringe pump and on-line transferred to the GC system via the transfer line. The SVE was opened a few seconds before the transfer started and closed 1.5 min after the end of the transfer in order to eliminate the ethyl acetate vapours without losing analytes. The GC temperature program and data acquisition were started 5.51 min after the SVE was closed to assure that solvent peak eluted.

Real samples were collected once a month in 2.5-l, precleaned, amber glass bottles and filtered through a 0.45-μm filter (MSI, Westboro, MA, USA). They were kept at 4°C in the dark until analysis.

3. Results and discussion

3.1. Chromatographic separation

The separation of the three pesticides studied was optimised by GC–MS by manually injecting 0.5 μl of a standard solution containing 25 mg l^{-1} of each pesticide in ethyl acetate into the on-column injector. The optimal conditions are described in Section 2.

The ions used to quantify the pesticides were selected from the spectrum obtained for each compound under EI ionization. In the full scan mode, only the base peak of each pesticide was selected for quantifying. Two ions were selected for each com-

pound so that the response under SIM mode could be acquired and quantified. The ions selected are shown in Table 1.

3.2. Transfer conditions

The transfer conditions such as transfer flow-rate, transfer temperature and SVE open time were optimised as described in a previous study [16], so that partially concurrent solvent evaporation (PCSE) could be used with an on-column interface. The optimal conditions found are described in Section 2.

3.3. SPE process

We selected some of the parameters that affect the SPE process by taking into account our previous experience in the field and the properties of the analytes studied. PLRP-S (polystyrene–polyvinylbenzene copolymer) was selected as the sorbent because of its ability to retain pesticides with similar characteristics to the compounds studied [15]. Ethyl acetate was selected because it desorbs analytes with a wide range of polarities from PLRP-S and it is compatible with the transfer conditions used. Before the elution step, 3 bar N_2 were passed through the precolumn for 30 min so that water would not destroy the deactivation of the retention gap [15].

Other parameters such as sample volume, solvent elution volume and sample pre-treatment were optimised step by step. First of all, the elution volume was optimised by preconcentrating 10 ml of a standard solution of 100 μg l^{-1} and eluting with different volumes of ethyl acetate (from 100 to 300 μl). A volume of 100 μl ethyl acetate was selected as the elution volume because recoveries were not greater when the volume was higher. However, the recoveries were very low (8% for dichlofluanid and Irgarol 1051 and 68% for 4-chloro-3-methylphenol). We assumed that this was due to the adsorption of the analytes in the system as was observed in a previous work which studied a different group of pesticides [16]. This also agrees with results from other authors when different compounds were studied [17]. Then, sample pre-treatment conditions were optimised to minimise the adsorption of the analytes in tubes and valves, with a sample volume of 10 ml and an elution volume of 100 μl. Recoveries were calculated by comparing the areas with the ones obtained with 100-μl loop injections. To test whether our hypothesis was true or not, different volumes of methanol were added to the sample before the enrichment step. The results are shown in Table 2. Methanol increased the recoveries of Irgarol 1051 and dichlofluanid, but the recovery of 4-chloro-3-methylphenol decreased with a high percentage of methanol due to methanol causing elution of the phenol. Ten percent of methanol was selected as the best condition, taking into account the recoveries of the three analytes. Then, acetic acid was also added to the sample before the preconcentration to ensure the analytes were in the neutral form and to increase the recoveries obtained by using 10% of methanol. As can be seen in Table 2, recoveries increased slightly for 4-chloro-3-methylphenol and dichlofluanid, while the recovery of Irgarol 1051 increased by about 20% when 1% of acetic acid was added. Then, 10% of methanol and 1% of acetic acid was added to the samples and standard solutions before the preconcentration in the next experiments.

The sample volume was optimised by preconcentrating different volumes (between 10 and 20 ml) of standard solutions (10% methanol and 1% acetic acid) at different concentration levels so that the theoretical final amount preconcentrated (1 μg) was kept constant. The elution was carried out with 100

Table 2
Recoveries obtained by adding different percentages of methanol and acetic acid to the samples before the preconcentration step

Compounds	Recovery (%)					
	0% acetic acid				1% acetic acid	
	Methanol				Methanol	
	0%	5%	10%	30%	0%	10%
4-Chloro-3-methylphenol	68	65	60	–	69	69
Dichlofluanid	8	29	60	61	42	67
Irgarol 1051	8	29	63	66	76	84

μl of ethyl acetate. The volume selected was 10 ml because higher volumes decreased the recoveries for all compounds, mainly for 4-chloro-3-methylphenol (with 15 ml, its recovery was 47%). Additional experiments demonstrated that no carryover effects were observed with the conditions selected.

3.4. Application to real samples

The performance of the method for real samples was tested in marina, fishing port and Ebro river water. Sea water was sampled along the Tarragona coast at five different points close to fishing ports and marinas for the period between March and June 1999.

Firstly, a blank of sea water was analysed. None of the peaks eluted at the same retention time as the analytes studied. The recoveries for sea water using the SPE–GC–MS method were similar to those obtained when standard solutions were analysed. When sea water spiked with different levels of analytes was analysed under full-scan acquisition, linearity was good between 0.5 and 20 or 30 μg l^{-1}, the correlation coefficients were higher than 0.996. The limits of detection were calculated taking into account a signal/noise ratio of 3 and were between 0.1 and 0.2 μg l^{-1}. The repeatability and reproducibility between days was also checked by analysing six samples of sea water spiked with 1 μg l^{-1} of each analyte. The results, expressed as relative standard deviation (RSD), varied between 5 and 19% for repeatability and between 10 and 23% for reproducibility. The results obtained for sea water under full-scan acquisition are shown in Table 1.

When SIM acquisition was used, the response for sea water was linear between 0.01 or 0.05 and 5 μg l^{-1} with correlation coefficients higher than 0.996. The limits of detection were between 0.01 and 0.02 μg l^{-1} and were calculated using the criteria described above. The repeatability and reproducibility were calculated by preconcentrating six sea water samples spiked with 0.1 μg l^{-1} of each analyte and the results were between 5 and 20% and 12 and 22%, respectively. The results obtained when SIM was used are also included in Table 1. Fig. 2 shows the chromatogram obtained from the analysis of an unspiked sea water sample and sea water spiked at 0.1 μg l^{-1} in the SIM acquisition mode.

As can be seen, the method developed and applied to sea water allowed the three antifouling compounds studied to be determined at 0.1–0.05 μg l^{-1} under SIM acquisition, meanwhile the method using SPME–GC–MS(SIM) described in a previous paper [14] has as a limit of quantitation of 0.2 μg l^{-1}. The method developed also uses an instrument which is simpler and less expensive than the method using SPE–LC-(APCI)–MS [15] and the spectra provide us more structural information. Limits of detection are higher but the sample volume required is 10 times lower.

Different marina and fishing port water samples collected from March to June along the coast from Tarragona to Cambrils were analysed. Irgarol 1051 was found in some samples collected in March, May or June in different marinas along the zone studied. Although the comparison of the spectrum obtained under full-scan acquisition and that of the standard allowed the identification of the analyte, its quantitation was done using SIM acquisition. The concentration of Irgarol 1051 was 0.05 μg l^{-1} with a relative standard deviation of 13% ($n=3$) in one sample collected in May, and for the rest the concentration was lower than their quantitation limit. Fig. 3 shows the extracted ion chromatogram (m/z 182) and the spectrum corresponding to Irgarol 1051 of one of those samples.

To check whether the method developed can be applied to a different matrix, Ebro river water was analysed and no peaks corresponding to the compounds studied were found. The recoveries, linearity of the response, correlation coefficients, limits of detection and repeatability and reproducibility between days were similar to those obtained for sea water. This demonstrated that the salt content of the matrix is not a factor affecting the performance of the method. Fig. 4 shows the extracted ion chromatogram of a Ebro river water sample spiked at 1 μg l^{-1} obtained under full-scan acquisition.

The precolumn packed with PLRP-S can analyse at least 100 real samples without any change in performance.

4. Conclusions

SPE has been on-line coupled to GC–MS through

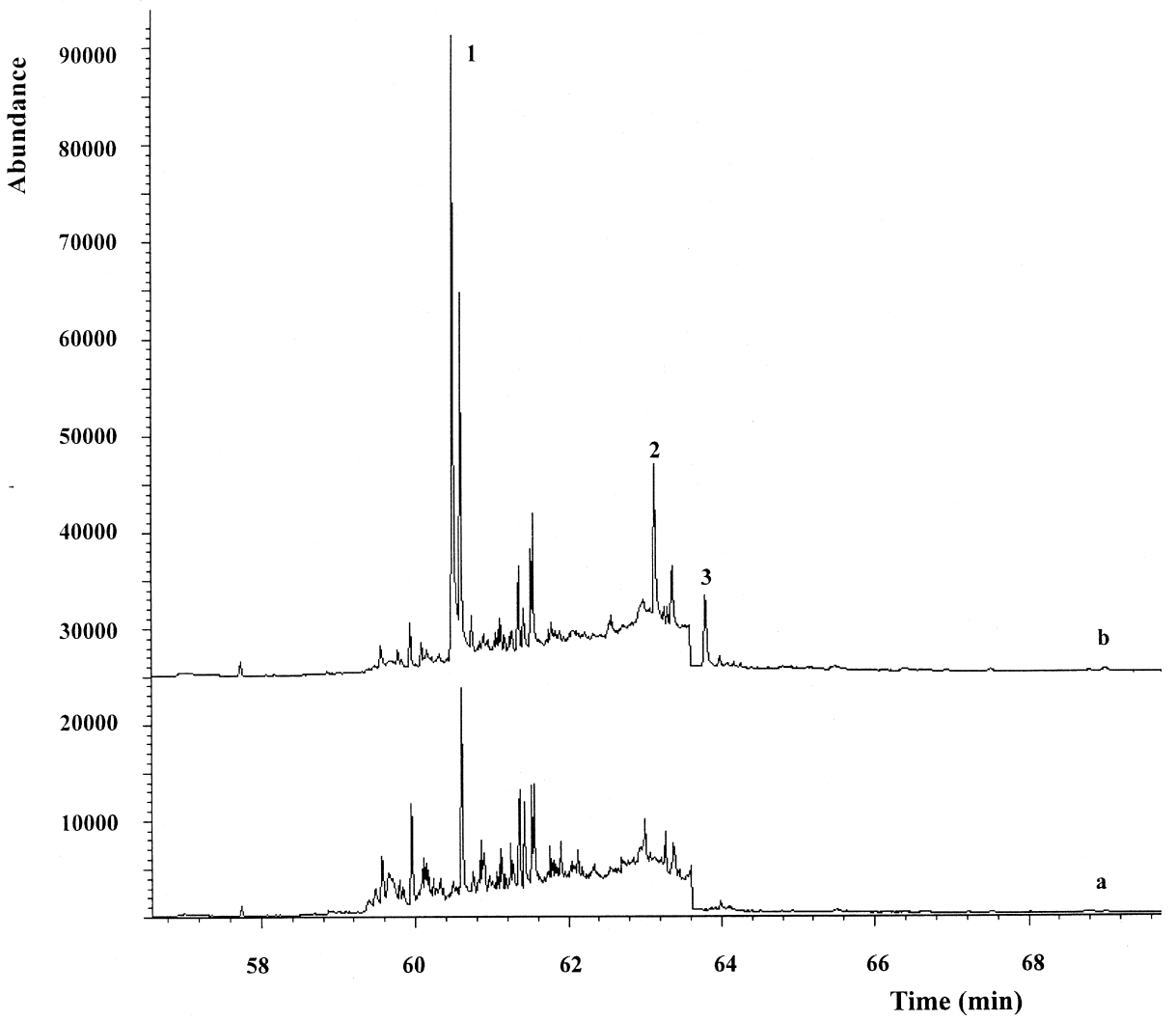

Fig. 2. Chromatograms obtained by on-line SPE–GC–MS (SIM) of: (a) 10 ml sea water; (b) 10 ml sea water spiked with 0.1 μg l^{-1}. Peaks: (1) 4-chloro-3-methylphenol, (2) dichlofluanid, (3) Irgarol 1051.

an on-column interface to determine a group of antifouling compounds in water samples.

Parameters affecting the SPE process have been optimised and 10% of methanol together with 1% acetic acid have been added to the samples prior the preconcentration step in order to minimise adsorption problems and enhance the recoveries of the analytes. Transfer conditions were: a syringe pump flow-rate of 47 μl min^{-1}, transfer temperature of 60°C and the SVE was closed 1.5 min after the end of the transfer.

The method developed allows the quantitation of all the analytes at levels around 0.05 μg l^{-1} by preconcentrating only 10 ml of sample and using SIM acquisition mode.

Irgarol 1051 has been found in some sea water at a concentration between 0.05 and 0.01 μg l^{-1}.

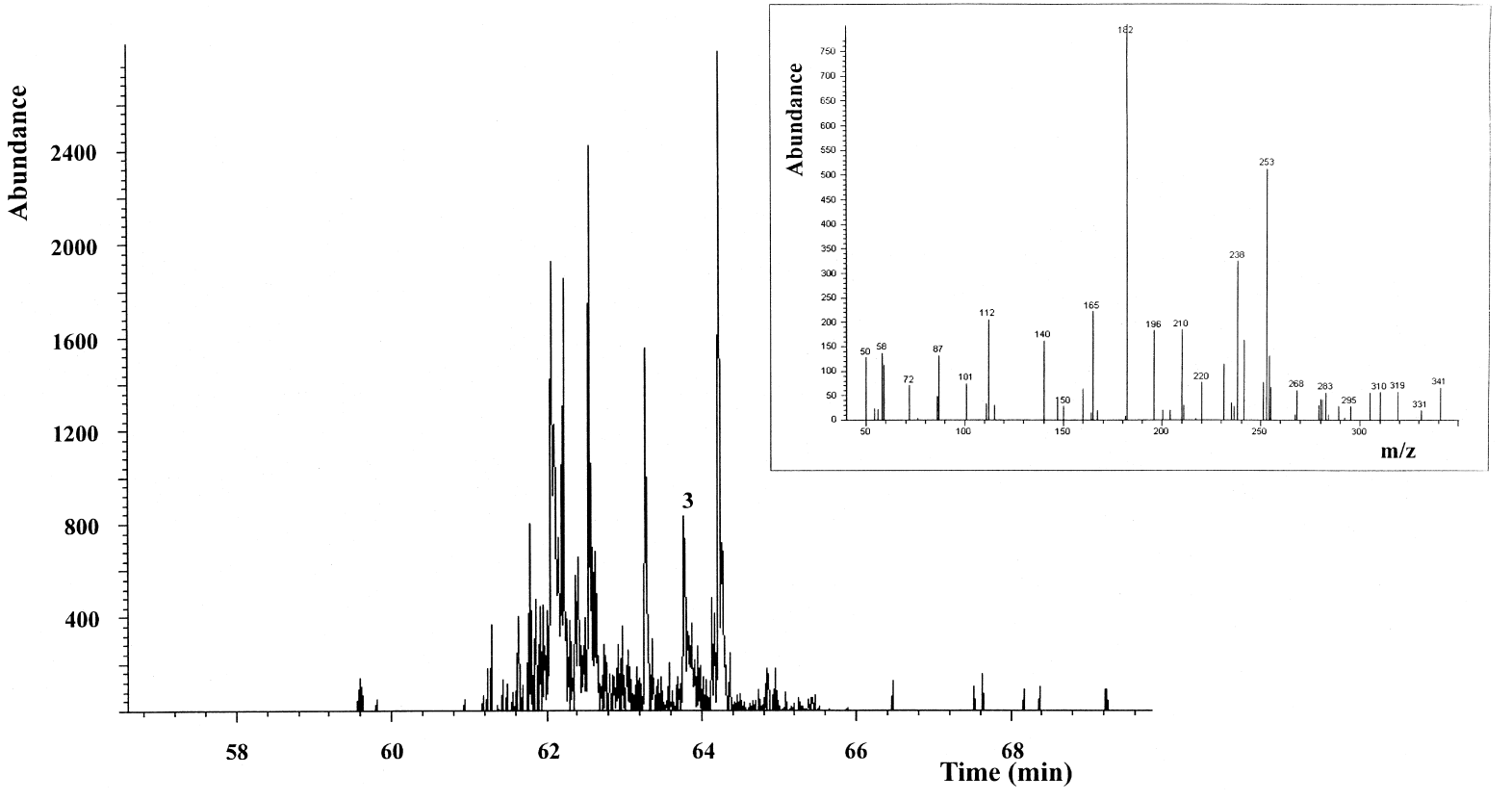

Fig. 3. Extracted ion chromatogram (m/z 182) of a marina sample. The insert shows the spectrum of Irgarol 1051.

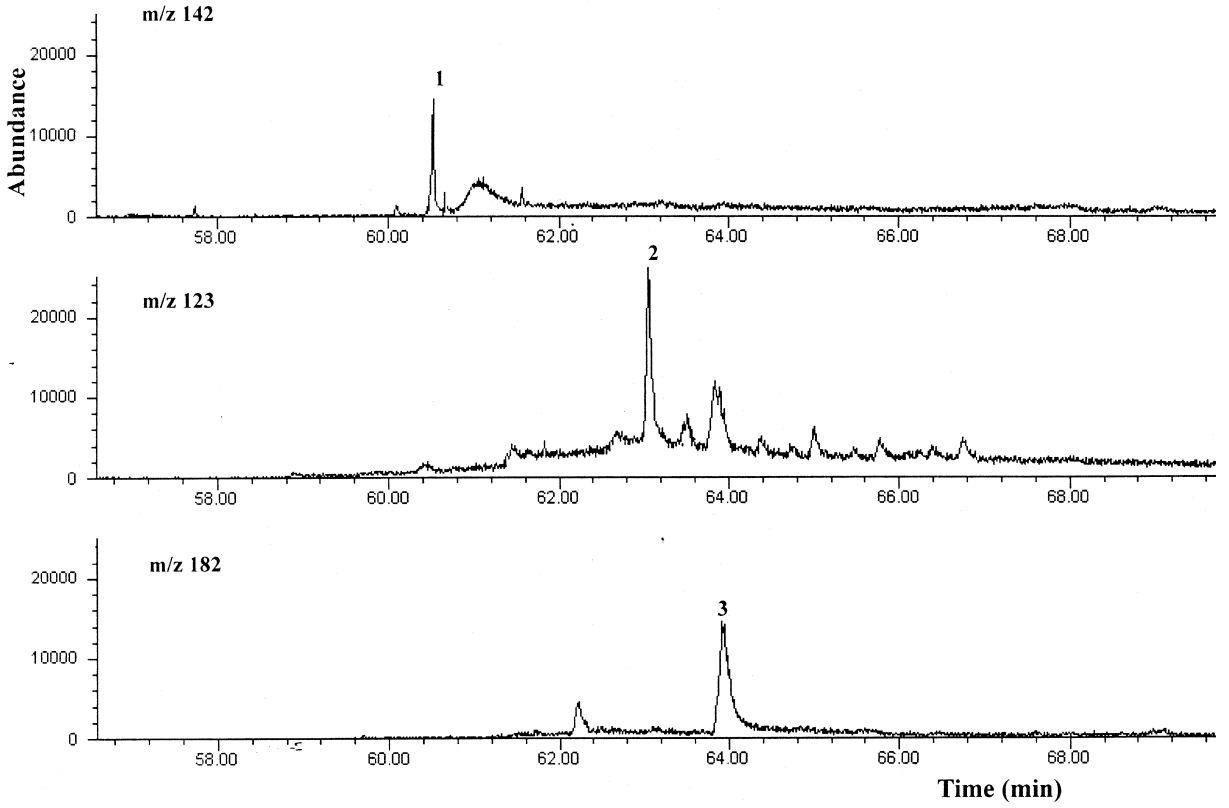

Fig. 4. Extracted ion chromatogram obtained by analysing an Ebro river water spiked at 1 μg l^{-1} by SPE–GC–MS. For peak assignation see Fig. 1.

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References

[1] N. Voulvoulis, M.D. Scrimshaw, J.N. Lester, Appl. Organometal. Chem. 13 (1999) 135.
[2] B. Dahl, H. Blanck, Mar. Pollut. Bull. 32 (1996) 342.
[3] A. Scarlett, M.E. Donkin, T.W. Fileman, P. Donkin, Mar. Pollut. Bull. 34 (1997) 645.
[4] I. Tolosa, J.W. Readman, A. Blaevoet, S. Ghilini, J. Bartocci, M. Horvat, Mar. Pollut. Bull. 32 (1996) 335.
[5] I. Tolosa, J.W. Readman, Anal. Chim. Acta 335 (1996) 267.
[6] I. Ferrer, B. Ballesteros, M.P. Marco, D. Barceló, Environ. Sci. Technol. 31 (1997) 3530.
[7] R.J.C.A. Steen, P.E.G. Leonards, U.A.Th. Brinkman, W.P. Cofino, J. Chromatogr. A 766 (1997) 153.
[8] S. Tólh, K. Becker-van Slooten, L. Spack, L.F. de Alencastro, J. Tarradellas, Bull. Environ. Contam. Toxicol. 57 (1996) 426.
[9] E. Pocurull, R.M. Marcé, F. Borrull, J. Chromatogr. A 738 (1996) 1.
[10] N. Voulvoulis, M.D. Scrimshaw, J.N. Lester, Chemosphere 38 (1999) 3503.
[11] B. Ballesteros, D. Barceló, F. Camps, M.P. Marco, Anal. Chim. Acta 347 (1997) 139.
[12] J. Penalva, M.A. González-Martínez, R. Puchades, A. Maquieira, M.P. Marco, D. Barceló, Anal. Chim. Acta 387 (1999) 227.
[13] J. Siebers, P. Mattusch, Chemosphere 33 (1996) 1597.
[14] A. Peñalver, E. Pocurull, F. Borrull, R.M. Marcé, J. Chromatogr. A 839 (1999) 253.
[15] I. Ferrer, D. Barceló, J. Chromatography A (in press).
[16] E. Pocurull, C. Aguilar, F. Borrull, R.M. Marcé, J. Chromatogr. A 818 (1998) 85.
[17] A.J.H. Louter, S. Ramalho, J.J. Vreuls, U.A.Th. Brinkman, J. Microcol. Sep. 8 (1996) 469.