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Solid-phase microextraction of the antifouling Irgarol 1051 and the fungicides dichlofluanid and 4-chloro-3-methylphenol in water samples

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Abstract

Three pesticides usually added to paint formulations, Irgarol 1051, dichlofluanid and 4-chloro-3-methylphenol, were determined by solid-phase microextraction (SPME) with 85-μm polyacrylate fibers and gas chromatography–mass spectrometry. The parameters affecting the SPME process (the pH, the addition of salt to the sample, and the time and temperature of the absorption step) were optimized. The method developed was applied to the analysis of water samples from Ebro river, marinas and fishing ports. The method enables these compounds to be detected at concentrations between 0.2 and 3.0 $\mu g\,l^{-1}$ under full scan conditions and between 0.05 and 0.08 $\mu g\ \ l^{-1}$ under SIM mode.

Keywords: Irgarol; Dichlofluanid; 4-Chloro-3-methylphenol

1. Introduction

Several organic compounds with different chemical characteristics, such as pesticides, are currently added to paint formulations. Irgarol 1051, 4-chloro-3-methylphenol and dichlofluanid are some of these compounds. Irgarol 1051 is a biocide agent which has recently been used in antifouling paints for boats and vessels to substitute organotin compounds whose use has been restricted by European Union regulations [1]. To date, only a few studies have been published about this compound in environmental samples [1–3] and the concentrations found in marina waters range from 100 to 700 $ng\,l^{-1}$ [4]. Dichlofluanid and 4-chloro-3-methylphenol are fungicides which are added to paints. Depending on where the paint is applied, these compounds can contaminate different environments, such as soil, water and air [2].

Pesticides are usually present at trace levels in aqueous samples. Consequently, the analysis of these compounds involves preconcentration or extraction techniques. Irgarol 1051 and 4-chloro-3-methylphenol are usually analyzed by liquid–liquid extraction (LLE) [5] or solid-phase extraction (SPE) [3,4,6,7] followed by gas chromatography (GC) [5,6] or high-performance liquid chromatography (HPLC) [3,6]. Immunochemical techniques (ELISA) [9] have also been applied to determine Irgarol 1051. Only a few studies have been published to date about

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dichlofluanid in environmental samples. This compound is frequently determined in air samples by using GC and different trapping sorbents [8].

In the last few years, a new extraction technique, solid-phase microextraction (SPME), has been introduced by Pawliszyn and co-workers [10,11]. It is a further advance in the isolation of organic contaminants from aqueous samples at trace levels because it is a good alternative to the conventional extraction techniques, LLE and SPE. This new extraction technique is very simple, solvent-free, fast and easily automated [12].

To date, SPME has been successfully applied to the analysis of a wide range of organic compounds such as pesticides [13,14], phenols [15] and volatile organic compounds (VOCs) [16,17] in different matrices. To our knowledge, SPME has not been tested for determining Irgarol 1051 and dichlofluanid.

2. Experimental

2.1. Reagents and standards

The pesticides studied were two fungicides, 4-chloro-3-methylphenol and dichlofluanid, and one antifouling, Irgarol 1051. The fungicides were purchased from Riedel-de Häen (Seelze-Hannover, Germany) and they were more than 98% pure. Irgarol 1051 was supplied by Ciba-Geigy (Barcelona, Spain) with a purity of 100%. A stock standard solution of 2000 mg l^{-1} of each compound was prepared in ethyl acetate. Working standard solutions were prepared by diluting the stock solutions with ethyl acetate. The stock and working standards were stored at 4°C in the refrigerator. Aqueous solutions (Milli-Q, tap and sea water) were prepared by spiking the water with an appropriate amount of the working solution.

Ethyl acetate was of PAR quality (for residue analysis) (Fisher Scientific, Leicestershire, UK). Water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Helium carrier gas (99.995% quality) was supplied by Carburos Metálicos (Tarragona, Spain). Sodium chloride with a purity of more than 99.5% was obtained from Probus (Barcelona, Spain).

2.2. Instrumentation

GC analyses were performed on a Hewlett-Packard 5890 Series II gas chromatograph (Palo Alto, CA, USA) equipped with a split/splitless injector and an HP5972 mass spectrometer. A Merlin microseal high pressure septum and an insert liner of 0.75 mm I.D., both from Hewlett-Packard, were used. Analytes were separated using a Hewlett-Packard HP-1 fused-silica capillary column (cross-linked 5% methyl silicone) of 30 m×0.25 mm with a phase thickness of 0.25 μm.

The optimized temperature program was as follows: the initial temperature was 80°C, which was increased to 250°C at 35°C min^{-1}. This temperature was held for 8 min. The total run time was 16.65 min. The detector and the injector temperatures were set at 280 and 250°C, respectively. The helium carrier gas was maintained at a flow-rate of 1.8 ml min^{-1}. The samples were injected in splitless mode and the splitter was opened after 4.5 min (delay time). The injection volume in direct injection was 2 μl.

The conditions for electron impact ionization (EI) were an ion energy of 70 eV and the mass range scanned was 50–350 m/z under full-scan acquisition mode. In the selected ion monitoring (SIM) acquisition mode, two peaks of each compound studied were monitored. The MS was tuned to m/z 69, 219 and 502 for EI corresponding to perfluorobutylamine (PFBA).

The data were acquired with the HP CHEMSTATION equipped with the mass spectral libraries Hppest and Wiley 138 which were used to compare the experimental spectra obtained.

2.3. SPME procedure

The SPME device and the 85-μm polyacrylate coated fibers were supplied by Supelco (Bellefonte, PA, USA). Before initial application, the fiber was conditioned in the hot port of the gas chromatograph at 300°C for 3 h, according to the instructions provided by the supplier. After the conditioning process, a fiber blank was run to confirm that there were no extraneous peaks which might have been due to contaminants introduced during the manufacture of the fiber.

For the SPME process, 3 ml of water samples spiked with an appropriate amount of each pesticide was introduced in 4-ml vials. The concentration of NaCl in the samples was 180 g l^{-1}, which is half the saturated concentration of NaCl in water, and the pH was not modified. In the extraction process, the fiber was directly immersed in the sample solution during 60 min at 60°C. The samples were heated with a heater unit from Selecta (Abrera, Spain) and continuously stirred magnetically. Finally, the compounds were thermally desorbed from the fiber in the gas chromatograph injector at 250°C for 2 min. In real water samples the desorption time was increased to 5 min to avoid carryover effects. The performance of the fiber was checked and at least 40 samples were analyzed with the same fiber.

Samples from Ebro river, marinas and fishing ports were filtered through a 0.45-μm membrane filter (MSI, Wetsboro, MA, USA) before analysis.

3. Results and discussion

3.1. Chromatographic separation

The separation of the three pesticides studied, Irgarol 1051, dichlofluanid and 4-chloro-3-methylphenol, was optimized before the parameters which affect the SPME process. The optimal conditions are reported in the experimental part.

The linearity of the response for each pesticide, the limits of detection (calculated by Winefordner and Long's method with the *K* value equal to 6 [18]), the repeatability and reproducibility of the response between days were studied under full scan and selected ion monitoring (SIM) by directly injecting 2 μl of standard solutions in ethyl acetate. In the full scan mode, only the base peak of each pesticide (182 for Irgarol 1051, 123 for dichlofluanid and 107 for 4-chloro-3-methylphenol) was selected for quantifying. Two ions were selected from the spectrum of each compound to quantify the response under SIM mode: 182 (100) and 253 (71) for Irgarol 1051; 123 (100) and 167 (37) for dichlofluanid; and 107 (100) and 142 (96) for 4-chloro-3-methylphenol; the values in parentheses give the relative abundance (%) of each peak in the spectrum.

3.2. Optimization of the SPME process

Taking into account the characteristic of the compounds to be determined and previous experience of our group [13], a polyacrylate fiber was selected for this study.

The effect of various parameters on the SPME process was evaluated. The main parameters, the sample pH, the addition of NaCl to the sample, the temperature of the absorption process and the absorption time, were optimized by using standard solutions containing 30 μg l^{-1} of each pesticide in Milli-Q water under full scan acquisition mode. The absorption conditions chosen for the optimization process were 45 min at 40 °C. The desorption conditions were 2 min at 250 °C [13].

3.2.1. Effect of the pH

Three pH values were tested: the pH of the sample was about 6 and it was modified to values of 2.5 and 4 by adding hydrochloric acid before the SPME process. No salt was added. The results are shown in Fig. 1a The pH of the sample solution had no significant effect on the extraction efficiency of dichlofluanid, but higher pH values improved the recovery of Irgarol 1051. For 4-chloro-3-methylphenol, the extraction was most efficient at pH 4, because the decrease in pH causes the acid–base equilibrium of the hydroxyl group to shift towards the neutral form, which has a higher affinity for the fiber [19]. On the basis of the results, the pH selected was pH 6 which means that the pH of the sample did not have to be modified.

3.2.2. Effect of salt addition

The effect of increasing the ionic strength of the sample was determined with standard solutions containing 0–360 g l^{-1} of sodium chloride. The data of Fig. 1b show that the expected salting-out effect was indeed observed for 4-chloro-3-methylphenol [15] but the effect of Irgarol 1051 and dichlorofluanid was rather limited; best results were obtained at 180 g l^{-1}, which was used in all further studies.

3.2.3. Effect of the temperature of the absorption process

Fig. 2a shows that the amount of compounds

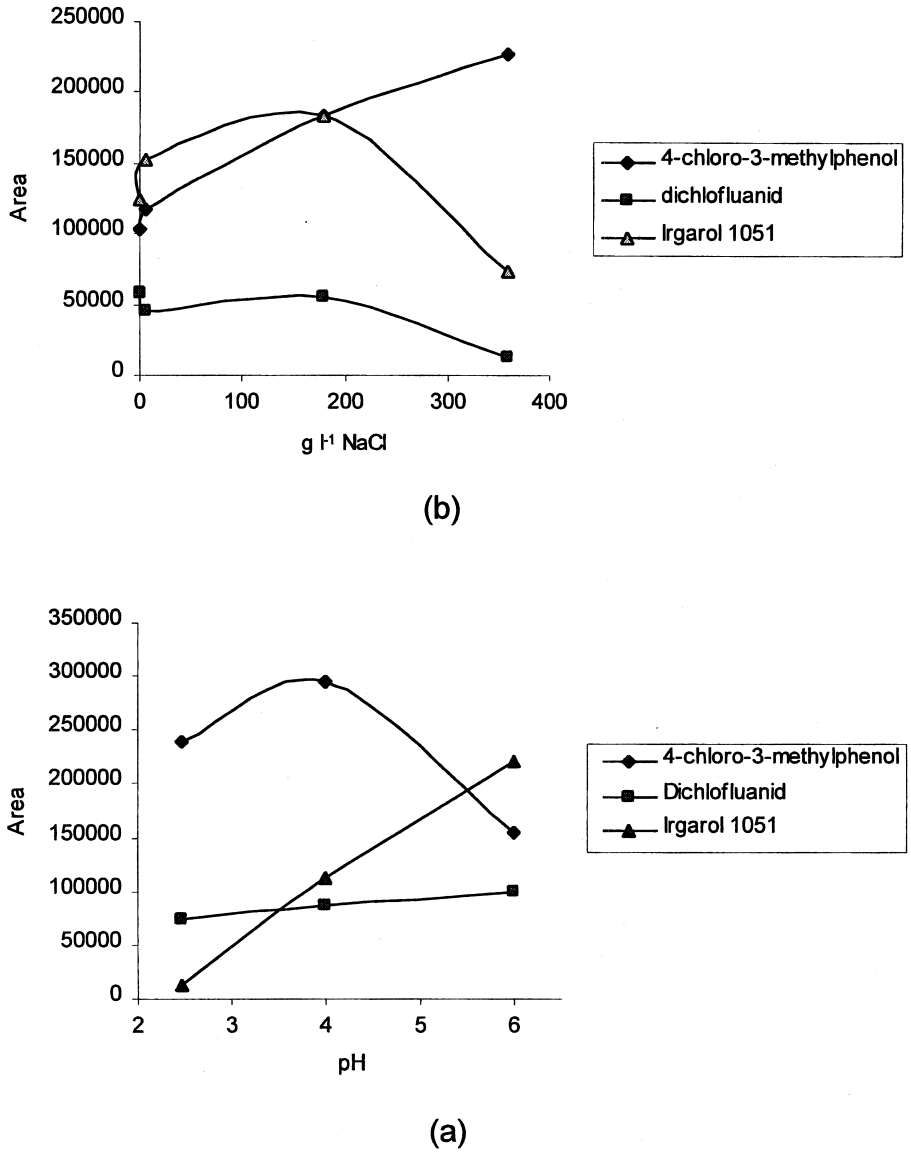

Fig. 1. Effect of varying the pH (a) and the NaCl addition to the sample (b) for the three pesticides. For experimental conditions, see Section 3.2.

extracted increases up at 60°C which was selected for further work. The decrease at higher temperatures can be attributed to the exothermic nature of the absorption step [12–14].

3.2.4. Effect of the absorption time

Absorption times were varied from 30 to 90 min at an absorption temperature of 60°C. Fig. 2b shows that the extraction efficiency depends on the compound studied. Equilibrium was reached within 60 min for all compounds and this absorption time was selected.

3.3. Analysis of real samples

Firstly, the performance of the method was tested

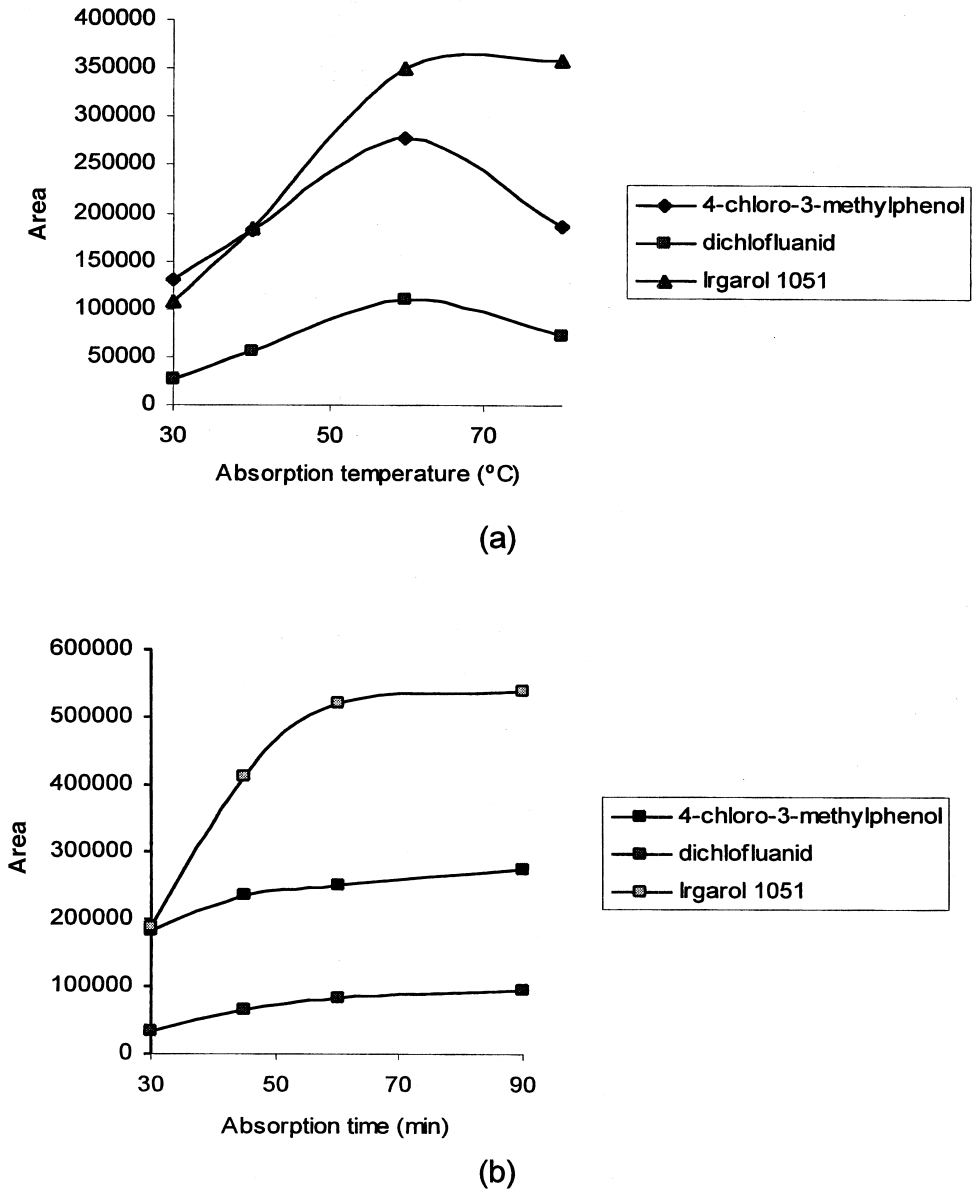

Fig. 2. Effect of the temperature (a) and the time (b) of the absorption process on the amount of pesticides extracted. For experimental conditions, see Section 3.2.

with marina water samples. NaCl was added to sea water samples taking into account the initial NaCl concentration of these samples. The desorption time was 5 min to avoid memory effects. First, a blank of Tarragona marina water sample in the full scan mode was analyzed in order to verify the presence of different peaks at the same retention time as the pesticides being studied. Some peaks appeared in the blank chromatogram but none appeared at the retention times of the pesticides, so this sample was used as a blank. The linearity, limits of detection, and the repeatability and the reproducibility of the

Table 1
Analytical data for SPME–GC–MS of target analytes in Tarragona marina water samples

Compounds	Full scan					SIM				
	Linearity range ($\mu g\ l^{-1}$)	r^2	LOD ($\mu g\ l^{-1}$)	Repeatability (%, $n=5$)[a]	Reproducibility (%, $n=5$)[a]	Linearity range ($\mu g\ l^{-1}$)	r^2	LOD ($\mu g\ l^{-1}$)	Repeatability (%, $n=5$)[b]	Reproducibility (%, $n=5$)[b]
4-Chloro-3-methylphenol	2–30	0.9971	0.5	12	16	0.2–10	0.9963	0.06	12	13
Dichlofluanid	10–30	0.9999	3.0	24	26	0.2–10	0.9910	0.08	18	19
Irgarol 1051	1–30	0.9947	0.2	17	19	0.2–10	0.9989	0.05	10	15

[a] Determined at 15 $\mu g\ l^{-1}$.
[b] Determined at 0.5 $\mu g\ l^{-1}$.

method were checked under full scan and SIM modes (Table 1). Although the levels which can be determined are low enough to detect Irgarol in marinas (typically 100–1700 ng l^{-1}) it does not enable low levels such as those which can be present in coastal and estuarine waters (1–40 ng l^{-1}). However, this level can be lowered by using more sensitive detection, such as tandem mass spectrometry [4]. Fig. 3 shows the chromatograms of a Tarragona marine water sample and the same sample spiked at 15 $\mu g\ l^{-1}$ of each pesticide.

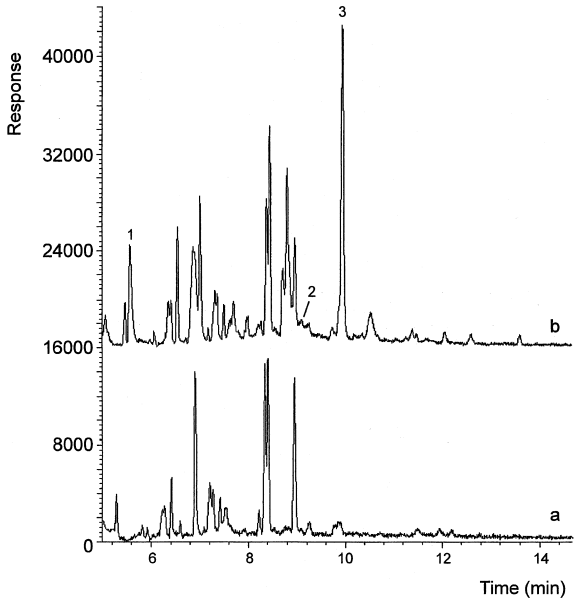

Fig. 3. Chromatograms obtained by SPME–GC–MS under full scan mode of acquisition of (a) Tarragona marina water and (b) Tarragona marina water spiked with a standard solution of pesticides at a concentration of 15 $\mu g\ l^{-1}$ level. Peaks: (1) 4-chloro-3-methylphenol, (2) dichlofluanid, (3) Irgarol 1051.

Various marina, fishing port and Ebro river water samples were also analyzed by SPME–GC–MS under full scan mode, and Irgarol 1051 was detected in some of them. Fig. 4 shows the total ion chromatogram obtained when a water sample from the fishing port in Cambrils (Tarragona) was analyzed by SPME–GC–MS under full scan acquisition mode. As can be observed, there is one peak that appears at the same retention time as Irgarol 1051 and the presence of this compound was confirmed by comparing the experimental spectrum and the corresponding standard. Fig. 4 also shows the extracted ion chromatogram of Irgarol 1051 and the insert corresponding to the spectrum of the peak. However, it could not be quantified because Irgarol 1051 was found in a concentration between the detection limit and the quantification limit of the method. Quantification was possible when the SIM acquisition mode was used and the concentration of Irgarol 1051 was found to be 0.3 $\mu g\ l^{-1}$.

4. Conclusions

SPME–GC can be used to rapidly determine Irgarol 1051, dichlofluanid and 4-chloro-3-methylphenol in different environmental waters. Optimum absorption required 60 min at 60°C. NaCl should be added to improve the extraction efficiency; the pH of the samples was not modified. SPME–GC–MS enables the compounds studied to be determined at 0.2 $\mu g\ l^{-1}$ in real water samples.

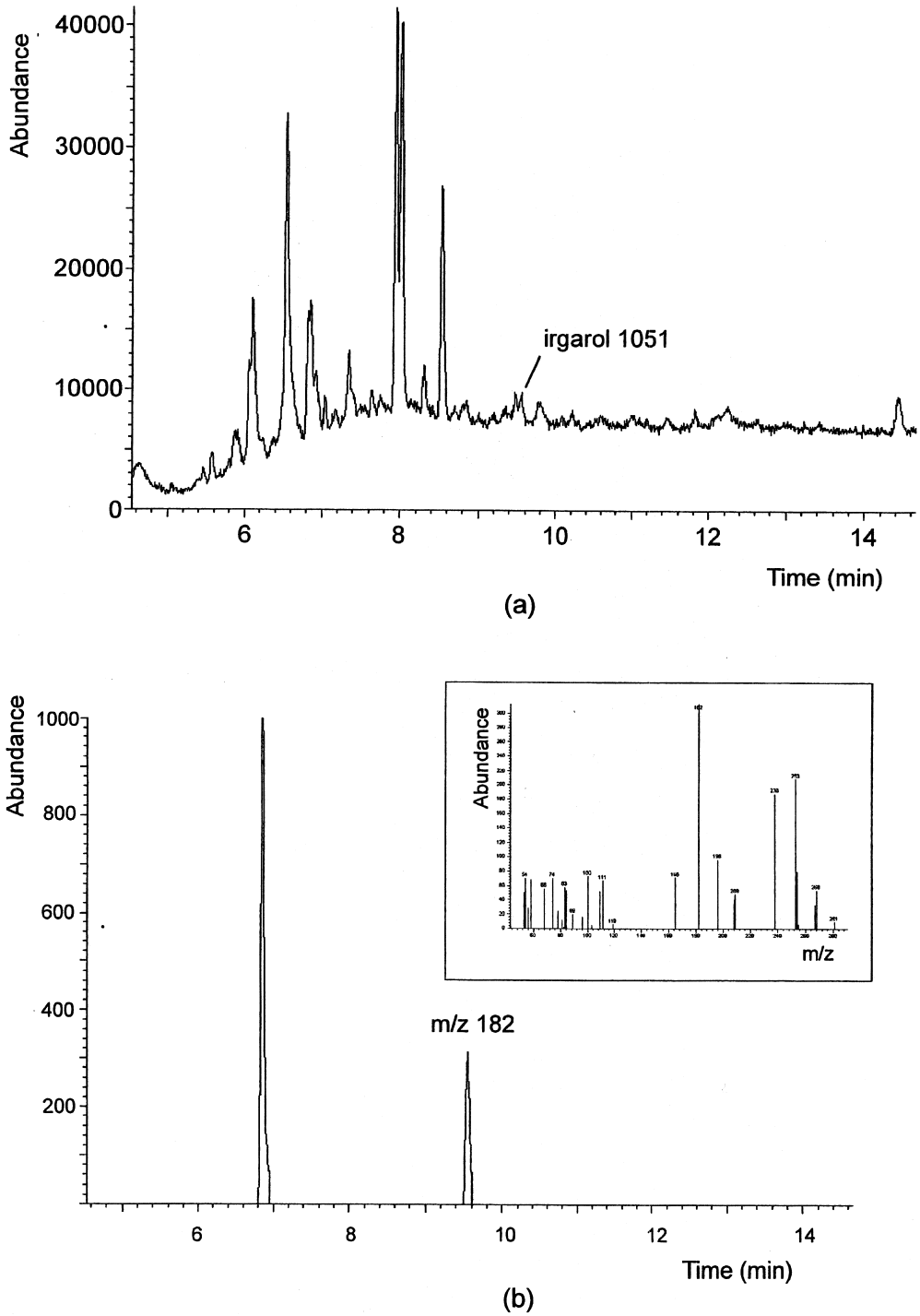

Fig. 4. (a) Full scan chromatogram obtained by SPME–GC–MS of a water sample from the fishing port in Cambrils (Tarragona), and (b) the extracted-ion chromatogram of Irgarol 1051 (m/z 182). The insert in b shows the spectrum of the Irgarol 1051 peak.

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