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Stir bar sorptive extraction and large volume injection gas chromatography to determine a group of endocrine disrupters in water samples

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Abstract

Stir bar sorptive extraction (SBSE) combined with gas chromatography (GC) with mass spectrometric detection (MS) has been applied to determine a group of suspected endocrine disrupters in water samples. One centimeter stir bars coated with PDMS were used to extract the analytes and then solvent desorption was carried out. The absorption and desorption parameters in SBSE were optimized and large volume injection was used with a programmed temperature vaporizer injector (PTV) in GC to enhance the sensitivity of the method. The linear range of some endocrine disrupters was between 0.05 and 5 μg l^{-1} and limits of detection were 0.01–0.24 μg l^{-1} under full scan acquisition mode. The repeatability and reproducibility of the method (n=5) for Ebro river water samples spiked at a level of 0.5 μg l^{-1} was below 13 and 23%, respectively. Recoveries between 42 and 96% were obtained with the exception of atrazine. The method was applied to analyze real water samples from the Ebro River and irrigation streams of Ebro Delta and some of the compounds studied (aldrin, dieldrin, 4,4′-DDE and 4,4′-DDT) were found in some of them between detection and quantification limits.

Keywords: Stir bar sorptive extraction; Large volume injection; Water analysis; Endocrine disrupters; Organochlorine compounds

1. Introduction

Interest in developing analytical methods for determining endocrine disrupting chemicals (EDCs) in the environment has increased considerably in recent years [1–4] because these compounds are suspected of disturbing the normal endocrine and reproductive functions of animals and humans [5]. A broad range of compounds including some pesticides, alkylphenol polyethoxylates (PAEs) and their metabolites, polychlorinated biphenyls (PCBs), some phthalate esters, bisphenol A, and some synthetic estrogens such as diethylstilbestrol, mestranol or 17α-ethynylestradiol have been reported to have endocrine disrupting effects [3–5]. The interest in the determination of pesticides is well known due to their adverse effects in the environment but now some of them are also considered endocrine disrupters [3–5].

Gas chromatography (GC) coupled to mass spectrometry (MS) [6–8] and high-performance liquid chromatography (HPLC) coupled to MS [3] or to

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ultraviolet detection (UV) [1,2] are the analytical techniques most frequently used. In some cases, biological techniques have also been applied to determine these compounds [9,10]. The analytical methods usually include a preconcentration technique for determining these compounds at the very low concentrations at which endocrine disrupters have adverse effects in the environment [1]. Solid-phase extraction (SPE) [1,3,7,8] is the most commonly used technique but solid-phase microextraction (SPME) has also been applied for the determination of these compounds [2,6]. Recently, Sandra and co-workers developed a new extraction technique based on the same extraction principles as SPME [11] but the sorbent, which is polydimethylsiloxane (PDMS), is placed on a stir bar. This technique is known as stir bar sorptive extraction (SBSE) and the coated stir bars are commercialized under the name of Twister. The amount of PDMS in the stir bar is higher than the amount on a SPME fiber so higher recoveries and therefore sensitivities are expected when SBSE is used. The stir bar is immersed in the sample or placed in the headspace (HS-SBSE) for a period of time at a fixed temperature and the analytes are extracted. SBSE can be used with both GC and HPLC but it is more frequently combined with GC because the desorption step is straightforward. In this case, the coated stir bar is usually introduced in a glass thermal desorption tube after extraction and it is placed in a commercialized thermal desorption unit mounted on the GC [11–14]. Another option is to use an organic solvent for desorption, usually when SBSE is followed by HPLC. Some studies have used SBSE with HPLC [15,16]. For example, Popp et al. [16] developed an off-line SBSE/HPLC method with solvent desorption for determining polycyclic aromatic hydrocarbons (PAHs) in water samples. To our knowledge, no applications of SBSE to determine the group of endocrine disrupters studied in the present paper have been published to date.

The main objective of this paper was to study the possibilities of SBSE and GC–MS using full scan acquisition mode for the determination of a group of compounds suspected to be endocrine disrupters in water samples. Another objective of this work was to determine whether solvent desorption in SBSE combined with large volume injection in gas chromatography could be an alternative to the more commonly used SBSE with thermal desorption.

2. Experimental

2.1. Reagents and standards

The endocrine disrupters studied were: hexachlorobenzene (HCB), atrazine, lindane, vinclozolin, malathion, aldrin, α-endosulfan, 4,4′-DDE, dieldrin, endrin and 4,4′-DDT. All the compounds except HCB were supplied by Riedel-de Häen (Seelze-Hannover, Germany) and the purity was higher than 99%. HCB was purchased from Fluka (Buchs, Switzerland) with a purity of 99%. The internal standard used was 1-chlorooctadecane and it was supplied by Aldrich (Steinheim, Germany) with a purity of 96%. A stock standard solution of 1000 mg l^{-1} of each compound was prepared in ethyl acetate from Merck (Darmstadt, Germany). Working standard solutions of 50 mg l^{-1} were prepared weekly in the same organic solvent. Stock and working standards were stored at 4 °C in a refrigerator. Aqueous solutions were prepared daily by diluting the working solution with Milli-Q water (Millipore, Bedford, MA, USA) or real water samples.

The sodium chloride (over 99.5% pure) which was added to the aqueous samples, was obtained from Riedel-de Häen (Seelze-Hannover, Germany). The pH of the aqueous samples was also adjusted with hydrochloric acid from Probus (Badalona, Spain). Isooctane from Merck was the organic solvent used to desorb the endocrine disrupters in the SBSE procedure.

2.2. Instrumentation

An Agilent 6890 gas chromatograph (Palo Alto, CA, USA) equipped with a split/splitless injector and a PTV injector was used. Detection was carried out with an Agilent 5973 mass spectrometer. The column used was an Agilent HP-5MS fused-silica capillary column (cross-linked 5% methyl silicone) of 30 m×0.25 mm I.D. with a phase thickness of 0.25 μm. A PTV injector using solvent vent mode and a liner of 7 cm×2 mm I.D. packed with 0.014 g of Tenax from Supelco (Bellefonte, PA, USA) were

used. Two six-port Valco valves (Houston, TX, USA) automatically controlled by the GC–MS software were used for the large volume injection in the gas chromatograph [20].

The stir bars (10 mm long×3.2 mm O.D.), coated with an extracting phase of PDMS (63 μl), are commercialized by Gerstel (Mülheim an der Ruhr, Germany). A stirrer and heater unit from Selecta (Abrera, Spain) was used to perform the SBSE process.

2.3. Optimum conditions

2.3.1. Chromatographic separation and detection

The optimized temperature program used for separation of analytes was as follows: the initial temperature was 60 °C, this temperature was held for 9.90 min and then it was increased to 270 °C at 30 °C min^{-1}. This temperature was held for 7 min. The total run time was 23.90 min and the solvent delay 14 min. The detector was set at 280 °C and the helium carrier gas was maintained at a flow rate of 1.2 ml min^{-1}.

The ion energy used for electron impact ionization (EI) in the mass spectrometer was 70 eV and the mass range scanned was 50–350 m/z under full-scan acquisition mode. The MS was tuned to m/z 69, 219 and 502 for EI corresponding to perfluorobutylamine (PFBA). Full-scan acquisition mode was used and the base peak of each pesticide (284 for HCB, 200 for atrazine, 181 for lindane, 212 for vinclozolin, 125 for malathion, 263 for aldrin and endrin, 79 for dieldrin, 195 for α-endosulfan, 246 for 4,4′-DDE, 235 for 4,4′-DDT and 85 for 1-chlorooctadecane, the internal standard), was selected for quantification.

2.3.2. SBSE procedure

Stir bars were conditioned by placing them in the split/splitless injector under He flow (1.2 ml min^{-1}) for 4 h at 300 °C. A blank run was carried out after the conditioning process to check that the sorbent had not caused any spurious peaks in the chromatogram.

Ten milliliters of an aqueous sample containing the endocrine disrupters were introduced in a vial. The concentration of NaCl in the aqueous sample was 10 g l^{-1} and the pH was not modified. The twister was immersed in the vial and it was stirred in the sample for 60 min at 50 °C and 1200 rpm. After this time, the stir bar was removed from the vial and the water remaining on the surface was dried with a lint-free tissue from Agilent Technologies. The analytes were desorbed by introducing the stir bar in a 4-ml vial, containing 1 ml of isooctane, in the stirrer unit (1200 rpm) for 30 min at room temperature. The internal standard, 1-chlorooctadecane, was added to the 1 ml of isooctane containing the endocrine disrupters to obtain a concentration of 25 μg l^{-1}. When real samples were analyzed, an aliquot of 200 μl of this sample was then injected in the PTV injector, which was at a temperature of 65 °C, at 47 μl min^{-1} by a syringe pump and the split valve was maintained opened at 50 ml min^{-1} for 2 min to remove the isooctane. After this time, the split valve was closed and the analytes retained in the Tenax were desorbed by increasing the temperature of the injector from 65 to 300 °C at 12 °C s^{-1}.

Real samples (Ebro river and irrigation streams of Ebro Delta water) were filtered through a 0.45-μm membrane filter (MSI, Wetsboro, MA, USA) before analysis.

3. Results and discussion

3.1. Optimization of the SBSE procedure

The various parameters affecting the SBSE process were optimized in order to increase the efficiency of the extraction and decrease the limits of detection of the method. In the extraction step, the time and temperature of extraction, sample pH, and addition of NaCl and organic solvent to the sample were optimized. In the solvent desorption step, the volume of organic solvent, time and temperature were optimized.

The organic solvent used for desorption and the internal standard were selected prior to the optimization of absorption and desorption steps in the SBSE process. Isooctane was used as organic solvent for desorption since it can be used with GC and it is compatible with the PDMS-coated stir bars [17]. We tried adding the internal standard (1-chlorooctadecane) before the SBSE process but the amount of 1-chlorooctadecane extracted was very low. For this reason, 1-chlorooctadecane was added to 1 ml of

isooctane after desorption to obtain a concentration of 25 μg l^{-1} of 1-chlorooctadecane. The optimization of the SBSE parameters was carried out by injecting 20 μl of the extract in the PTV injector. PTV parameters were set at the same values as 200 μl were injected except for the split valve time which was 25 s due to the lower volume of solvent to remove.

3.1.1. Optimization of the absorption process

To optimize absorption time, 10 ml Milli-Q water spiked at 5 μg l^{-1} of endocrine disrupters was used and the absorption temperature was set to 50 °C. The sample pH was not modified and NaCl was not added. Desorption was carried out in a magnetic stirrer unit (1200 rpm) by placing the stir bar with the analytes in a vial containing 1 ml of isooctane for 15 min at room temperature (25 °C). Different times between 15 and 60 min were tested and the results showed that the amount of analyte extracted increased with the absorption time. Times higher than 60 min were not tested because of the increase in the time of analysis. An absorption time of 60 min was selected for further analysis because it was a good compromise between time of analysis and response.

After the absorption time had been fixed, the absorption temperature was optimized by maintaining the other parameters (pH, NaCl) at the values previously used. A range of temperatures from room temperature (25 °C) to 70 °C was tested and results were best at 50 °C so this was the temperature selected for the next experiments.

The effect of modifying the pH and the addition of NaCl to the sample were also studied. Several pH values were tested (2, 4.8 and 6) but results were best when the pH of the sample was not modified (pH 4.8) so this was the pH selected. NaCl concentrations ranging from no salt addition to 360 g l^{-1} (NaCl saturation conditions) were tested and Fig. 1 shows how the amount extracted evolved when the salt concentration in the aqueous sample increased. As can be seen, the recoveries were higher at 10–15 g l^{-1} of NaCl for most of the compounds studied. A NaCl concentration of 10 g l^{-1} was selected for further experiments by taking into account the recoveries of all the analytes studied.

The effect of adding an organic solvent to the

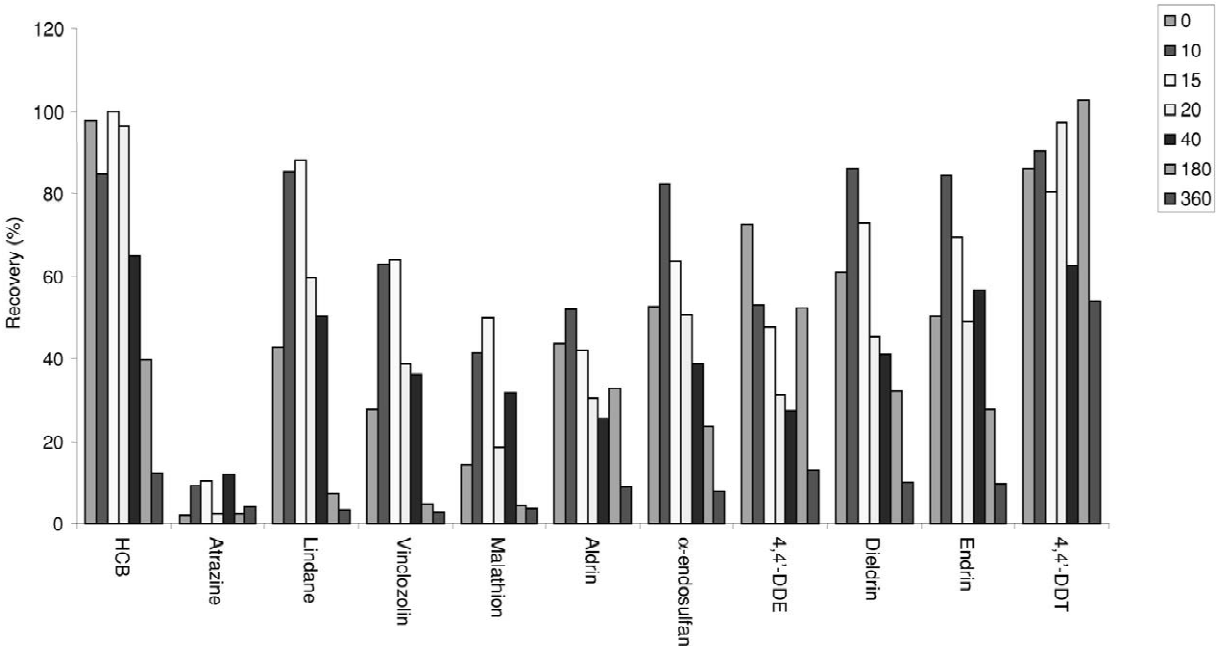


Fig. 1. Recoveries ($n=2$) of endocrine disruptors (%) at different NaCl concentrations (g l^{-1}) in the aqueous sample at a spiking level of 5 μg l^{-1}.

aqueous sample before extraction was also evaluated. Some authors [18,19] have observed that adding small amounts of organic solvents to the aqueous sample prevents compounds from being retained in the walls of the vials during extraction and therefore improves recovery. Various amounts of acetonitrile were added to the aqueous sample (0–5%). The recovery of compounds eluting last was not affected when the percentage of acetonitrile increased but for most compounds, the recoveries slightly decreased. For this reason, acetonitrile was not added in further experiments.

3.1.2. Optimization of desorption process

Various periods of time (5–60 min) were tested and the amount of analytes extracted did not increase at times higher than 30 min so this desorption time was selected for further experiments.

The effect of the volume of isooctane on the desorption of analytes was also studied in a range from 0.5 to 5 ml. The recoveries remained constant at volumes higher than 1 ml so we continued using this desorption volume in the SBSE/LVI-GC–MS method.

To optimize desorption temperature, we tested temperatures between room temperature (25 °C) and 50 °C. Results were best at room temperature so this was the desorption temperature used in the next experiments. In order to determine whether there was carryover in the stir bar after desorption, a second desorption was carried out. No peaks corresponding to the endocrine disrupters were observed in the chromatogram so analytes were fully desorbed under these conditions.

Sonication was also tested to see whether it was more effective than magnetic stirring and whether it could decrease the desorption time. The results obtained with sonication were worse than those obtained with magnetic stirring so magnetic stirring was used for desorption.

3.2. Performance of the SBSE/LVI-GC–MS method

Once the parameters of both the absorption and desorption processes had been optimized, the performance of the method was validated with Milli-Q water samples using SBSE/LVI-GC–MS. We injected an aliquot of 200 μl of the extract in order to decrease detection limits.

When Milli-Q water was spiked with different levels of endocrine disruptors under full scan acquisition mode, linearity was good for most compounds between 0.05 and 5 $\mu g\ l^{-1}$ (it was calculated using 10 standard solutions with a concentration between this range). The limits of detection (LODs) were calculated using the Winefordner and Long method [21], with a K value equal to 6, and they were between 0.01 and 0.2 $\mu g\ l^{-1}$. The repeatability of the method was determined by performing five extractions for Milli-Q water with a concentration of 0.5 $\mu g\ l^{-1}$ of endocrine disruptors and relative standard

Table 1
Linear range, determination coefficients, limits of detection, repeatability, reproducibility and recoveries for Ebro River water samples by SBSE/LVI-GC–MS

Compound	Linear range ($\mu g\ l^{-1}$)	r^2	LOD ($\mu g\ l^{-1}$)	Repeatability ($n=5$)[a]	Reproducibility ($n=5$)[a]	Recovery (%)[a]
Hexachlorobenzene	0.05–5	0.9979	0.01	13	20	85
Atrazine	0.2–5	0.9884	0.06	6	17	9
Lindane	0.1–5	0.9994	0.03	10	23	85
Vinclozolin	0.05–5	0.9991	0.02	11	21	63
Malathion	0.2–5	0.9969	0.05	2	5	42
Aldrin	0.1–5	0.9951	0.08	3	4	52
α-Endosulfane	0.05–5	0.9977	0.02	8	18	82
4,4′-DDE	0.5–2.5	0.9826	0.20	6	8	53
Dieldrin	0.05–5	0.9965	0.02	3	11	86
Endrin	0.1–5	0.9963	0.03	9	13	84
4,4′-DDT	0.5–5	0.9880	0.24	13	19	96

[a] Determined at a concentration of endocrine disrupters of 0.5 $\mu g\ l^{-1}$.

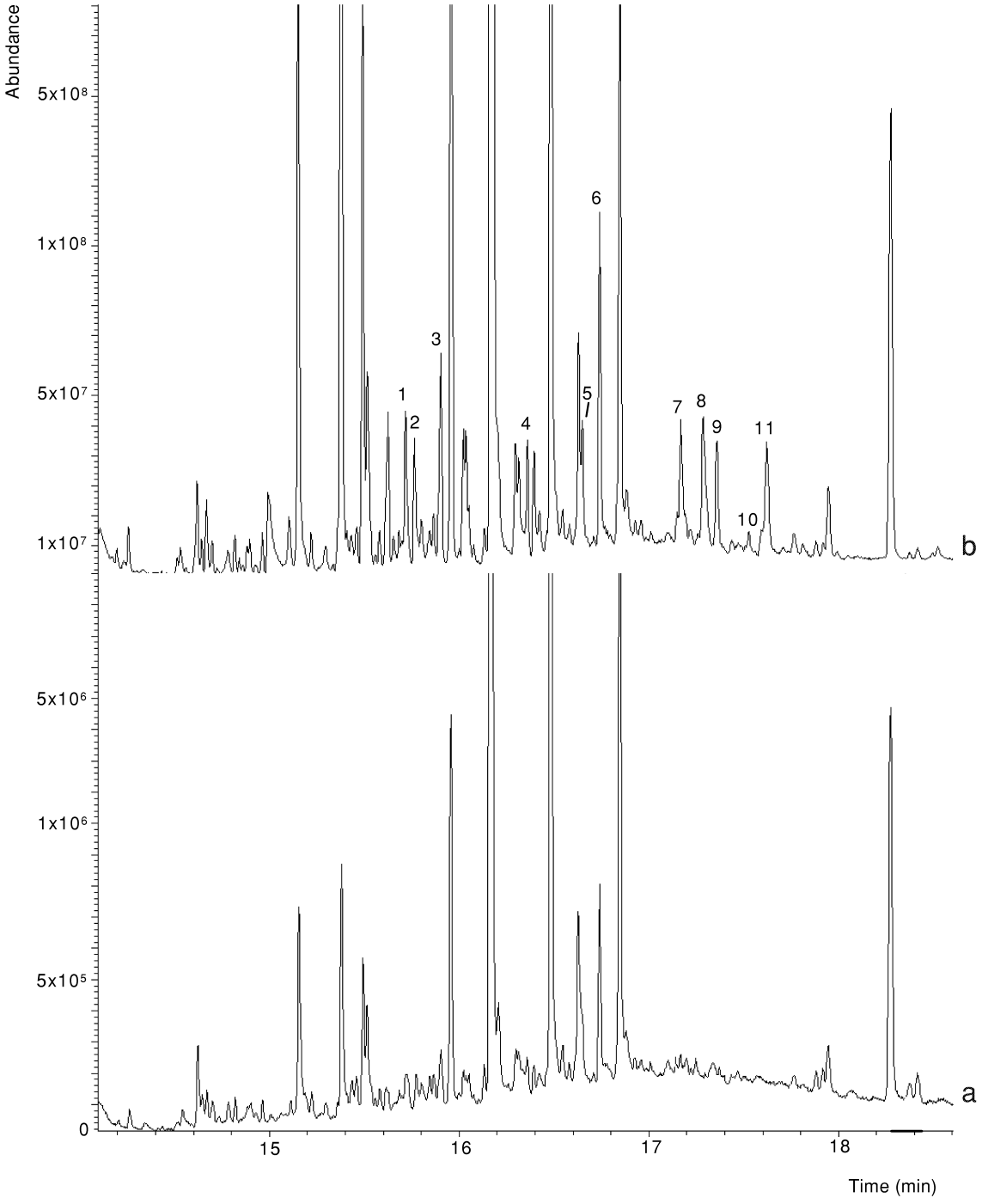
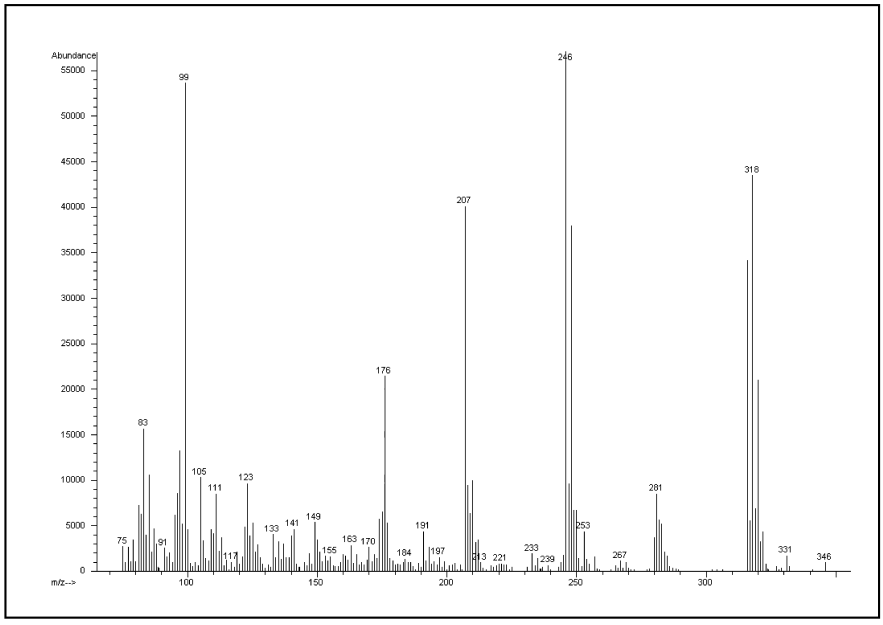


Fig. 2. Chromatograms of: (a) Ebro River water; (b) Ebro River water spiked with 0.5 μg l^{-1} of endocrine disruptors. Peak assignment: (1) Hexachlorobenzene; (2) Atrazine; (3) Lindane; (4) Vinclozolin; (5) Malathion; (6) Aldrin; (7) α-Endosulfan; (8) 4,4′-DDE; (9) Dieldrin; (10) Endrin; (11) 4,4′-DDT.



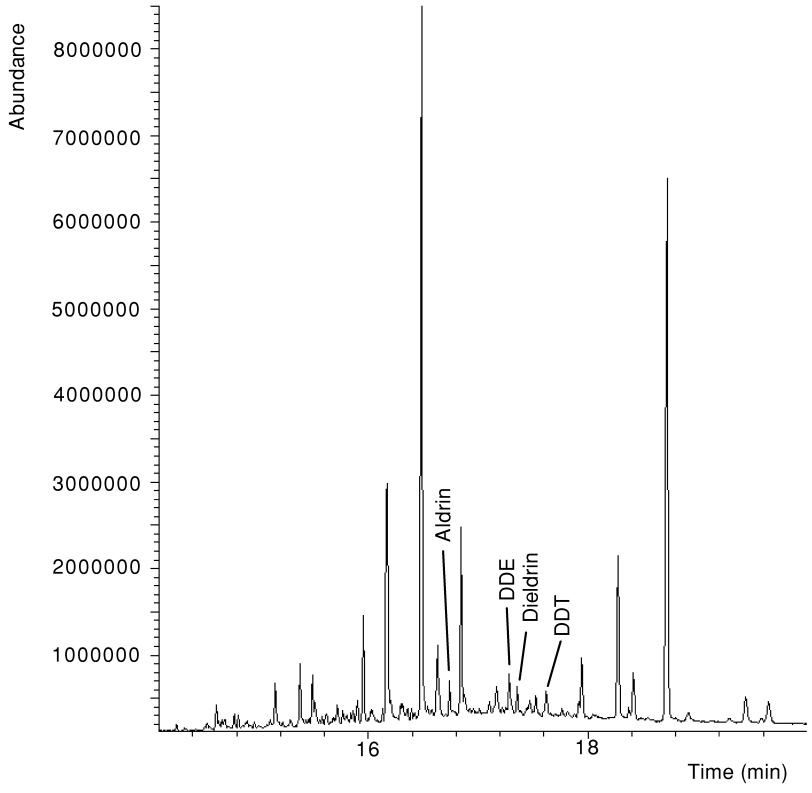


Fig. 3. Chromatogram obtained by SBSE/LVI-GC–MS of 10 ml of water sample from an irrigation stream in the Ebro Delta. The insert shows the spectrum of 4,4′-DDT.

deviations, RSD (%), were between 2 and 10%. The reproducibility of the method was also checked at the same concentrations as repeatability and RSD values ($n=5$) were between 3 and 15%.

The performance of the method was also checked with Ebro River water samples. First, an Ebro River sample was analyzed and no peak was found at the same retention times of the compounds studied. Table 1 shows the results with the SBSE/LVI-GC–MS for Ebro River water using full scan acquisition mode. The slopes of calibration curves were statistically comparable (*F*- and *t*-tests were used with $\alpha=0.05$) with those obtained with Milli-Q water so matrix interference was not significant. The limits of detection of the method can be decreased if selected ion monitoring (SIM) acquisition mode is used in the mass spectrometry detector, but we used full scan mode because it identifies and quantifies the analytes in the same analysis. Fig. 2 shows the chromatograms of a water sample from Ebro River unspiked and spiked with the endocrine disrupters. As can be seen in Fig. 2, some peaks appeared in the blank chromatogram at the same retention time as endocrine disrupters but the spectra were different.

Some of the compounds included in this study were determined in a previous work by our group [22] using SPME–GC–MS. The limits of detection in full scan mode of acquisition were similar to that obtained using the SBSE/LVI-GC–MS method. It should be taken into account that in SPME, all the compounds desorbed are introduced in the gas chromatograph for separation whereas in the SBSE method developed here, only 200 μl of 1000 μl was injected.

3.3. Analysis of real samples

Real water samples from different points along Ebro River and irrigation streams were analyzed with the SBSE/LVI-GC–MS method. In the chromatograms of some of the Ebro river water samples, some peaks appeared at the retention time of aldrin, dieldrin, 4,4′-DDT and 4,4′-DDE. They were assigned by comparing the sample spectrum and the standard solution spectra. Nowadays, these compounds are banned by an EU directive [23] but the fact that they were extensively used for decades has meant that they can still be found in water samples. For instance, Fig. 3 shows the total ion chromatogram (TIC) obtained under full scan acquisition mode for one of the water samples from an irrigation stream in the Ebro Delta. The insert in Fig. 3 shows the spectrum corresponding to the peak of 4,4′-DDT (match factor: 99%). These compounds were found at concentrations between detection and quantification limits of the method so they could not be quantified.

4. Conclusions

SBSE with solvent desorption and combined with large volume injection in GC can be used to determine a group of pesticides considered to be endocrine disrupters in water samples from different origins. With the SBSE/LVI-GC–MS method developed, the compounds studied can be determined at low $\mu g\ l^{-1}$ levels in real water samples. The parameters that affect both absorption and desorption procedures in SBSE must be optimized to improve the efficiency of the method.

Solvent desorption combined with large volume injection in GC has proved to be a useful alternative when a thermal desorption unit is not available.

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