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Solid-phase microextraction coupled to high-performance liquid chromatography to determine phenolic compounds in water samples ☆

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Abstract

Solid-phase microextraction (SPME) coupled to high-performance liquid chromatography (HPLC) with ultraviolet (UV) and electrochemical detection (ED) has been applied to determine 11 phenolic compounds considered priority pollutants by the US Environmental Protection Agency. 85 μm polyacrylate fibers were used to extract the analytes from the aqueous samples. Two different designs of the liquid chromatograph were compared in combination with SPME. Dynamic and static modes of desorption in both HPLC designs were compared and the variables affecting both absorption and desorption processes in SPME–HPLC were optimized. Static desorption in both HPLC systems showed better recoveries for the phenolic compounds. The performance of the SPME–HPLC–UV–ED method was evaluated with river water and wastewater samples. The method enabled the determination of phenolic compounds at low levels in these water samples.
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1. Introduction

Phenolic compounds, which are used in several industrial processes, are some of the most important contaminants present in the environment [1]. They are used to manufacture chemicals such as pesticides, in pulp processing, and as preservatives for woods, textiles and leather [1,2]. As a result, they are often found in waters [3,4], soils [5] and sediments [5,6]. Phenolic compounds, especially chlorophenols, are known for their toxicity and persistence in the environment [1–3]. For this reason, some of them have been included in the lists of priority pollutants of several countries and they are subject to legislation [3]. The European Union, for example, has set the maximum total and individual phenol permitted concentrations in water used for human consumption at 0.5 μg l^{-1}, and 0.1 μg l^{-1}, respectively [7].

High-performance liquid chromatography (HPLC) [8–10] is frequently used for the analysis of phenolic

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compounds because, unlike in gas chromatography (GC) no derivatization of compounds is needed [4–6,11–15]. In HPLC, electrochemical detection (ED) can be used to determine phenols at lower concentrations than ultraviolet absorbance detection, although UV detection is more sensitive for some nitrophenols [8]. To reach the concentration levels required by legislation, conventional methods of determining phenolic compounds involve preconcentration. Solid-phase extraction (SPE) is the most frequently used preconcentration technique for phenolic compounds [8–10,16].

A more recent extraction technique, solid-phase microextraction (SPME), was introduced by Pawliszyn [17]. SPME consists of an absorption and a desorption step. In the absorption step, a coated fused-silica fiber extracts the analytes from the sample matrix. In the desorption step, the analytes are desorbed from the fiber and introduced into the analytical column for separation. Usually, SPME is combined with GC, placing the fiber in the hot injector of the gas chromatograph, where the analytes are thermally desorbed. SPME and HPLC were first coupled in 1995 [18]; the system has been commercially available since 1996. An organic solvent (static desorption) or the mobile phase (dynamic desorption) is used to desorb the analytes from the SPME fiber. To date, SPME has been successfully used to determine phenols and nitrophenols in water [4,12,13,15] and soil [6,14,19,20] samples. Some of these papers have included a derivatization step in the analytical method to enable the GC determination of some of the compounds [12,13].

The aim of this paper is to develop an SPME–HPLC method using 85 μm polyacrylate fibers, to determine in water samples 11 phenols that the US Environmental Protection Agency (EPA) listed as priority pollutants. It is also an objective of this paper to compare two liquid chromatograph designs and the dynamic and static modes of desorption. These designs differ in the elution of the compounds retained in the SPME fiber. In one of the designs, the mobile phase desorbs the analytes. In the other, only the organic solvent of the mobile phase acts as the desorbing agent. The method developed has been applied for the analysis of real water samples from the Ebro river and from wastewater treatment plants.

2. Experimental

2.1. Reagents and standards

The compounds studied were the 11 phenolic EPA priority pollutants: phenol (Ph), 4-nitrophenol (4-NP), 2,4-dinitrophenol (2,4-DNP), 2-chlorophenol (2-CP), 2-nitrophenol (2-NP), 2,4-dimethylphenol (2,4-DMP), 2-methyl-4,6-dinitrophenol (2-M-4,6-DNP), 4-chloro-3-methylphenol (4-C-3-MP), 2,4-dichlorophenol (2,4-DCP), 2,4,6-trichlorophenol (2,4,6-TCP) and pentachlorophenol (PCP). All compounds were purchased from Aldrich (Steinheim, Germany), except PCP, which was purchased from Jansen (Geel, Belgium). A stock standard solution of 2000 mg l^{-1} of each compound was prepared in methanol. Working standard solutions of 100 mg l^{-1} were prepared weekly in methanol. Stock and working standards were stored at 4 °C in the refrigerator. The aqueous solutions were prepared daily by diluting the working solution with water (Milli-Q and real water samples). Sodium chloride (over 99.5% pure), was obtained from Riedel-de Häen (Seelze-Hannover, Germany), and hydrochloric acid from Probus (Badalona, Spain).

HPLC-grade methanol from SDS (Peypin, France) and Milli-Q quality water (Millipore, Bedford, MA, USA), adjusted to pH 2.8 with acetic acid from Probus, were used in the preparation of the mobile phase. To adjust the ionic strength of the mobile phase, potassium chloride, supplied by BDH (Poole, UK), was added.

2.2. Instrumentation

The SPME device, the 85 μm polyacrylate fibers and the SPME–HPLC interface were purchased from Supelco (Bellefonte, PA, USA). Two chromatographic systems were used. The first was a Hewlett-Packard (HP) 1050 liquid chromatograph (Palo Alto, CA, USA) with a UV spectrophotometric detector, the second was assembled of two LC-10AD$_{VP}$ pumps, a DGU-14A degasser and a CTO-6AS oven from Shimadzu (Tokyo, Japan) with two detectors connected in series, an HP-1100 UV detector and a HP-1049A electrochemical detector, both from Hewlett-Packard. The Shimadzu HPLC system was a

modification of a conventional liquid chromatograph in which the mixing chamber was placed after the injection valve to ensure that only the organic solvent of mobile phase passes through the injection valve. Chromatographic data were collected and recorded by an HP-3365 Series II Chemstation. The separation was performed on an 25×0.4 cm I.D. Spherisorb ODS2 column packed with 5 μm particles (Teknokroma, Barcelona, Spain), in both chromatographic systems.

2.3. Chromatographic separation

The separation conditions were taken from a previous paper by our group [21] and were the same for both chromatographic systems. The mobile phase consisted of Milli-Q water [containing 1% (v/v) acetic acid and 0.5 g l^{-1} of KCl] as solvent A, and methanol as solvent B. The flow-rate of the mobile phase was 1 ml min^{-1}; the gradient profile was 25% B at 0 min, 50% B at 25 min, 100% B at 30 min, followed by isocratic elution for 5 min. The mobile phase was returned to its initial composition in 5 min. The oven temperature was set at 65 °C.

In the UV spectrophotometric detector, all compounds were detected at 280 nm, except PCP, which was detected at 302 nm. The potential used in the electrochemical detector was 1.0 V [21]. This detector worked in the amperometric mode, so the eluent had to contain KCl (0.5 g l^{-1}). A glassy carbon electrode and a solid state Ag/AgCl reference electrode were used. The surface of the electrode was electrochemically cleaned once a day by applying a cyclic treatment with alternate potentials. The working electrode was polished in the conventional way once a week.

2.4. SPME procedure

Before their first use, the 85 μm polyacrylate fibers were conditioned in the desorption chamber of the SPME–HPLC interface for 30 min, according to the supplier's instructions. After conditioning, a fiber blank was run. SPME was carried out by introducing 3.5 ml of aqueous samples into 4-ml vials. The samples were saturated with NaCl (360 g l^{-1}) and their pH was adjusted with HCl to 2.5 [4,20]. The 85 μm polyacrylate fiber was then immersed in the sample for 30 min at 50 °C. The samples were heated and stirred with a magnetic stirrer and heater unit from Selecta (Abrera, Spain) at a constant speed of 1400 rpm. The analytes were desorbed from the fiber and introduced into the chromatographic system by the commercial SPME–HPLC interface. The fiber was cleaned with Milli-Q water after each analysis to avoid damage due to crystallization of NaCl. At least 20 samples were analyzed by the same fiber before it was damaged by the double-tapered ferrule.

Real samples (from river and wastewater treatment plant) were filtered through a 0.45 μm nylon membrane filter (Whatman, Maidstone, UK) before analysis.

3. Results and discussion

3.1. Optimization of desorption process

Polyacrylate-coated fibers were selected for the SPME because good results were obtained with them previously for phenolic compounds [4,5,11–13]. Two modes of desorption are possible in SPME–HPLC: dynamic desorption and static desorption. In the dynamic mode, analytes are desorbed from the fiber by the moving stream of the mobile phase. In the static mode, the fiber is kept in the desorption chamber, filled with an organic solvent or the mobile phase, for a period of time.

The SPME–HPLC interface connected to the two HPLC systems (Shimadzu and Hewlett-Packard) were evaluated in both the dynamic and static modes of desorption with water samples containing the phenolic compounds at 0.14 mg l^{-1}. An absorption time of 20 min and a temperature of 45 °C were initially selected for the SPME absorption process [4].

In the first, conventional HPLC design (HP-1050), the mixing chamber was placed before the SPME desorption chamber (see Fig. 1a). First, the dynamic desorption mode was tested. The fiber, loaded with the analytes, was introduced into the desorption chamber, the valve was immediately switched from the load to the inject position and mobile phase at 1 ml min^{-1} was passed through the desorption

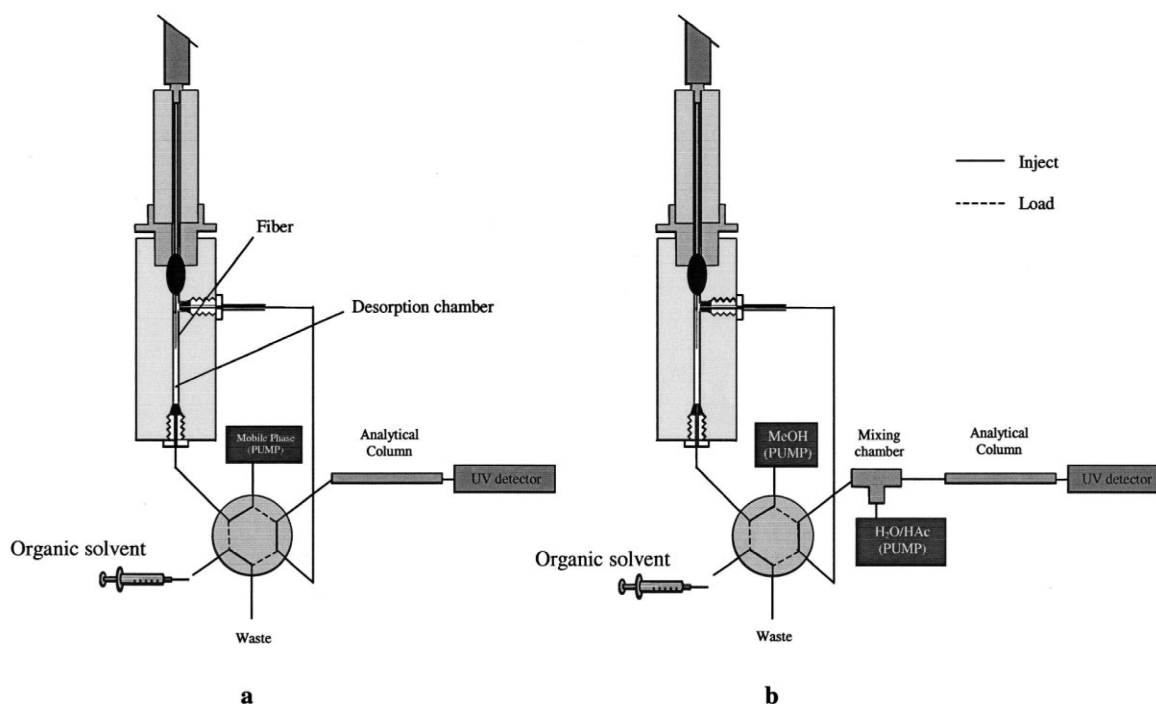

Fig. 1. Experimental set-up of the SPME–HPLC designs: (a) SPME interface connected to the HP-1050 HPLC system; (b) SPME interface connected to the Shimadzu HPLC system.

chamber for 5 min to effect complete desorption. The valve was then returned to the load position and the fiber was removed from the desorption chamber. The recoveries for the phenolic compounds in the dynamic mode are shown in Table 1. Although these values are low, they are typical in SPME. Phenol was not recovered under these conditions. In the static mode, the fiber was also placed in the desorp-

Table 1
Recoveries (%) obtained by extraction of 3.5 ml of Milli-Q water samples spiked with 0.14 mg l^{-1} for the two liquid chromatograph designs

Compound	HP-1050				Shimadzu		
	Dynamic	Static			Dynamic	Static	
		Mobile phase[a]	100% Methanol	100% Acetonitrile		100% Methanol	100% Acetonitrile
Ph	–	–	–	–	0.2	0.9	1.8
4-NP	0.8	1.0	1.7	1.2	0.7	1.8	2.2
2,4-DNP	0.6	0.9	1.6	1.4	1.4	2.1	2.2
2-CP	1.4	2.5	4.1	3.8	2.6	4.1	3.7
2-NP	0.7	0.6	2.2	2.3	1.7	2.3	2.1
2,4-DMP	2.1	2.8	5.7	6.7	4.7	5.7	5.4
2-M-4,6-DNP	*	0.8	4.9	*	3.1	5.9	5.3
4-C-3-MP	*	2.6	4.0	*	7.1	7.5	6.6
2,4-DCP	2.2	3.3	8.6	10.3	10.1	10.2	9.1
2,4,6-TCP	2.8	4.2	12.6	12.4	12.3	18.3	18.3
PCP	3.1	5.1	14.3	16.9	21.9	29.9	29.5

* Coelution of peaks.
[a] Initial composition of mobile phase (methanol–Milli-Q water, 25:75).

tion chamber but the valve was switched from the load position to the inject position only after 5 min.

Several experiments were carried out in the static mode. First, the desorption chamber was filled with the initial composition of the mobile phase (MeOH–Milli-Q water, 25:75). Five minutes were enough to desorb all the phenolic compounds. Phenol was not recovered in the static mode either, but the peak areas for the other phenolic compounds increased. We also evaluated static desorption using methanol and acetonitrile. They were introduced into the desorption chamber with a conventional HPLC syringe (see Fig. 1a). Table 1 shows that the results in the static desorption mode were best when an organic solvent was used: methanol and acetonitrile provided similar results and, in both cases, fronting peaks were obtained. This was probably due to an inefficient mixing between the mobile phase and the organic solvent introduced into the desorption chamber, because there were no fronting peaks when desorption chamber was full of mobile phase.

In the second HPLC design tested (Fig. 1b), the mixing chamber was placed after the desorption chamber so only the organic solvent (methanol) of the mobile phase passed through the desorption chamber. The peaks were not distorted. Again, desorption was studied in both the static and dynamic modes using methanol at a flow-rate of 0.250 ml min^{-1} [mobile phase flow-rate (25% aqueous methanol): 1 ml min^{-1}] in both modes. An additional experiment was carried out with acetonitrile in the desorption chamber to compare with the results from the HP-1050 liquid chromatograph. Peaks were not distorted. Table 1 shows that the second HPLC design was better than the HP 1050 SPME system in both the static and dynamic modes for the more apolar compounds. Recoveries were better in the static mode than in the dynamic mode. Since methanol and acetonitrile provided similar results, methanol, the organic solvent of the mobile phase, was selected for further experiments.

Desorption times ranging from 1 to 5 min were tested. The results are shown in Fig. 2. A desorption time of 2 min was selected because after this time recoveries did not increase significantly and in a subsequent analysis no peaks appeared at the re-

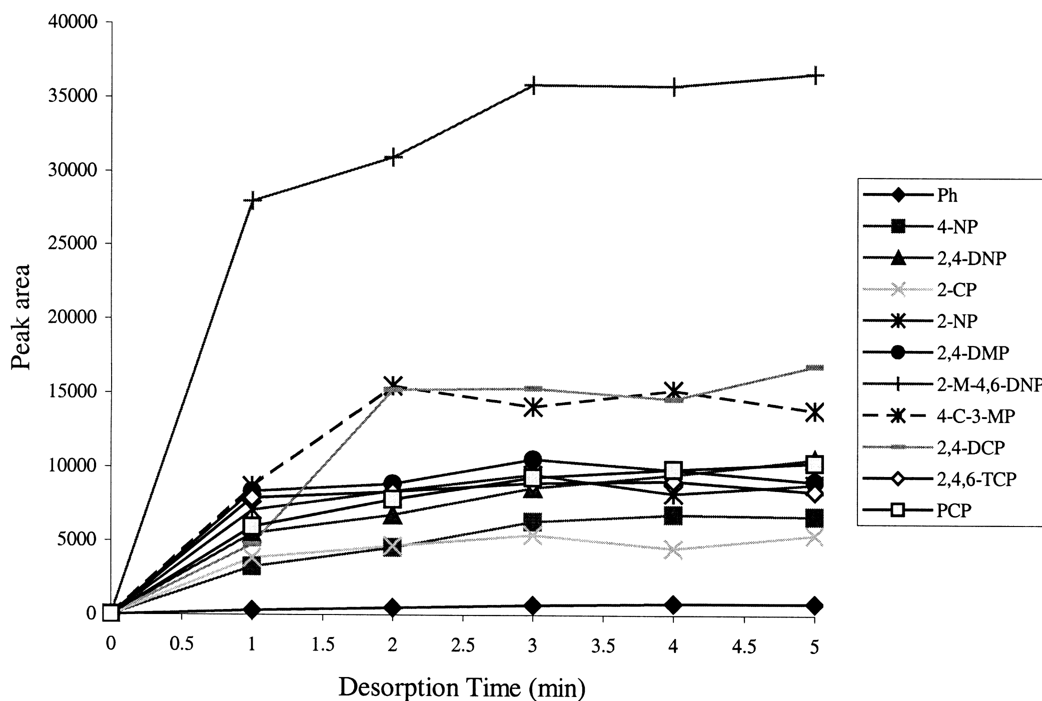

Fig. 2. Optimization of desorption time in the static mode for the Shimazdu HPLC design.

tention time of the phenolic compounds. After 2 min, the fiber could be removed from the SPME–HPLC interface and prepared for a new run.

3.2. Optimization of the absorption process

The factors affecting the SPME absorption process were optimized: i.e., the time (5–40 min) and temperature of absorption (25–60 °C), the addition of NaCl to the sample (0–360 g l^{-1}) and the pH of the sample (0.5–5). The concentration of phenolic compounds in Milli-Q water samples was maintained at 0.14 mg l^{-1} and the desorption parameters were fixed at the previously optimized values. The best results were obtained at 30 min; 50 °C, 360 g l^{-1} (saturated solution) and pH 2.5, allowing the analysis of a new sample every 47 min.

3.3. Performance of the SPME–HPLC method

Once the SPME parameters had been optimized, the method was checked by analyzing 3.5 ml Milli-Q water samples spiked with the compounds. An electrochemical detector was connected in series to the ultraviolet detector to enable low levels of some phenolic compounds to be determined [8]. However, some nitrophenols (2,4-DNP and 2-M-4,6-DNP) showed better responses with the ultraviolet detector, and so the two detectors connected in series were used. Also, the ratio of the responses from the two detectors was used to confirm the presence of these compounds in real water samples.

The linearity of the response was checked in the 0.005 to 30 mg l^{-1} range in the ultraviolet detector, and in the 0.05 to 100 μg l^{-1} range of standard solutions in the electrochemical detector. The linearity in both cases was good (R^2>0.9925) for most compounds, except phenol, for which it was 0.1–30 mg l^{-1} when UV was used, and 2,4-DNP and 2-M-4,6-DNP, for which it was 10–100 μg l^{-1} when ED was used. The limits of detection of the method, calculated by the Long and Winefordner criterion with a K of 6 [22], were between 1.0 and 18 μg l^{-1} for UV detection, and between 0.01 and 12 μg l^{-1} for ED. The repeatability and the reproducibility between days, calculated as relative standard deviation (RSD, n=5), were determined with Milli-Q water spiked with 1 μg l^{-1} for ED and with 0.1 mg l^{-1} for UV detection. In ED, the RSDs were under 12.2 and 15.3% for repeatability and reproducibility, respectively. In UV detection, the RSDs were under 13.2 and 18.5%, respectively.

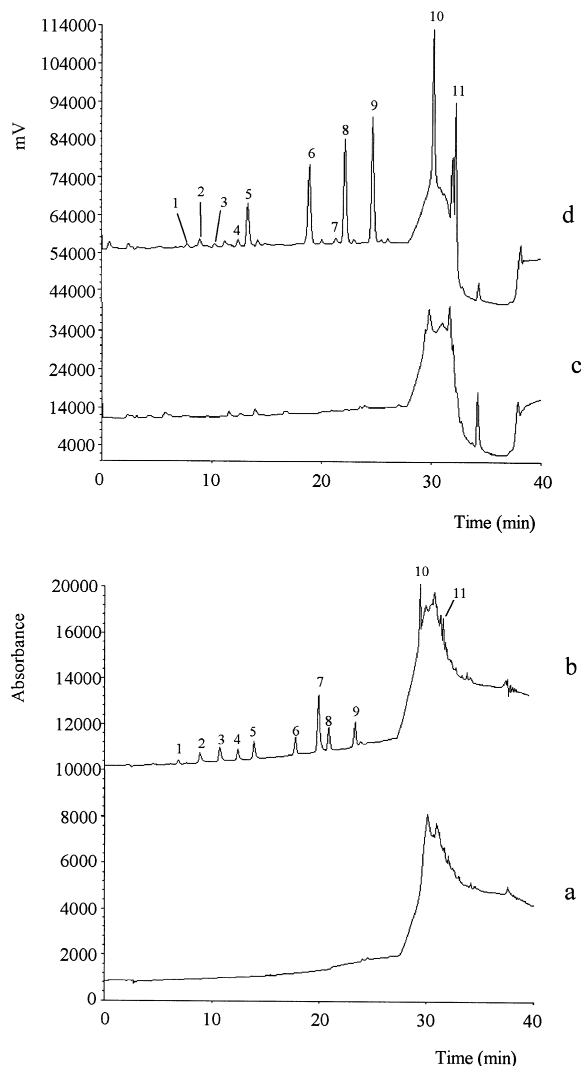

Fig. 3. Chromatograms obtained by SPME–HPLC–UV–ED of 3.5 ml of Ebro river water using (a, b) UV detection and (c, d) ED in the static mode of desorption. (a, c) Unspiked Ebro river water; (b) Ebro river water spiked with 0.1 mg l^{-1} of each compound; (d) Ebro river water spiked with 1 μg l^{-1} of each compound. Peaks: (1) Ph; (2) 4-NP; (3) 2,4-DNP; (4) 2-CP; (5) 2-NP; (6) 2,4-DMP; (7) 2-M-4,6-DNP; (8) 4-C-3-MP; (9) 2,4-DCP; (10) 2,4,6-TCP; (11) PCP.

Table 2
Linear range, determination coefficients, limits of detection and repeatability and reproducibility for Ebro river water by SPME–HPLC–UV–ED

Compound	UV detection					ED detection				
	Linear range (mg l^{-1})	R^2	LOD (μg l^{-1})	RSD (%)[a]	RSD (%)[b]	Linear range (μg l^{-1})	R^2	LOD (μg l^{-1})	RSD (%)[a]	RSD (%)[b]
Ph	0.1–30	0.9982	23	9.9	20.1	0.5–100	0.9982	0.1	13.2	14.2
4-NP	0.01–30	0.9961	3.6	8.9	9.9	0.2–100	0.9991	0.05	9.1	11.9
2,4-DNP	0.01–30	0.9979	4.1	14.0	14.4	50–100	0.9982	15	15.3	17.2
2-CP	0.01–30	0.9947	3.9	14.5	14.8	0.2–100	0.9956	0.06	6.8	9.3
2-NP	0.005–30	0.9933	1.6	11.3	12.2	0.05–100	0.9967	0.03	11.6	11.9
2,4-DMP	0.005–30	0.9966	1.8	11.9	12.3	0.05–100	0.9994	0.015	7.5	9.1
2-M-4,6-DNP	0.002–30	0.9981	0.4	12.7	14.6	10–100	0.9960	4.5	13.1	14.6
4-C-3-MP	0.01–30	0.9956	2.7	14.3	15.7	0.05–100	0.9985	0.013	6.4	11.2
2,4-DCP	0.01–30	0.9981	3.8	15.1	15.5	0.05–100	0.9929	0.02	7.1	13.4
2,4,6-TCP	0.005–30	0.9902	2.0	14.7	14.9	0.05–100	0.9936	0.017	9.6	11.9
PCP	0.01–30	0.9977	2.9	18.1	20.3	0.1–100	0.9967	0.05	4.2	8.1

[a] Under repeatability conditions ($n=5$).
[b] Under reproducibility between days conditions ($n=5$).

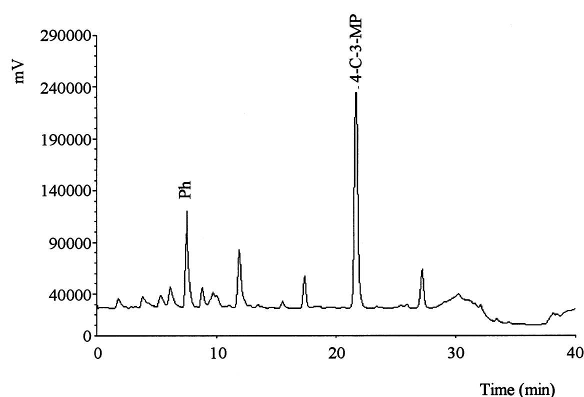

Fig. 4. Chromatograms obtained by the SPME–HPLC–ED method by extracting 3.5 ml of a wastewater sample.

3.4. Application to real samples

The method was validated with Ebro river water samples. The maximum content of humic and fulvic acids in these waters is 10 mg l^{-1} [23]. First, a blank of the Ebro river water was run to verify the absence of peaks at the retention time of phenolic compounds: no interference was found as shown in Fig. 3. The linear range, the limits of detection, and the repeatability and reproducibility of the method were also determined with Ebro river water, in the same way as with Milli-Q water. The results, shown in Table 2, were similar to those for Milli-Q water samples indicating that the presence of matrix components, such as humic and fulvic acids, did not interfere in the extraction of phenolic compounds. Fig. 3 also shows the chromatograms for the Ebro river water sample spiked with 1 $\mu g\ l^{-1}$ for ED, and 0.1 mg l^{-1} for UV detection.

Several wastewater samples were also analyzed. Fig. 4 shows the chromatogram of one of these samples. Phenol and 4-chloro-3-methylphenol were quantified using the calibration curves for ED because, for UV detection, the responses were between the limit of quantification and the limit of detection. The concentrations were 87 $\mu g\ l^{-1}$ for phenol and 7.4 $\mu g\ l^{-1}$ for 4-chloro-3-methylphenol.

4. Conclusions

Eleven phenolic EPA priority pollutants were successfully determined of SPME–HPLC in real water samples. One sample could be analyzed every 47 min.

The modified HPLC design, in which the desorption chamber for SPME was placed before the mixing chamber of the mobile phase, provided better results than coupling the SPME interface to the conventional HPLC design. With this system, recoveries were higher and the peaks were not distorted.

Acknowledgements

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