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Solid-phase microextraction coupled to liquid chromatography for the analysis of phenolic compounds in water

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Abstract

Solid-phase microextraction (SPME) coupled to high-performance liquid chromatography (HPLC) has been applied to the analysis of priority pollutant phenolic compounds in water samples. Two types of polar fibers [50 μm Carbowax–templated resin (CW–TPR) and 60 μm polydimethylsiloxane–divinylbenzene (PDMS–DVB)] were evaluated. The effects of equilibration time and ionic strength of samples on the adsorption step were studied. The parameters affecting the desorption process, such as desorption mode, solvent composition and desorption time, were optimized. The developed method was used to determine the phenols in spiked river water samples collected in the Douro River, Portugal. Detection limits of 1–10 μg l^{-1} were achieved under the optimized conditions.

Keywords: Water analysis; Solid-phase microextraction; Phenolic compounds

1. Introduction

Phenolic compounds are a group of organic pollutants present in the environment as a result of various processes such as industrial, biogeochemical and as pesticide degradation products [1]. Due to their toxicity and persistence, a number of phenolic compounds have been included in the legislation. In this respect, the European Union (EU) has included the phenols cited below in its Directive 76/464/EEC concerning dangerous substances discharged into the aquatic environment: 2-amino-4-chlorophenol, 4-chloro-3-methylphenol, 2-chlorophenol, 3-chlorophenol, 4-chlorophenol, pentachlorophenol and trichlorophenols. The US Environmental Protection Agency (EPA) list of priority pollutants also includes 11 phenolic compounds. Some of them are included in the EU directive, but others are not, such as 2-nitrophenol, 4-nitrophenol, 2,4-dinitrophenol, 4,6-dinitro-2-methylphenol, 2,4-dichlorophenol, 2,4-dimethylphenol and 2,4,6-trichlorophenol. The Directive 75/440/EEC states that the maximal concentration of phenolic compounds in surface water for drinking purposes should be 1–10 μg l^{-1} [2]. Current official analytical methods, US EPA 604 [3], 625 [4] (acid extractable section) and 8041 [5], are based on liquid–liquid extraction (LLE), followed by gas chromatography (GC) using several detection methods, like electron-capture detection (ECD) and mass spectrometry (MS). However, the direct analysis of phenols by GC is problematic and GC is usually performed after derivatization. Moreover, the need for cleaner procedures led to sample prepara-

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tion procedures based on solid-phase extraction (SPE) in order to avoid the manipulation of large volumes of toxic organic solvents. More recently, solid-phase microextraction (SPME) has been used. In this extraction method, sorbent-coated silica fibers are used to extract analytes from aqueous and gaseous samples. In water samples, the fiber is usually immersed to extract the analytes and the fibers are then directly transferred to the injector of a chromatograph, where the analytes are desorbed and subsequently separated and quantified [6,7]. This process is significantly simpler than conventional techniques, thereby reducing analyte loss during extraction. To date, two kinds of coating have been tested for the analysis of phenols by SPME–GC, polydimethylsiloxane (PDMS) [8,9] and polyacrylate (PA) [8–13]. Results obtained with the PDMS fiber were not satisfactory [8], due to the relative non-polar nature of this fiber, whereas the more polar PA fiber has been found suitable for the extraction of phenols from water [10–13] and soils [14].

However, the analysis of phenolic compounds by SPME–liquid chromatography (LC) has not been reported so far. In this paper we present the first application of SPME–high-performance liquid chromatography (HPLC) to the analysis of phenolic compounds. Two types of polar fibers were used, 60 μm polydimethylsiloxane–divinylbenzene (PDMS–DVB) and 50 μm Carbowax–templated resin (CW–TPR). The parameters of the desorption procedure such as desorption mode, composition of solvent for desorption, the period that the fiber was flushed by the mobile phase, the duration of the fiber soaking and the composition and flow-rate of the mobile phase during the desorption period, were studied and optimized. The effects of the properties of analytes and fiber coatings, pH, ionic strength and carry-over of samples were also investigated. The analytical performance of the method is also presented and discussed.

2. Experimental

2.1. Chemicals

Phenol (P), 4-nitrophenol (4-NP), 2-nitrophenol (2-NP), 2,4-dimethylphenol (2,4-DMP), 4-chloro-3-methylphenol (4-C-3MP), 2,4-dichlorophenol (2,4-DCP) and 2,4,6-trichlorophenol (2,4,6-TCP) were purchased from Merck (Darmstadt, Germany) and 2-chlorophenol (2-CP) and pentachlorophenol (PCP) were obtained from Sigma–Aldrich (St. Louis, MO, USA). They were >99% purity.

Stock standard solutions (500 mg l^{-1}) of each phenol were prepared by dissolving the compounds in acetonitrile. A mixture standard solution, containing 50 mg l^{-1} of each phenol, was also prepared in acetonitrile. These solutions were stored in dark glass bottles at 4°C. Working standard solutions were freshly prepared by dilution in water.

A 60 μm PMDS–DVB, and a 50 μm CW–TPR SPME fiber from Supelco were used. Before first use, fibers were conditioned with initial mobile phase (A–B, 70:30) directly in the HPLC system until a stable baseline was obtained.

Acetonitrile and water of HPLC grade (Merck) were used throughout. All other reagents were of analytical-reagent grade.

The glassware used for experiments was previously soaked in sodium dichromate–sulfuric acid mixture for 24 h and rinsed with doubly-deionized water.

2.2. Apparatus

A HPLC system assembled from modular components (Varian, Palo Alto, CA, USA) was used. It consisted of a 9012Q Model gradient pump, a column oven with a temperature control module, a six-port Rheodyne 7125 manual injector and a 9065 PolyChrom photodiode array detector. Data were collected by a personal computer using Varian Star software.

A manual SPME fiber holder and a SPME–HPLC interface from Supelco (Bellefonte, PA, USA) were used. The SPME–HPLC interface consisted of a six-port injection valve and a 70-μl desorption chamber, which replaced the injection loop in the HPLC system. It can operate in both dynamic and static desorption modes. For static elution, the fiber is introduced into the desorption chamber, which has been filled with the desorption solvent, and after a soaking period, the fiber is removed and the valve is switched to the INJECT position. Desorbed analytes are delivered to the LC column by the mobile phase flow. For dynamic desorption, the fiber is also

introduced into the desorption chamber with the valve in the LOAD position, and then the valve is switched to the INJECT position, allowing the mobile phase to pass through the desorption chamber. Analytes are desorbed from the fiber into the mobile phase stream and carried to the LC column. After the desorption period, the valve is returned to the LOAD position and the fiber is withdrawn.

An agitation platform from Variomag (Munich, Germany) and a Crison GLP 21 pHmeter (Alella, Barcelona, Spain) equipped with an Ag/AgCl combined glass electrode, Crison 52-02, were used.

2.3. Procedures

2.3.1. Chromatographic separation

The separation was performed on a Hypersil Green Env column (150×4.6 mm, 5 μm) (Hypersil, Cheshire, UK) equipped with the corresponding guard column, using a binary gradient elution. Mobile phase A was a 1% aqueous acetic acid solution and mobile phase B a 1% acetic acid solution in acetonitrile. Both solutions were filtered through a 0.22-μm nylon membrane filter (MSI, Westboro, MA, USA). Eluent solvent consisted of A–B (70:30) for 7 min, followed by gradient elution from 30 to 70% of mobile phase B in 3 min, a hold time of 5 min and a post-time of 3 min to back to initial conditions. The mobile phase flow-rate was set at 1.0 ml min^{-1} and the chromatograph was set at 35°C.

To improve sensitivity, quantitation was done using the optimum wavelength for each compound: P at 268 nm, 4-NP at 311 nm, 2-NP, 2-CP 2,4-DMP, 4-C-3MP, 2,4-DCP and 2,4,6-TCP at 278 nm, and PCP at 302 nm.

2.3.2. Solid-phase microextraction

A 4-ml of standard solutions or water samples previously acidified to pH 2 with hydrochloric acid and saturated with sodium chloride were transferred to 4.5-ml amber glass vials. The fiber was immersed into the sample for 40 min with magnetic stirring (600 cycles per minute) at room temperature. Then the fiber was placed in the desorption chamber, previously filled with the initial mobile phase (A–B, 70:30) and held inside the chamber for 2 min. The mobile phase flow-rate was set to 0.25 ml min^{-1} and the injection valve switched to the INJECT position, without removing the fiber, thus allowing the mobile phase to pass through the desorption chamber for 2 min. Then the injection valve was switched to LOAD and the mobile phase re-established at the normal flow-rate of 1 ml min^{-1}.

In order to avoid carry-over, the fiber was held inside the desorption chamber for 5 min and flushed twice with 500 μl of mobile phase (A–B, 70:30). After each desorption, the fiber was dried under room conditions for 3 min before the following extraction. The fiber can be used repeatedly achieving consistent results for a minimum of 50 extraction cycles.

3. Results and discussion

3.1. Chromatographic separation

The chromatographic separation of phenolic compounds is usually performed with reversed-phase C_{18} silica-based columns and mobile phases consisting of water–methanol or water–acetonitrile mixtures with acetic acid–acetate or phosphate buffer. The initial conditions for the chromatographic separation, which were selected from literature data [15], consisted of a gradient elution using water–acetonitrile (ACN), both acidified with 1% of acetic acid, from ACN–water (30:70, v/v) in isocratic mode for 15 min to 100% ACN in 15 min. Although satisfactory separation was achieved, the gradient program was modified in order to reduce analysis time. A constant composition, at 30% ACN, from start to minute 7 and a linear gradient to 70% ACN in 3 min, which provided good separation at reasonable retention times, were the selected conditions.

3.2. Solid-phase microextraction

Two types of polar fibers, PDMS–DVB and CW–TPR, were evaluated for the direct extraction of phenols from a water matrix using SPME–LC.

To optimize the desorption process, several experiments were carried out from standard solutions in the same experimental absorption conditions, pH adjusted to 2 with hydrochloric acid, 25°C and no

salt added. The pH was adjusted to 2 to ensure that all phenols were in their neutral form, which increased affinity for the fiber coating. After immersion of the fiber into the sample solution for 30 min, it was introduced into the desorption chamber. Dynamic and static desorption modes were used.

Elution was first performed in the static mode, which allows the application of various solvents for desorption, and several acetonitrile–water mixtures were tested at a constant desorption time of 2 min. Although analyte recoveries increased with acetonitrile content (Fig. 1), especially for the less polar PCP, acetonitrile percentages higher than 30% resulted in broad chromatographic peaks for the earlier eluting compounds and in a dramatic shortening of the fiber life. Therefore, the initial chromatographic mobile phase (30% acetonitrile) was chosen as the desorption solvent. The effect of soaking time, from 1 to 5 min, was also evaluated and the obtained results indicate that, for both fibers, desorption equilibrium was almost reached in 2 min, since no significant differences in peak areas were observed from 2 to 5 min. However, because of the low volume of the desorption chamber, analytes were not

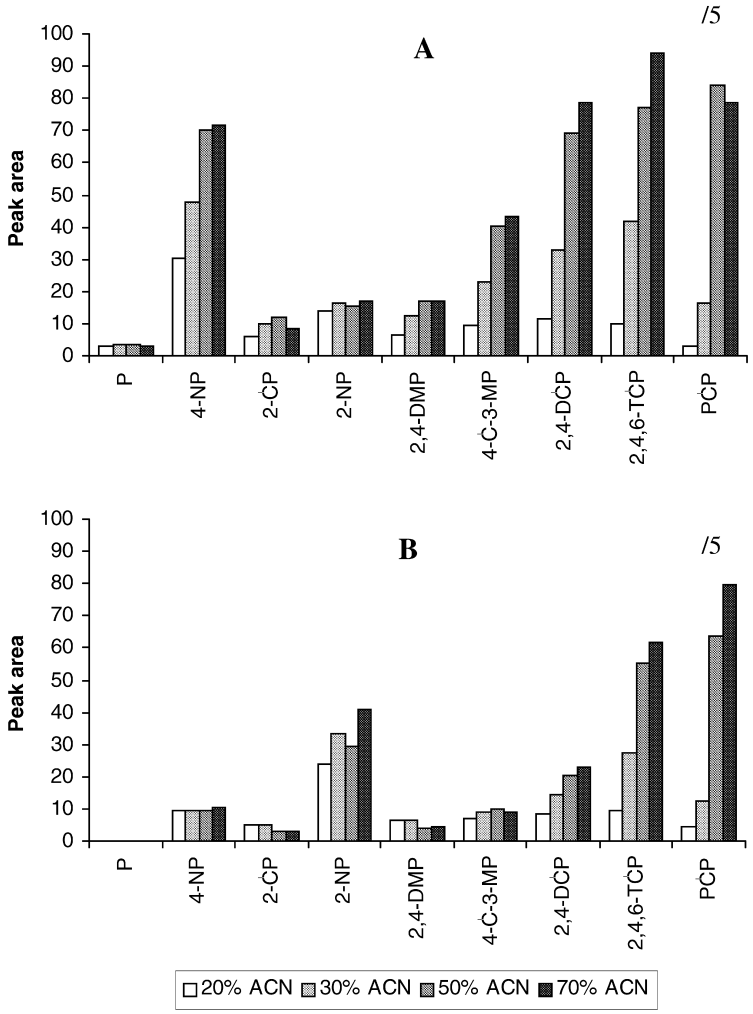

Fig. 1. Effect of acetonitrile content on the desorption solvent. Absorption time: 30 min. Desorption mode: static, 2 min. (A) CW–TPR fiber, (B) PDMS–DVB fiber. Peak areas for PCP are fivefold divided.

totally desorbed after equilibrium was reached, but partially remained in the fiber, as shown by a subsequent desorption.

When dynamic desorption was used, the chromatogram strongly depended on the flow-rate of the mobile phase during desorption. A flow-rate of 1 ml min^{-1} led to dramatic band broadening and peak tailing, which must be due to slow desorption of the analytes from the fiber into the mobile phase. A significant improvement in peak shape was observed by decreasing the flow-rate from 1 to 0.25 ml min^{-1} during desorption; however, none of the obtained chromatograms were satisfactory.

To overcome the drawbacks mentioned, a third approach was assayed. Desorption was first performed by soaking the fiber in the mobile phase for 2 min (static mode) and then allowing the mobile phase (0.25 ml min^{-1}) to pass through the chamber (dynamic mode) to desorb the analytes remaining in the fiber after equilibration. This provided sharper peaks when compared with the dynamic mode for the earlier eluting compounds, and higher peak areas when compared with the static mode for the less polar analytes (Table 1). The effect of dynamic desorption time on recoveries was also studied and no effect was observed from 2 min.

Some of the phenols studied (mainly P, 2-CP, and 2,4-DMP) gave low extraction efficiencies regardless of the eluent composition, elution mode or soaking period. This behavior appears to be due to the low amount of these more water-soluble compounds extracted by the stationary phase in the adsorption step, rather than to low efficiencies in the desorption step. Thus, the adsorption step was optimized.

Electrolytes are usually added to the samples in SPME experiments to improve extraction of organics from aqueous solutions. In particular, the addition of sodium chloride enhances the extraction of phenols with a PA fiber using SPME–GC, as reported by Buchholz and Pawliszyn [8]. Initial experiments adding 30 g l^{-1} NaCl hardly affected extraction efficiency. Nevertheless, at the saturation level, extraction with the CW–TPR fiber was enhanced by a factor ranging from 2.5 to 16 for all the analytes (Table 2), except PCP, which was more extracted when no salt was added. This apparently anomalous result may be due to the slower diffusion through the saturated salt solution compared to pure water. Further experiments confirmed that under the extraction condition used here (30 min) less than 50% of the equilibrium amount of PCP was absorbed on the fiber. The less polar coating PDMS–DVB extracted lower amounts of phenols than the CW–TPR fiber, which was thus selected.

The effect of extraction time on the amount of analyte extracted is shown in Fig. 2. The extraction time profile depends on the individual phenol and seems to be related with analyte polarity. The most polar compounds (P, 2-CP and 2-NP), reached equilibrium in 10–20 min, whereas 4-NP, 2-CP, 2-NP and 2,4-DMP did in 40 min and the less polar phenols (2,4-DCP, 2,4,6-TCP and PCP) require much longer equilibration times (80 min). Since SPME allows precise determinations at non-equilibrium conditions when extraction time and mixing

Table 1
Peak areas obtained with the two fibers in both elution modes

Compound	65 μm PDMS–DVB		50 μm CW–TPR	
	Static[a]	Dynamic[b]	Static[a]	Dynamic[b]
P	–	–	3.2	3.0
4-NP	5.2	7.5	48	50
2-CP	3.7	3.2	9.7	13
2-NP	33	38	16	20
2,4-DMP	5.8	6	13	11
4-C-3-MP	9.2	11	23	37
2,4-DCP	15	21	33	57
2,4,6-TCP	30	43	42	57
PCP	61	155	83	142

[a] Two minutes for equilibration.
[b] Two minutes for equilibration plus 2 min dynamic elution.

Table 2
Effect of salt on the extraction of phenols with the 50 μm CW–TPR fiber ($n=3$)

Compound	pH 2	pH 2+saturated with NaCl	Factor increase
P	3	30	10.0
4-NP	50	380	7.6
2-CP	13	156	12.0
2-NP	20	124	6.2
2,4-DMP	11	177	16.1
4-C-3-MP	37	200	5.4
2,4-DCP	57	217	3.8
2,4,6-TCP	57	142	2.5
PCP	142	71	0.5

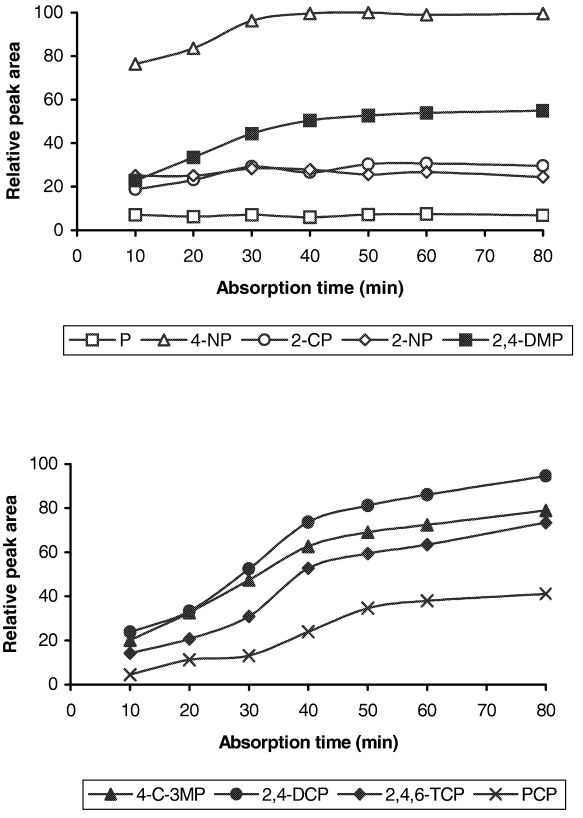

Fig. 2. Absorption–time profile for the CW–TPR fiber. See Section 2.3 for desorption conditions.

conditions are controlled, 40 min was selected for further experiments.

Fig. 3a shows a chromatogram obtained from a standard solution containing 100 μg l^{-1} of each phenol in the selected extraction conditions, recorded at a wavelength of 278 nm. The percent extraction yield of each phenol, i.e., the percent of analytes in a sample transferred to the LC, are presented in Table 3. Phenol itself and the nitro-substituted phenols were the least extracted analytes, whereas recoveries for methyl- and chloro-substituted phenols ranged between 6 and 15%, except for PCP. A comparison between these data and those reported by Buchholz and Pawliszyn [8] for the PA fiber under similar extraction conditions, shows that CW–TPR fiber lead to somewhat higher recoveries than PA coating, except for 2,4,6-TCP and especially TCP, which have much higher affinity towards the PA coating. The low value obtained for PCP in our study is

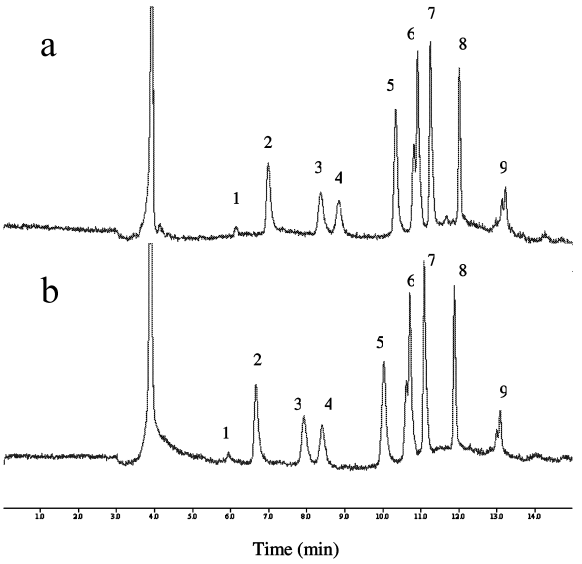

Fig. 3. SPME–LC–UV (278 nm) chromatograms of (a) a standard mixture of phenolic compounds containing 100 μg l^{-1} of each phenol and (b) a river water sample spiked with 100 μg l^{-1} of each compound extracted with the CW–TPR fiber. Absorption time: 40 min. See Section 2.3 for desorption conditions. Peaks: (1) phenol, (2) 4-nitrophenol, (3) 2-chlorophenol, (4) 2-nitrophenol, (5) 2,4-dimethylphenol, (6) 4-chloro-3-methylphenol, (7) 2,4-dichlorophenol, (8) 2,4,6-trichlorophenol and (9) pentachlorophenol.

probably related to its lower solubility in the desorption solvent, which caused some of the adsorbed analyte to remain in the fiber after desorption. To

Table 3
Limits of detection, precisions and percent extraction yields for the analysis of phenolic compounds in pure water with the CW–TPR fiber

Compound	LOD[a]	RSD[b] (%)	% Extraction[c]
P	10	4.0	1.3
4-NP	1	0.8	3.6
2-CP	5	0.7	6.4
2-NP	6	5.6	1.7
2,4-DMP	3	1.6	11
4-C-3-MP	2	4.2	15
2,4-DCP	3	4.6	16
2,4,6-TCP	4	3.3	12
PCP	5	12.0	3.0

All concentrations expressed as μg l^{-1}.

[a] Limits of detection are calculated as three times the standard deviation of seven replicate runs.

[b] Data obtained by extraction in seven replicates at 100 μg l^{-1}.

[c] Data obtained by extraction in three replicates at 100 μg l^{-1}.

avoid carry-over effect the fiber was flushed twice for 5 min with 500 μl of mobile phase (A–B, 70:30).

3.3. Analytical performance of the method

Figures of merit for the nine phenols are summarized in Table 3. There is a linear correlation between peak area and concentration for each phenol from 10 to 1000 μg l^{-1} (the highest concentration tested). Precision was determined from a set of seven water samples spiked at 100 μg l^{-1}. For most of the analytes, relative standard deviation (RSD) was lower than 5%. Detection limits, calculated as three times the standard deviation of baseline from seven replicate runs, ranged between 1 and 10 μg l^{-1}. These values are about 10 times higher than those obtained by on-line solid-phase extraction (SPE)–LC–UV, where almost quantitative retention of phenols was achieved [15–17].

Finally, the SPME–LC method was applied to the analysis of a river water sample from the Douro River in Porto (Portugal). The sample was collected in a 2.5-l glass bottle, filtered through a 0.45-μm nylon membrane, acidified to pH 2 with hydrochloric acid and stored at 4°C. Aliquots of 4 ml were saturated with sodium chloride and analyzed by SPME–HPLC. Direct analysis revealed no measurable phenolic compound and so the sample was spiked with various amounts, from 10 to 1000 μg l^{-1}, of each phenol. A chromatogram obtained from a SPME extraction of a river water sample spiked with 100 μg l^{-1} of phenols is shown in Fig. 3b. The differences in the slopes of the calibration curves between pure water and river water were lower than 5% except for 2-NP (Table 4). This suggests that recovery is independent of the matrix of the sample solution and quantification can thus be performed using a external calibration curve. However, the detection limits attained with diode array detection (DAD) are not low enough for the analysis of phenols in natural waters at the levels established in legislation, and a more sensitive detection system, such as MS, must be used.

Table 4
Slopes and correlation coefficients of calibration curves[a] in pure water and river water

Compound	Pure water		River water	
	Slope	R^2	Slope	R^2
P	2.92	0.997	2.94	0.987
4-NP	45.98	0.998	43.70	0.994
2-CP	14.66	0.997	13.86	0.990
2-NP	12.57	0.996	11.20	0.967
2,4-DMP	26.44	0.999	26.15	0.997
4-C-3-MP	29.34	0.993	29.14	0.996
2,4-DCP	30.58	0.999	30.15	0.996
2,4,6-TCP	19.86	0.998	19.72	0.995
PCP	5.65	0.974	5.41	0.932

Conditions as described in Section 2.3.

[a] Calibration curves with the following concentrations: 10, 50, 100, 250, 500 and 1000 μg l^{-1} for the CW–TPR fiber.

4. Conclusions

SPME–LC–DAD was evaluated for the analysis of priority phenolic compounds in water samples. Although the most polar fiber coating CW–TPR led to higher extraction recovery than PDMS–DVB, only about 1–16% of the phenols were extracted under optimum conditions. Limits of detection using UV range from 1 to 10 μg l^{-1}, which are not low enough to analyze phenols in natural waters. However, the method can be used with other detectors, such as MS, which would provide suitable detection limits.

Our studies demonstrate that the low affinity of these relatively polar compounds to the available fiber coatings makes SPME inferior in terms of sensitivity when compared with alternative approaches, such on-line SPE–LC.

However, SPME is attractive since it is simple, solvent-less and suitable for small sample volumes and for on-site analysis. As SPME–LC is still in the development stage, we can expect new fibers that overcome the above mentioned drawbacks to become available in the future.

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