Implementation of Solid-Phase Microextraction with Micellar Desorption Method for Priority Phenolic Compound Determination in Natural Waters

M.E. Torres Padrón, C. Mahugo Santana, Z. Sosa Ferrera, and **J.J. Santana Rodríguez***
University of Las Palmas de Gran Canaria, Department of Chemistry

Abstract

Eleven phenolic compounds considered by the Environmental Protection Agency to be priority pollutants are extracted and determined in different water samples. The method involves the extraction and clean-up step of target compounds by solid-phase microextraction and micellar desorption (SPME–MD) and a second step of determination by liquid chromatography with diode array detection. Different fibers and surfactants are evaluated for the analysis of these target analytes in water samples. In the optimum conditions for the SPME process, recoveries for the target compounds are between 80% and 109%; relative standard deviations are lower than 10%, and detection limits are in the range 0.3–3.5 ng/mL. The main advantages of this method are the combination of time and efficiency, safety, and an environmentally friendly process for sample extraction prior to instrumental determination. This demonstrates that SPME–MD can be used as an alternative to traditional methods for the extraction and determination of priority phenolic compounds in natural waters from different origins.

Introduction

Phenols are some of the most important contaminants present in the environment. They are introduced into the environment by different sources: directly, as industrial effluents; and indirectly, as conversion products from natural and synthetic chemicals, including pesticides (1).

Because most phenolic compounds exhibit a high degree of toxicity and adverse effects upon biota and humans, the European Union, the World Health Organization (WHO), and the US Environmental Protection Agency (EPA) have determined maximum permissible levels of phenols in surface, ground, and drinking waters, and some of these compounds have been listed as priority pollutants.

Legislation is very strict with respect to the quality required for surface waters or to the protection of groundwater against pollution caused by certain dangerous substances (2,3). New directives indicate the need to measure such priority compounds (4).

Until now, official analytical methods for these compounds have been based on liquid–liquid extraction followed by gas chromatography (GC) (5–7). Alternative analytical techniques for the determination of these compounds include high-performance liquid chromatography (HPLC) coupled with different detection systems (8–11). However, because the concentration of these phenols in water samples is rather low, it is necessary to apply a pre-concentration step prior to the chromatographic analysis. Thus, sample handling continues to play a basic role in environmental analysis. In fact, in previous years, a lot of effort has been devoted to develop processes of sample extraction and clean-up that are safer, faster, and with minimal solvent use.

Solid-phase microextraction (SPME) is a simple and effective adsorption and desorption technique for different organic compounds in different matrices.

SPME–GC and SPME–GC–mass spectrometry have been readily used for the extraction and determination of phenols (12–14). On the other hand, SPME–HPLC has been used to extract, pre-concentrate, and determine phenolic compounds, mainly in water samples (15,16). Desorption may be made in an appropriate desorption chamber, using an organic solvent. However, this process has some disadvantages. When elution is performed in the static mode, analytes are not totally desorbed in the first elution, so they partially remain in the fiber and must be eluted in subsequent desorptions. Dynamic desorption usually results in broad chromatographic peaks due to slow desorption of the analytes from the fiber into the mobile phase. Desorption may be also made using off-line organic solvent desorption. However, analyte responses are lower and apolar analytes have worse resolution, with broadening of the chromatographic peaks (15). For that, SPME–HPLC has limited application, due to the need to optimize desorption conditions.

In order to achieve the greatest efficiencies in the extraction of the 11 priority phenolic compounds listed by the EPA, and to make improvements to the chromatograms, we implemented an SPME method with a new desorption mode using a micellar

* Author to whom correspondence should be addressed: Department of Chemistry, Faculty of Marine Sciences, University of Las Palmas de Gran Canaria, 35017, Las Palmas de Gran Canaria, Spain; email jsantana@dqui.ulpgc.es.

medium as desorbing agent (MD) and it was combined with HPLC. Micellar media have been widely used in separation science to dissolve highly hydrophobic analytes, due to the properties of micelles. Specifically, non-ionic surfactants have been widely used for the extraction of different organic substances from different types of matrices (17–22), which demonstrates their high potential as extractants. Moreover, their compatibility with aqueous–organic mobile phase in chromatographic analysis facilitates these kinds of applications. The use of surfactants allows us to establish an environmentally friendly method without the use of organic solvents, as they are contamination sources.

In this paper, a method for the extraction and determination of 11 priority phenolic compounds in environmental liquid samples is implemented using SPME–MD–HPLC. We investigated the use of four different surfactants: Polyoxyethylene 10 lauryl ether (POLE), Polyoxyethylene 9 lauryl ether (Polidocanol), Polyoxyethylene 6 lauryl ether ($C_{12}E_6$), and Hexadecyltrimethylammonium bromide (HTAB) for the desorption of the analytes from the fiber. The method was applied to the extraction and determination of target analytes in various types of spiked natural waters. The results obtained in this study are shown and discussed.

Experimental

Reagents

The compounds studied were 11 phenolic EPA priority pollutants. They are listed in Table I (numbers and abbreviations identify the compounds in Figures). All compounds were obtained from Sigma-Aldrich (Madrid, Spain), and their stock solutions (1000 µg/mL) were prepared by dissolving appropriate amounts of the commercial products in methanol and stored in glass-stoppered bottles at 4°C. Ultra-high-quality water obtained by a Milli-Q (Millipore, Milford, MA) water purification system was used to prepare the mobile phase and the working aqueous standard solutions. Appropriate volumes of the stock solutions were diluted to prepare the solutions' target phenolic compounds at 200 ng/mL. The standard certified mixture of phenolic compounds no. 219010200 was obtained from the Laboratories Dr. Ehrenstorfer (Augsburg, Germany) and provided by CYMIT Química, S.L. (Barcelona, Spain).

The surfactants used in this study (POLE, Polidocanol, $C_{12}E_6$, and HTAB) were obtained from Sigma-Aldrich (Madrid, Spain) and prepared in de-ionized water.

Methanol used to dissolve standards and to prepare the mobile phase was HPLC-grade and was obtained from Panreac Química (Barcelona, Spain). It was filtered through a 0.22-µm acetate membrane filter.

Five commercially available fibers were purchased from Supelco and evaluated: carbowax-TPR/100 (CW-TPR), polydimethylsiloxane (PDMS), polydimethylsiloxane–divinylbenzene (PDMS–DVB) (60 and 65 µm), polyacrylate (PA), and a 75 µm carboxen–PDMS. Before use, the fibers were conditioned with methanol according to supplier instructions. Specifically, each day prior to analysis, the fibers were washed in water to eliminate salt excess and conditioned in methanol, and dried before the extractions.

Instrumentation and chromatographic conditions

The chromatograph system consisted of two Waters 510 pumps (Waters Associates, Milford, MA) fitted with a Waters injector Rheodyne model 7725 with a 50 µL sample loop to optimize the external micellar desorption and a Waters 996 Photodiode Array detection (DAD) system to detect the target compounds. The system and the data management were controlled by Millenium software from Waters (Waters Cromatografía, Barcelona, Spain). The stationary-phase column was a Waters Nova-Pak C18, 3.9 × 150 mm, 4 µm particle diameter.

The initial mobile phase was methanol–water (with 1% acetic acid) (30:70 v/v) up to 100% methanol in 20 min at a flow rate of 1.0 mL/min. In order to quantitate all compounds under study, the analytes were detected at their maximum wavelengths. The retention time and the wavelength for each compound are listed in Table I.

SPME–MD

SPME was carried out by introducing 4 mL of aqueous samples, containing 200 ng/mL of target phenols, into glass vials. The samples were saturated with NaCl (30% w/v), and their pH was adjusted with acetic acid 1% (v/v) to 2.7. The fiber was then immersed in the sample for 40 min. During the extraction, the samples were heated to 40°C and stirred with a magnetic stirrer at a constant speed of 1400 rpm. After the extraction step was developed under the optimum conditions, the fiber loaded with the analytes was introduced for 10 min into a conical glass insert of 100-µL, contained in a 4-mL glass vial with 60 µL of different surfactants. The external setup is illustrated in Figure 1.

Quantitation of the compounds was performed in the range of 10–300 ng/mL for the studied analytes by injecting 50 µL of the surfactant solution into the LC.

Table I. List of Priority Phenolic Compounds, Retention Times (t_R), and Detection Wavelengths (λ)

No.	Compound	Abbrevation	t_R (min)	λ (nm)
1	Phenol	PH	4.1	270
2	4-Nitrophenol	4-NP	6.6	315
3	2,4-Dinitrophenol	2,4-DNP	7.7	270
4	2- Nitrophenol	2-NP	9.0	280
5	2-Chlorophenol	2-CP	9.7	280
6	2,4-Dimethylphenol	2,4-DMP	13.1	280
7	4,6-Dinitro-ortho-cresol	4,6-DNOC	13.5	270
8	4-Chloro-meta-cresol	4-CMC	14.5	280
9	2,4-Dichlorophenol	2,4-DCP	15.1	290
10	2,4,6-Trichlorophenol	2,4,6-TCP	16.7	290
11	Pentachlorophenol	PCP	19.3	303

After each desorption, the fibers were cleaned with Milli-Q water and then methanol to avoid damage due to the use of NaCl. Also, they were dried before the next use. Blanks were run to confirm the absence of carryover.

Spiked water samples

Prior to the analysis, seawater, filtered sewage water, and groundwater were successively filtered through a 0.22-µm cellulose acetate filter, stored in the dark, and refrigerated until analysis. Fifty nanogram/milliliters of each phenolic compound were spiked in 4 mL of a water sample and analyzed according to the procedure described, using the 65-µm PDMS–DVB fiber. Three replicate analyses of each water sample were carried out.

Statistical analysis

Experimental designs were performed using Statgraphics Plus software, version 5.1 (Manugistic, Rockville, MD). Statistical tests were done using SPSS 11.0 (Chicago, IL).

Results and Discussion

To optimize the extraction process, we used a spiked Milli-Q water sample with a concentration of 200 ng/mL of each analyte.

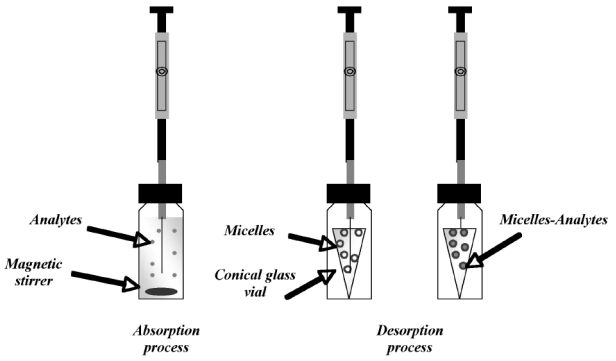

Figure 1. Scheme of SPME with MD procedure.

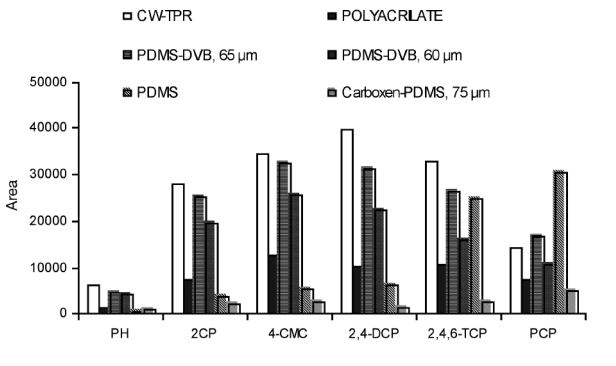

Figure 2. Relative extraction efficiencies of some studied phenolic priority compounds with different fibers. Milli-Q water containing 200 ng/mL was used for each analyte. Extraction and chromatographic conditions specified in the text.

According to our previous experiences (23) and the results of other researchers (24,25), the acidification and salting out effects enhance the extraction efficiency of phenolic compounds from aqueous samples. It is necessary to acidify the sample to ensure that all compounds are in their neutral forms, which have greater affinities for the polar coating. Moreover, the addition of an inert salt to water increases the distribution constants of the compounds between water sample and the fiber, and improves the extraction efficiency. Due to this, water samples were acidified at pH 2.7 with acetic acid, and ionic strength was fixed at 30% (w/v) NaCl to increase the extraction recovery.

Optimization of SPME–MD

To achieve an efficient extraction of the compounds from the water samples using SPME, several experimental parameters must be evaluated. To ensure extraction efficiency of analytes from a sample, the amount of analyte extracted depends on the polarity and thickness of the stationary phase (fiber evaluation), the time and temperature of the extraction needed to reach equilibrium between the sample matrix and the coating of the fiber (time and temperature extraction), stirring of the sample, and volume of the sample to extract the analytes. With respect to the desorption process with micellar media, the nature and concentration of the surfactant must also be optimized. All experiments were carried out in triplicate, and the average peak area was counted for comparison.

Fiber evaluation

A preliminary and qualitative assay was performed to evaluate the extraction efficiency of different fibers: PA, CW–TPR, PDMS–DVB 60 and 65 µm, Carboxen–PDMS 75 µm, and PDMS. The initial experimental conditions were: absorption time, 40 min; temperature, 40°C at constant stirring speed of 550 rpm; and 10 min of desorption in a desorption volume of 60 µL of 5% (v/v) POLE. These were the optimum conditions obtained in a previous work with a CW-TPR fiber (23).

Relative extracting efficiencies of some studied phenolic priority compounds with these fibers are shown in Figure 2. As can be seen, all the fibers were suitable for all analytes, except Carboxen–PDMS and PA. For the most of the compounds, the PDMS fiber gave the worst extraction efficiency, except for 2,4,6-

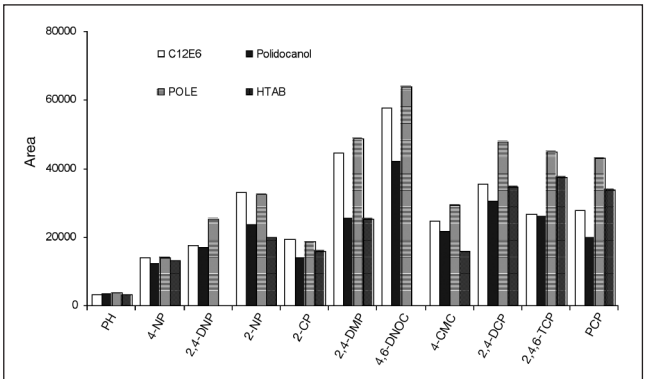

Figure 3. Response of analytes to the presence of different surfactants in the desorption process. Milli-Q water containing 200 ng/mL was used for each analyte. Extraction and chromatographic conditions specified in the text.

TCP and PCP, which presented good relative responses using this fiber. The PDMS fiber is the least polar of the studied fibers and these results are in agreement with this characteristic.

The best results were obtained with the most polar fiber, CW–TPR, but we chose the PDMS–DVB (65 µm) fiber because it showed good extraction ability with most polar compounds. Moreover, the CW-TPR fiber was successfully used in a previous study for chlorophenol analysis (23).

Stirring of the sample

Stirring of the solution improves the mass transference between the aqueous phase and the fiber, and therefore, the equilibrium can be achieved more rapidly.

In this study, the water samples were continually agitated at two different stirring rates of 500 and 1400 rpm. The results obtained indicate that the signal increases with the stirring rate for the most substituted phenols. However, the most polar compounds did not present a meaningful improvement. Moreover, when the extraction process was carried out at 1400 rpm, the relative standard deviation (RSD) was lower than 10% for most of the phenolic compounds. For that, we chose this stirring speed for subsequent studies.

Volume of the sample

Generally, the amount of analyte extracted is proportional to the sample volume. Hence, the sensitivity of the method can be improved by increasing the volume of the sample. However, a much longer equilibration time is required because the extraction rate is controlled by the diffusion of analytes from the sample matrix to the fiber.

Few studies report the effect of this parameter on the SPME process (26,27). In order to select the optimum sample volume, three different volumes of 4, 8, and 12 mL were assayed. In general, the data obtained demonstrate that an increase in the sample volume does not significantly improve the extraction efficiency in the target analytes. This can be due to the fact that when the sample volume increases, more agitation is required. However, an increase in the agitation rate could affect the method precision. For that, we chose a sample volume of 4 mL.

In summary, the optimum extraction parameters for the studied 11 priority phenolic compounds were: sample volume, 4 mL; absorption time, 40 min; temperature, 40°C; 30% (w/v) NaCl; pH adjusted at 2.7; and an agitation rate of 1400 rpm.

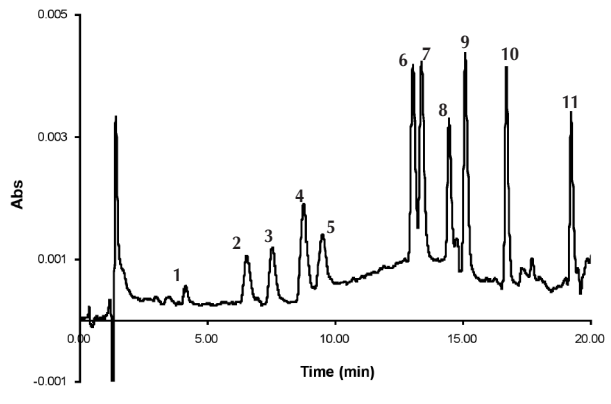

Figure 4. Chromatogram of an eleven priority phenolic mixture (200 ng/mL for each analyte) in Milli-Q water under SPME–MD optimum conditions. The numbering refers to Table I. Chromatographic conditions specified in the text.

Optimization of external MD process

The nature and concentration of the surfactant may be important parameters for desorption efficiency. Hence, a correct choice of the surfactant is fundamental to obtain a satisfactory desorption process. When selecting the surfactant, consideration should be given to the function of its interaction with the analytes and matrix. In addition, the surfactant phase must be compatible with the water–organic mobile phase usually employed in HPLC.

In order to study the influence of the nature of the surfactant in the desorption efficiencies, we used four different surfactants in the desorption of 11 priority phenolic pollutants: POLE, Polidocanol, $C_{12}E_6$, and HTAB. The first three are non-ionic and the last one is cationic.

In a previous study, we found that the best value of the percentage of surfactant was 5% (v/v) (23). Therefore, this surfactant concentration was chosen. In the same way, a study of surfactant volume showed that 60 µL of surfactant was enough to ensure that the fiber is fully immersed in the micellar medium and allows a complete desorption. The process was carried out in

Table II. Analytical Characteristics of the Proposed SPME-MD–HPLC Method*

	Extraction efficiency† (%)	LOD‡ (ng/mL)	RSD§ (%)
PH	85.8	1.4	1.0
4-NP	104.9	4.8	6.6
2,4-DNP	81.4	1.9	5.7
2-NP	84.5	1.2	9.6
2-CP	109.1	3.5	7.4
2,4-DMP	84.5	0.7	8.7
4,6-DNOC	80.2	1.5	8.9
4-CMC	85.8	2.7	8.3
2,4-DCP	85.8	1.5	3.3
2,4,6-TCP	87.5	0.9	9.3
PCP	84.7	0.3	7.5

* Linear dynamic ranges described in the text.
† $n = 6$ (50 ng/mL of priority phenolic compounds mix)
‡ Detection limits are calculated as signal-to-noise ratio of three.
§ Relative standard deviation ($n = 6$).

Table III. Results Obtained in the Application of SPME–MD–HPLC Method to a Certified Chlorophenol Mixture* Using PDMS-DVB, 65 µm Fiber

Compounds	2-CP (ng/mL)	CMC (ng/mL)	2,4-DCP (ng/mL)	2,4,6-TCP (ng/mL)	PCP (ng/mL)
Obtained Concentration†	50.5 ± 2.9	55.0 ± 8.3	42.3 ± 3.6	46.9 ± 1.2	45.2 ± 0.7

* Concentration certified for each analyte: 50 ng/mL.
† Mean of three determinations.

a 100-µL conical glass vial with 60 µL of surfactant at 5% (v/v), after extraction conditions were previously optimized.

The results obtained are shown in Figure 3. As it can be seen, all phenolic derivatives can be desorbed by the four surfactants, except 2,4-DNP and 4,6-DNOC, which could not be detected when HTAB was used. In general, the use of non-ionic surfactants enhances the desorption efficiency, increasing with the surfactant polarity. So, the use of the most polar (POLE) allows the best desorption efficiency for the target analytes.

With respect to desorption time, the analytes can be affected by different partition equilibria between the fiber and micellar media. In previous studies, we obtained that a desorption time of 10 min was enough to obtain a good extraction efficiency, expressed as the area of the chromatographic peak.

In summary, to implement SPME–MD to determine 11 priority phenolic compounds in liquid samples, we chose a concentration of 5% (v/v) POLE, a desorption volume of 60 µL, and 10 min desorption time to be the best conditions, for obtaining the highest relative responses of studied analytes. In a previous work (23), we compared conventional SPME and SPME–MD. The results obtained demonstrated that the use of SPME–MD permits the determination of analytes of different polarities, whereas the use of methanol in conventional SPME does not allow the determination of the most polar analytes.

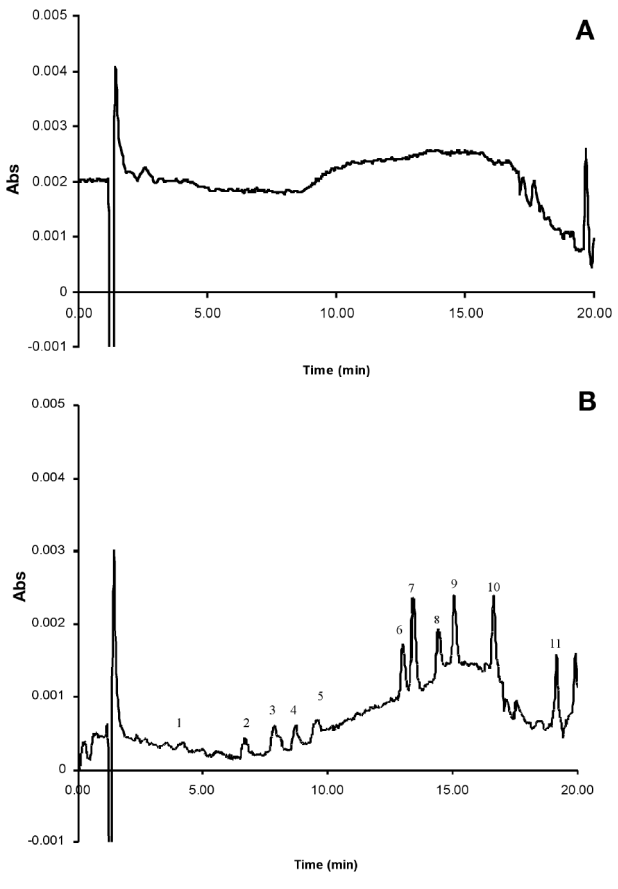

Figure 5. Chromatogram of blank (A) and spiked (B) groundwater sample (50 ng/mL for each analyte) analyzed by SPME–MD–HPLC using a 65-µm PDMS–DVB fiber. The numbering refers to Table I. Chromatographic conditions specified in the text.

Figure 4 shows the chromatogram obtained of a mixture of selected phenolic derivatives in Milli-Q water solution with a concentration of 200 ng/mL for each analyte, under the optimum conditions of SPME–MD. It can be observed that the optimized desorption leads to well-defined peaks and a stable baseline.

Analytical performance of the method

To evaluate the performance of the SPME–MD–HPLC method, the figures of merit were studied. The linear range was investigated between 10–300 ng/mL for the phenolic analytes and each point of the calibration curve was obtained from the mean value of three area measurements. Linear relationships were obtained between peak areas and the analyte concentrations. Table II shows the data obtained. In all cases, the correlation coefficients were greater than 0.99, and the recoveries were in the range of 81–109% for the different analytes. The precision of the method was determined by performing six replicates of water samples with 50 ng/mL of each phenolic derivative under the optimum conditions. Reproducibility, expressed as % RSD, was lower than 10% in all cases. Detection limits were calculated from the signal-to-noise ratio of the individual peaks, assuming a minimum detectable signal-to-noise level of 3 (28). LODs obtained for the different phenols varied in the range 0.3–4.8 ng/mL. These values are lower than those obtained with other methods.

To demonstrate the reliability of the quantitation by the optimized SPME–MD coupled with HPLC, we applied the method to a certified reference mixture of phenols containing five of the 11 priority phenols (2-CP, 4-CMC, 2,4-DCP, 2,4,6-TCP, and PCP) in acetonitrile. The analytes were diluted to a final concentration of 50 ng/mL prior to their extraction. It can be seen in Table III that the data obtained are very close to the reference values.

Analysis of water samples

Several spiked water samples of different origin (seawater, sewage, and ground water) were analysed by SPME–MD coupled to HPLC procedure under optimized conditions in order to study

Table IV. Average of Recovery Percentages and Standard Deviation (x ± SD) (n = 3) Obtained for Different Real Water Samples Spiked with 50 ng/mL of Priority Phenolic Compounds*

Compound	Filtered Sewage	Seawater	Groundwater
PH	103.0 ± 10.0	118.2 ± 4.6	91.7 ± 2.0
4-NP	98.6 ± 3.1	113.5 ± 7.7	103.7 ± 7.0
2,4-DNP	88.9 ± 8.3	113.2 ± 3.1	84.2 ± 10.2
2- NP	78.4 ± 14.0	72.0 ± 5.1	63.1 ± 3.9
2-CP	86.2 ± 3.7	98.8 ± 2.3	97.5 ± 8.0
2,4-DMP	57.1 ± 7.8	91.1 ± 9.1	86.8 ± 9.0
4,6-DNOC	98.7 ± 27.0	118.8 ± 8.3	106.7 ± 9.0
4-CMC	91.3 ± 14.0	101.1 ± 6.7	104.6 ± 8.0
2,4-DCP	61.8 ± 12.0	88.8 ± 6.4	105.6 ± 4.0
2,4,6-TCP	105.0 ± 3.2	109.8 ± 5.9	114.5 ± 10
PCP	57.3 ± 0.6	116.1 ± 7.8	120.2 ± 14

* Mean of three determinations.

the influence of matrix effects on the extraction. First, a blank of real samples was run to verify the absence of peaks at the retention time of the compounds under study. No interfering peaks appeared in the chromatogram corresponding to the blank analysis (Figure 5A). Samples were spiked with a concentration of 50 ng/mL of each phenolic compound. Figure 5B shows the chromatogram obtained for the extracted priority phenolic compounds from a spiked ground water sample and analysed by SPME–MD–HPLC using a PDMS–DVB, 65 µm fiber.

The relative recovery results for the analyzed samples are shown in Table IV. Percentage recoveries were determined as the ratio between the extracted amount (calculated from calibration curves of standards) and the initial amount added to the real sample. Each value corresponds to the mean obtained from three measurements.

The data obtained for different spiked water samples with the studied analytes demonstrate that, for the most phenols, the relative recoveries were higher than 80%, except for 2,4-DMP, 2,4-DCP, and PCP in filtered sewage waters. These three low results could be due to the presence of organic matter, such as humic acids, where the analytes could be adsorbed (29). Therefore, we can say, in general, that the matrix has no pronounced effect on the SPME–MD–HPLC analysis of phenols in water samples.

Conclusions

An SPME–MD method was implemented for the extraction and determination of 11 priority phenolic compounds in water samples. The use of surfactants in the desorption process allows an important sample pre-concentration and presents some advantages like less toxicity and reduction in price, with respect to the organic solvents used in the conventional SPME procedure. Moreover, the most commonly used surfactants are commercially available and compatible with the hydro-alcoholic mobile phase used in HPLC analysis. This allows the determination of the most polar compounds, which can not be determined using organic solvents. Therefore, SPME–MD is a useful tool to desorb analytes of different polarities in environmental liquid samples.

The proposed method is easy to use, precise, reproducible, and linear for the target analytes. A simple calibration curve method based on simple aqueous standards can be used. Recoveries of the studied compounds were higher than 80%. Finally, the optimized procedure was successfully applied to the analysis of phenolic derivatives in different water matrices.

The experimental results presented clearly demonstrate that the combination of SPME-MD and HPLC is found to be very suitable for the determination of this kind of compound in water samples.

Acknowledgments

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