Talanta 148 (2016) 292–300

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Preconcentration of polar phenolic compounds from water samples and soil extract by liquid-phase microextraction and determination via liquid chromatography with ultraviolet detection

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ARTICLE INFO

Article history:
Received 1 August 2015
Received in revised form
30 October 2015
Accepted 1 November 2015
Available online 3 November 2015

Keywords:
Phenol
Cresols
Solvent microextraction
Preconcentration
Waters
Soil extract

ABSTRACT

This work proposes a liquid-phase microextraction (LPME) method to extract the highly polar compounds phenol (Ph), *o*-cresol (*o*-Cr), *m*-cresol (*m*-Cr), *p*-cresol (*p*-Cr), and 2,4-dimethylphenol (2,4-DMP) from aqueous matrices. The first extraction step of the LPME method employed a common volumetric flask and *n*-octanol, and the second extraction step used NaOH as the acceptor phase. The optimized extraction conditions were 900 μL of *n*-octanol as the extraction solvent, NaOH at 0.60 mol L^{-1} as the acceptor phase, an extraction time of 5.0 min, HCl at 0.01 mol L^{-1} and NaCl at 20.0% as the donor phase, and an extraction temperature of 20.0 °C. The analysis of 50.0 mL of aqueous sample, pretreated under the optimized LPME conditions, afforded a limit of detection (LOD) between 0.3 and 3.5 μg L^{-1}, a limit of quantification (LOQ) between 1.2 and 11.6 μg L^{-1}, and a linear range from 2.50 to 50.0 μg L^{-1} for Ph, *o*-Cr, *m*-Cr and *p*-Cr and from 12.5 to 250 μg L^{-1} for 2,4-DMP. The proposed LPME method was a successful sample preparation strategy, and allowed for precise and accurate quantification of polar phenolic compounds in aqueous matrices such as tap water, river water, groundwater, and seawater, and also in a soil extract. The recovery values ranged from 72.5% to 126.0%, and the relative standard deviation was between 0.3 and 11.5%.

1. Introduction

Phenolic compounds are toxic substances that occur naturally in the environment, in plants and food [1]; they may also originate from decomposition of the lignin present in wood and humic substances [2]. This class of compounds has been employed as precursors and components of numerous chemicals in the industrial production of paper, detergents, polymers, pharmaceuticals, adhesives, explosives, phenolic resins, and petrochemical products [3]. However, several phenolic compounds present unpleasant organoleptic characteristics, toxicological effects or are highly persistent in the environment, which has placed them among the main contaminants in waters and soils. Indeed, the United States Environmental Protection Agency (US EPA) considers some of these compounds as priority pollutants and allows maximum total phenols concentrations of 1.0 μg L^{-1} and 100 μg kg^{-1} in drinking water and agricultural soils, respectively [4].

The toxicity and environmental issues associated with phenolic compounds have required the development of analytical techniques to quantify these substances. Gas chromatography (GC) [5–7] and liquid chromatography (LC) [8–11] are the main techniques employed in this scenario. The polar features and low volatility of phenolic compounds have favored the use of LC with ultraviolet detection (LC–UV) or coupled to mass spectrometry (LC–MS), because the use of LC avoids the need of derivatization processes [8,9,12]. Nonetheless, LC, especially LC–UV, usually presents higher limits of detection and requires sample enrichment before the determination of phenolic compounds in water samples [13].

Conventional methods like liquid–liquid extraction (LLE) and solid phase extraction (SPE) can aid in the preparation of aqueous samples for the determination of phenol content. However, these methods, particularly LLE, are time-consuming and tedious and they demand the use of large volumes of toxic solvents. To overcome these drawbacks, chemical analysts have turned to SPE-based miniaturized techniques such as solvent-free solid-phase

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http://dx.doi.org/10.1016/j.talanta.2015.11.004
0039-9140/

microextraction (SPME) [14,15] and stir bar sorptive extraction (SBSE) [16,17] to extract and preconcentrate phenolic species in water samples. Nevertheless, SPME and SBSE are relatively expensive, their coupling to LC is difficult, and additional steps like desorption, evaporation, and reconstitution are necessary prior to analysis [11]. In addition, the sorbent phases are fragile, susceptible to carryover, and they have a limited lifetime [5].

The limitations inherent to LLE, SPE, and even SPME and SBSE have encouraged investigations into miniaturized LLE pretreatment approaches based on solvent microextraction, the so-called liquid phase microextraction (LPME) techniques. LPME generally employs between 1 and 1000 μL of an acceptor organic solvent, which is immiscible with water, to extract the analyte from the aqueous phase, that is, the donor phase. It is possible to use LPME with GC, LC, and capillary electrophoresis (CE). The most common LPME categories include single-drop microextraction (SDME), dispersive liquid–liquid microextraction (DLLME), and hollow-fiber liquid phase microextraction (HF-LPME). The relevance of LPME techniques has motivated the publication of a number of detailed reviews [18–22]. SDME seems to be more appropriate to prepare samples for the determination of phenolic species and other analytes by GC [20], although some researchers have reported on the use of HF-LPME for this purpose [23,24]. Other authors have opted to use DLLME to pretreat samples for the determination of phenolic compounds [8,11,25–30]. To extract phenols from water, it is essential to acidify the aqueous matrix, in order to obtain the molecular form of the analytes and improve their extraction. Nevertheless, phenolic compounds present a wide range of hydrophobicity, which impacts their recovery; the log K_{OW} values vary from 1.46 for phenol to 5.12 for pentachlorophenol, and by using DLLME, recovery values of 13.1 and 81.8% for phenol and pentachlorophenol were observed, respectively [11]. On the basis of these data, it seems that the extraction of more polar phenolic compounds from water is not a straightforward task, which is probably the reason why most of the work based on LPME has described attractive results only for the more hydrophobic compounds, mainly chlorophenols [8,11,25,27–30]. For the more polar phenolic compounds, even the best extraction conditions for conventional DLLME provide the extraction of a small amount of the analyte, not to mention that they require higher sample volumes. In an attempt to achieve an extraction method capable of processing a larger sample volume (100 mL), Zhang et al. [12] used a special glass device. These authors designated the procedure as two-step LPME for the extraction of nitrophenols, chlorophenols, and phenol and obtained very satisfactory results.

Notwithstanding the several LPME procedures available to prepare samples for the determination of nitrophenols and chlorophenols, no reports have been published about the microextraction of cresols. Hence, the present study aimed to develop a two-stage method for the microextraction of phenol and cresols from water matrices. The first stage consisted of solvent extraction employing a simple commercial volumetric flask and *n*-octanol, whereas the second stage was comprised of an alkaline extraction. After the determination of the best conditions for analysis, the LPME method was successfully employed for the quantification of phenol, *o*-cresol, *m*-cresol, *p*-cresol and 2,4-dimethylphenol in different environmental water samples. It is important to note that these analytes were investigated in order to evaluate the possibility of water or soil contamination. Therefore, for the first time determination of the highly polar compounds, such as phenol and cresols, in a soil extract sample was carried out by LPME.

2. Experimental

2.1. Reagents, solutions, and materials

The phenol (Ph), *o*-cresol (*o*-Cr), *m*-cresol (*m*-Cr), *p*-cresol (*p*-Cr), and 2,4-dimethylphenol (2,4-DMP) standards were acquired from Sigma-Aldrich (St. Louis, USA) at purity greater than 99.0%. Methanol (HPLC grade) was purchased from J.T. Baker (Mexico City, Mexico). Acetonitrile (HPLC grade) was supplied by Carlo Erba (Rodano, Italy) and was used for the composition of the mobile phase. The analytical grade solvents, *n*-hexane, cyclohexane, butyl acetate, and *n*-octanol were obtained from Sigma-Aldrich (St. Louis, USA). All other reagents employed in the current study were of analytical grade and acquired from Merck, Carlo Erba, or J.T. Baker. High purity water (resistivity of 18 MΩ cm) was obtained with the aid of a reverse osmosis system from Quimis (Diadema, SP, Brazil), model Q842-210, followed by purification with a Simplicity UV water purifying system from Millipore (Molsheim, France). A Hanna potentiometer, model pH 21, coupled to an Ag/AgCl combined glass electrode was used to measure the pH values.

All of the glassware was kept in a 2.5% (v/v) alkaline detergent solution for at least 24 h, washed with water obtained from the reverse osmosis system, then with high purity water, and dried in a dust-free environment. Stock standard solutions at a concentration of 1000 mg L^{-1} were prepared in methanol and stored in amber glass vials at 4 °C. Working solutions containing the five phenolic compounds at concentrations ranging between 2.50 and 250 μg L^{-1} were prepared on a daily basis by dilution of the stock standard solution with high purity water. Before the chromatographic determinations, the standards and the samples were filtered through disposable 0.45 μm PTFE membranes with a diameter of 25 mm (from Millipore).

2.2. Chromatographic conditions

A liquid chromatography system from Waters (Milford, MA, USA) was employed, equipped with a quaternary pump (Waters 600E), a degasser (In Line AF), a thermostatted column compartment (Module II), an automatic sampler (20 μL, Waters 717 Plus), and a diode-array detector (Waters 2998). The signals were acquired at 270 nm with the aid of the software Empower 2. An octadecylsilane (C18) column from Kromasil AzkoNobel (Bohus, Sweden) (250 mm × 4.6 mm, particle size of 5 μm) was connected to a C18 guard column and employed at 40 °C. The mobile phase consisting of a 60:40 mixture of 1.0% acetic acid and acetonitrile was filtered through a 0.45 μm PTFE membrane (Millipore) and used in the isocratic mode at a flow rate of 1.0 mL min^{-1}.

2.3. Microextraction procedure

Fig. 1 illustrates the procedure employed for the microextraction and describes the optimized analytical conditions.

First, a solution containing *Ph*, *o*-Cr, *m*-Cr, *p*-Cr, and 2,4-DMP at 100.0 μg L^{-1} was employed to establish the best extraction conditions. The following parameters were evaluated, in triplicate: solvent (*n*-hexane, cyclohexane, butyl acetate, and *n*-octanol), NaOH concentration in the acceptor phase (0.10, 0.15, 0.25, 0.40, and 0.60 mol L^{-1}), stirring time (2.5, 5.0, 10.0, 20.0, and 30.0 min), volume of *n*-octanol in the extraction phase (100, 300, 500, 700, and 900 μL), HCl concentration in the donor phase (0.01, 0.05, 0.10, and 1.00 mol L^{-1}), extraction temperature (5.0, 10.0, 20.0, 30.0, 40.0, 50.0, and 60.0 ± 0.2 °C), and NaCl concentration in the donor phase (2.50, 5.00, 10.0, and 20.0%). The temperature was controlled by insertion of the volumetric flask in a glass jacket connected to a thermostatic bath (Fig. 1). Volumes of 25, 50, and 100 mL of the standard solution were also tested.

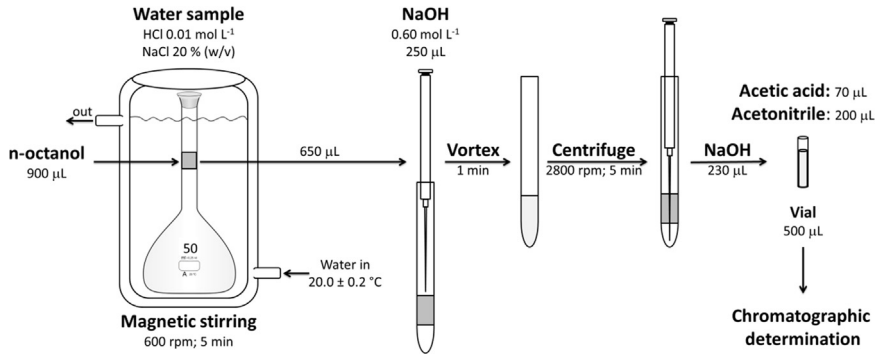

Fig. 1. Schematic procedure using a commercial volumetric flask for the extraction by LPME, and the description of the optimized analysis conditions.

After establishing the best analytical conditions, extraction involved the use of a standard solution containing the five phenolic compounds at a concentration of 250 μg L^{-1}. The HCl concentration was adjusted to 0.01 mol L^{-1}, 20.0% of NaCl (w/v) was added, and 50.0 mL of the resulting solution was transferred to a volumetric flask, as shown in Fig. 1. A 900 μL aliquot of *n*-octanol was added, and the solution was submitted to magnetic stirring at 600 rpm and 20 °C for 5 min. The solution was allowed to rest for 5 min. After phase separation, a 650 μL aliquot of the *n*-octanol phase was transferred to a glass centrifuge tube. A 250 μL aliquot of NaOH solution at 0.60 mol L^{-1} was mixed in a vortex for 1 min and centrifuged at 2800 rpm for 5 min. A 230 μL aliquot of the aqueous phase was withdrawn and placed in a 500 μL volumetric tube containing 70 μL concentrated acetic acid and 200 μL acetonitrile. This procedure was repeated for the analytes at concentrations of 2.50, 5.00, 12.5, 25.0, 50.0, and 125 μg L^{-1}, in triplicate, to construct the analytical curves. Similar experiments were carried out for the blank analysis.

2.4. Application of the method to real samples

The proposed method was employed for the analysis of environmental water samples: seawater, river water, tap water, and groundwater. The samples were filtered through a 0.45 μm cellulose nitrate membrane (Sartorius®) before the microextraction.

A sample of aqueous soil extract was also analyzed, using 6.00 g of soil stirred with 180 mL of $CaCl_2$ solution at 0.01 mol L^{-1}, at 150 rpm and 25 °C, for 24 h. After resting for 10 min, the supernatant was filtered through a 0.45 μm cellulose nitrate membrane (Sartorius®) and subjected to the microextraction method. Additional details about the soil extract have been reported in previous papers [31,32].

The water samples and the soil extract were fortified with the phenolic compounds at concentrations of 3.00, 15.0, and 40.0 μg L^{-1}, to evaluate the accuracy and precision of the microextraction method.

3. Results and discussion

3.1. Preliminary considerations

Several experiments were conducted in order to determine the optimal conditions to separate the target phenolic compounds by LC. The use of higher water and lower acetonitrile proportions (higher polarity) in the initial elution process, or gradient elution under different conditions failed to separate *m*-Cr and *p*-Cr. Despite this drawback, the Empresa Brasileira de Pesquisa Agropecuária (Embrapa) was interested in this study, and therefore, the work was carried out considering the mixture of *m*-Cr and *p*-Cr using isocratic elution, since the chromatographic peaks of both compounds were not separated by gradient elution. Because of the relatively low concentrations expected for the target analytes in real samples, the isocratic elution is advantageous, since it does not require baseline stabilization. The optimized chromatographic conditions afforded retention times of 5.69, 7.79, 8.53, and 12.5 min for Ph, *m*-Cr+*p*-Cr, *o*-Cr, and 2,4-DMP, respectively.

Because of the physicochemical characteristics of the target analytes, such as log K_{OW} values (25 °C) of 1.46, 1.98, 1.94, 1.95 and 2.40 and water solubility (g L^{-1}, 20 °C) of 93.0, 23.0, 23.5, 25.0 and 5.0 for Ph, *m*-Cr, *p*-Cr, *o*-Cr, and 2,4-DMP, respectively, the extraction of these compounds is not easy, in contrast to the more hydrophobic chlorophenols. As environmental water samples usually contain low concentrations of phenolic species, it is more appropriate to process larger sample volumes [12]. DLLME procedures commonly employ sample volumes ranging between 1.5 [25] and 10 mL [11]. In the present work, the use of commercial volumetric flasks facilitated the separation of the organic phase and the operation of the magnetic stirrer at its maximum speed (600 rpm), which improved the extraction efficiency [33]. The vortex time was fixed at 1 min, and the centrifugation speed was set at 2800 rpm, for 5 min. Initially, sample volumes of 25.0, 50.0, and 100 mL were pretreated by the DLLME procedure, and the sample volumes of 25.0 and 50.0 mL presented the best repeatability. Hence, a sample volume of 50.0 mL was selected, due to a better limit of detection, and analyte concentrations of 100 μg L^{-1} were employed to optimize the microextraction conditions. The volume of the acceptor phase (NaOH) was fixed at 250 μL. All determinations were performed in triplicate, and the results are depicted between Figs. 2 and 8.

3.2. Selection of the solvent for extraction

On the basis of previous literature about microextraction [19,20,27,33], solvents with different polarities, like *n*-hexane, cyclohexane, butyl acetate, and *n*-octanol, were investigated in order to achieve the best extraction capacity (Fig. 2).

Cyclohexane and *n*-hexane did not provide a satisfactory extraction of the analytes, not even for the less polar 2,4-DMP. Butyl acetate provided an improved extraction of the analytes, except for 2,4-DMP. The more polar *n*-octanol afforded the best extraction results, probably because its hydroxyl group favored hydrogen bonding. Different mixtures of these solvents were also tested, as an attempt to improve extraction of the less polar 2,4-DMP. The *n*-octanol remained the best option, based on the larger peak areas. Indeed, other authors have successfully employed this solvent in different LPME approaches to extract a number of phenolic compounds [9,12,29].

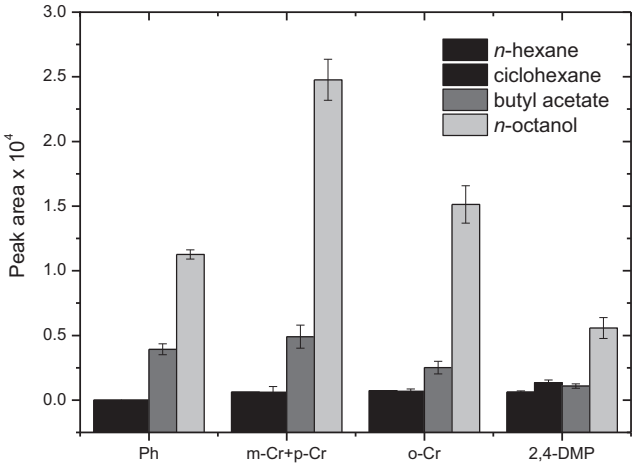

Fig. 2. Selection of the solvent for extraction of the phenolic compounds. Extraction time: 20 min; solvent volume: 500 μL; NaOH concentration (acceptor phase): 0.10 mol L^{-1}.

3.3. NaOH concentration

The acceptor solution, which removes the target phenolic compounds from the *n*-octanol phase, ought to be alkaline. This solution should ionize the phenolic compounds and maximize their solubility in the aqueous medium. Aqueous NaOH solutions have found application as acceptor solutions for the LPME of phenolic compounds [9,12,13,24,27]. Fig. 3 shows how NaOH concentrations between 0.01 and 0.60 mol L^{-1} influenced the microextraction process of the target analytes.

In the case of phenol, the results were practically the same regardless of the NaOH concentration. For the other analytes, NaOH concentrations higher than 0.10 mol L^{-1}, which is the concentration that is usually employed for LPME [9,12,24], led to superior results. The peak areas obtained for *m*-Cr+*p*-Cr and for *o*-Cr at NaOH concentrations ranging between 0.40 and 0.60 mol L^{-1} decreased slightly, whereas NaOH at 0.60 mol L^{-1} yielded a relatively higher signal for 2,4-DMP. Based on these results, a NaOH concentration of 0.60 mol L^{-1} was chosen for further experiments.

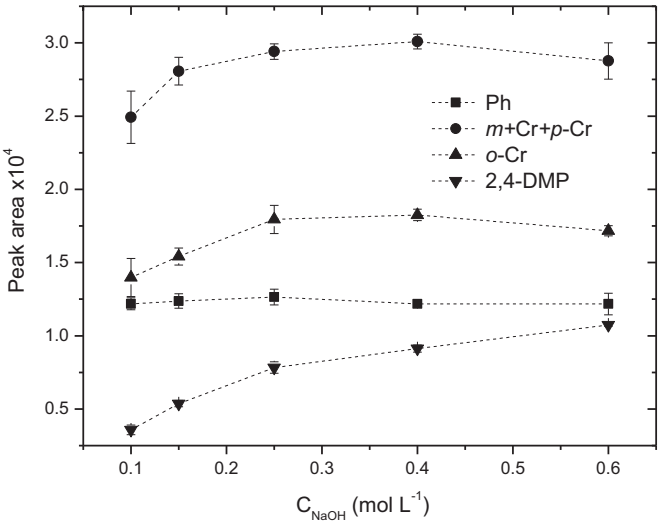

Fig. 3. Evaluation of the NaOH concentration in the acceptor phase for the extraction of the phenolic compounds. Extraction time: 20 min; *n*-octanol volume: 500 μL.

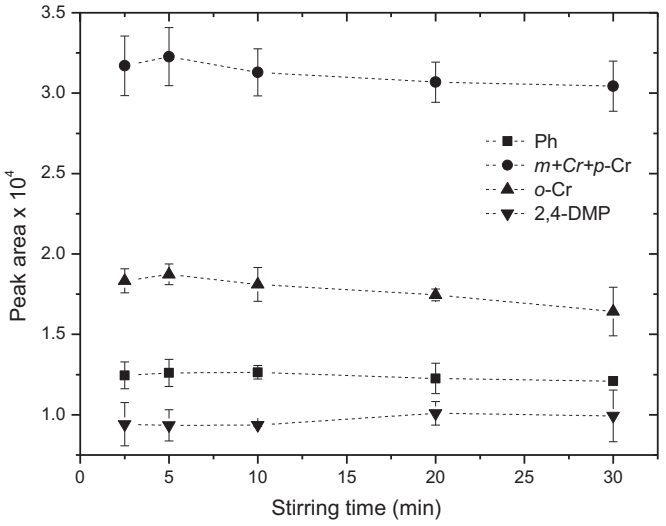

Fig. 4. Effect of the extraction time for determination of the phenolic compounds. *n*-octanol volume: 500 μL; NaOH concentration (acceptor phase): 0.60 mol L^{-1}.

3.4. Extraction time

The extraction time is an important factor in LPME, since shorter times provide higher analytical frequency, but afford lower analytical signals. The results of the magnetic stirring performed between 2.5 and 30 min are shown in Fig. 4.

Longer extraction times of 20 and 30 min only yielded higher peak areas for 2,4-DMP. In regard to the other analytes, the differences were not significant, and average peak areas were slightly higher at 5 min as compared with longer extraction times. Since intense agitation should promote more efficient mass transfer, the high rotation speed of 600 rpm may have been sufficient to establish an apparent equilibrium even at shorter stirring times. In addition, the high affinity of the analytes for water may have reduced the time necessary for the extraction process to reach equilibrium and could account for the longer extraction time required for the more apolar 2,4-DMP. Similar results for phenol, nitrophenols, and *p*-chlorophenol were observed in the literature [12] although in this case higher peak areas arose at 10 min. The results of the present study led to the selection of a 5 min extraction time for further experiments.

3.5. n-Octanol volume

Fig. 5 shows the results for *n*-octanol volumes ranging from 100 to 900 μL.

m-Cr+*p*-Cr, *o*-Cr, and Ph exhibited similar profiles: larger *n*-octanol volumes increased the peak areas, which tended to stabilize at higher solvent volumes. The solvent volume is an important parameter for LPME procedures because it affects microextraction from the donor phase [27]. Higher *n*-octanol volumes should afford better extraction efficiency, but at the same time they could cause a dilution, with a consequent reduction in the peak areas. In fact, the literature reported an analogous result and stated that the best *n*-octanol volume to extract phenolic compounds was 800 μL [12]. On the contrary, for 2,4-DMP, a divergent behavior was verified, 900 μL of *n*-octanol provided lower peak areas. Because 2,4-DMP presents higher affinity for the organic phase (log K_{OW} 2.40) and lower water solubility (5.0 g L^{-1}) in comparison with the other target compounds, it should be easier for *n*-octanol to extract 2,4-DMP from the donor aqueous solution, and thus higher peak areas would be expected. However, less efficient removal of 2,4-DMP from the organic phase may have

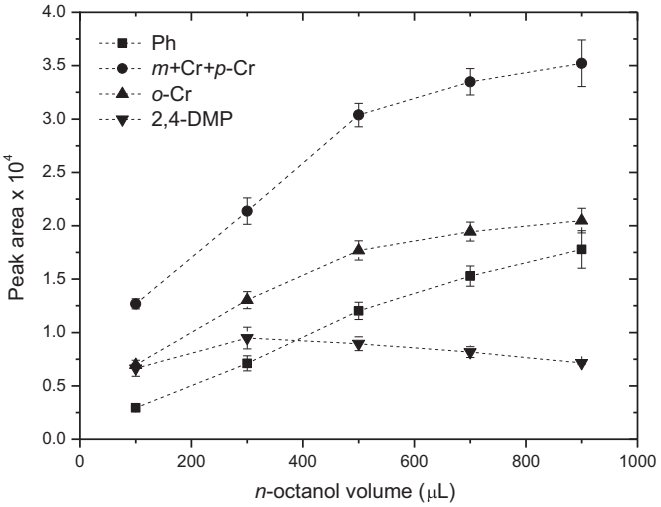

Fig. 5. Evaluation of *n*-octanol volume for the extraction of the phenolic compounds. Extraction time: 5 min; NaOH concentration (acceptor phase): 0.60 mol L^{-1}.

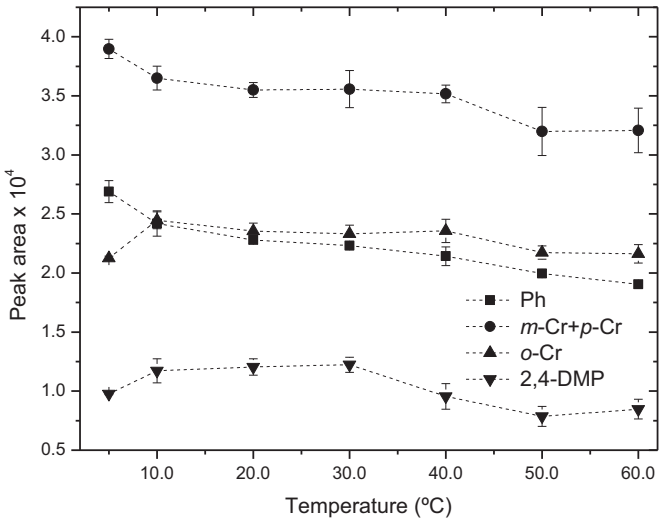

Fig. 7. Effect of the temperature for the extraction of the phenolic compounds. Extraction time: 5 min; *n*-octanol volume: 900 μL; HCl concentration: 0.01 mol L^{-1} (donor phase); NaOH concentration (acceptor phase): 0.60 mol L^{-1}.

occurred even in the alkaline medium. Despite the superior 2,4-DMP extraction by *n*-octanol, it is likely that a competitive effect could occur between this solvent and the alkaline aqueous phase, explaining the lower peak areas for 2,4-DMP in comparison with the other analytes, although this hypothesis was not investigated in the present work. In spite of the lower extraction efficiency in the case of 2,4-DMP, an *n*-octanol volume of 900 μL was chosen for the next steps.

3.6. HCl concentration

Because the presence of phenolic compounds in the protonated form culminates in better extraction efficiency, the pH of the aqueous solution (donor) is a relevant variable in microextraction techniques. In the specialized literature, the consensus is that acidic media provides better extraction during LPME [9,12,13,24,25,27]. Fig. 6 displays the results concerning the HCl concentration in the donor phase during the microextraction of the target analytes.

Despite the high standard deviation values, especially for *m*-Cr+*p*-Cr, reduction in the pH values did not impact the peak area

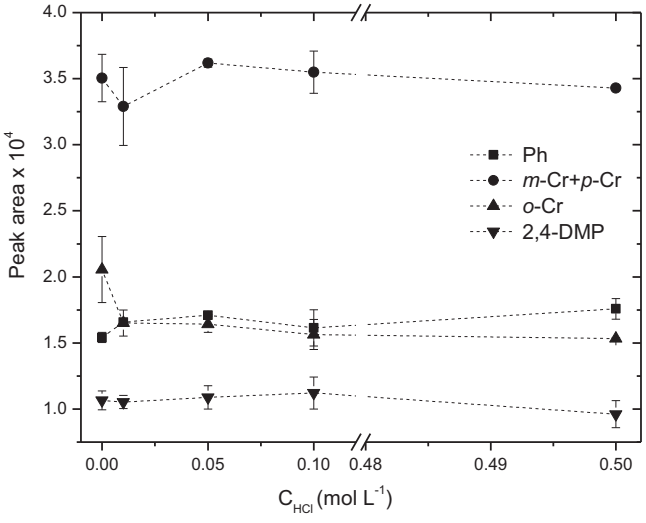

Fig. 6. Evaluation of the HCl concentration for the extraction of the phenolic compounds. Extraction time: 5 min; *n*-octanol volume: 900 μL; NaOH concentration (acceptor phase): 0.60 mol L^{-1}.

signals significantly. This aspect was probably related to the high pK_a values of the target analytes, which varied between 9.89 (Ph) and 10.6 (2,4-DMP). On the basis of these values, all the species should be protonated even in neutral pH conditions. Fan et al. [25] reported similar results for pH values lying between 3 and 8, and these authors selected pH 6 for the donor phase. Other works have described that pH 2.0 was the best value for the donor phase in other LPME procedures [9,12,27]. Thus, HCl at 0.01 mol L^{-1} was adopted in the subsequent experiments.

3.7. Temperature

The previous experiments were conducted at room temperature. Although temperature affects equilibrium and mass transfer during solvent extraction procedures, few works have investigated temperature effects on the extraction of these analytes [5,10], and for this reason, this study was carried out. The results of a study of temperatures between 5.0 and 60.0 °C (± 0.2 °C) with the aid of a glass jacket and a thermostatic bath are shown at the Fig. 7.

The peak areas of *o*-cresol and 2,4-DMP increased from 5 to 10 °C. It is likely that better mass transfer to the *n*-octanol phase took place [5,10]. In general, the peak areas of all the analytes decreased between 10 and 60 °C, although a relatively constant signal was observed between 10 and 30 °C for 2,4-DMP. The reduced extraction efficiency at higher temperatures was in contrast to literature results [5], but it agreed with results achieved for the microextraction of nitrophenols [10], which were better between 20 and 30 °C and decreased between 40 and 60 °C. A rise in temperature should enhance the extraction of the analytes from the aqueous phase; however, *n*-octanol is more soluble in water at higher temperatures, which could decrease the extraction of the analytes. Although a temperature of 10 °C afforded better extraction of Ph and *o*-Cr, the temperature of 20 °C was easier to control, and this temperature was selected for the other experiments.

3.8. Ionic strength

Increased ionic strength in the aqueous sample causes the classical “salting-out” effect, which is noteworthy for highly polar organic compounds [5] during LPME or conventional LLE. In the presence of salts, the water molecules prefer to hydrate the ionic salt. Consequently, fewer water molecules are available to solubilize the polar organic species, which reduces their solubility in

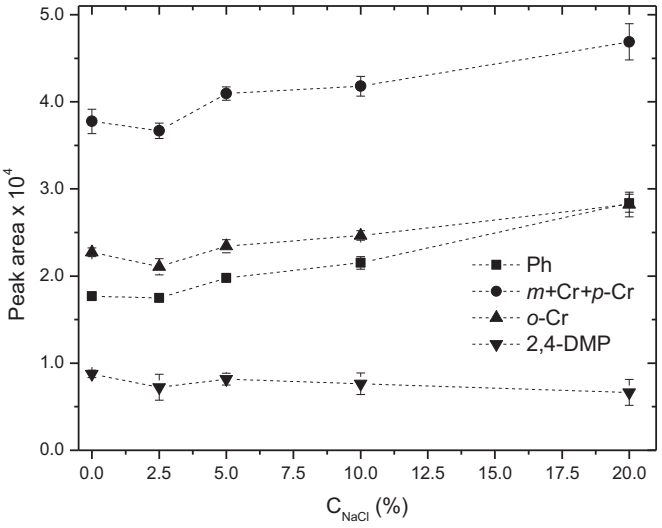

Fig. 8. Evaluation of the NaCl concentration for the extraction of the phenolic compounds. Extraction time: 5 min; *n*-octanol volume: 900 μL; HCl concentration: 0.01 mol L^{-1} (donor phase); NaOH concentration (acceptor phase): 0.60 mol L^{-1}; temperature: 20.0 °C.

Table 1
Performance parameters obtained for the microextraction method.

Analyte	Intercept[a]	Slope[a]	Range	LOQ	LOD	r^2	EF[b]
			μg L^{-1}				
Ph	−49 ± 10	76 ± 4	2.50–50.0	1.4	0.4	0.9995	15
m-Cr+*p*-Cr	−34 ± 7	57 ± 5	2.50–50.0	1.2	0.3	0.9993	70
o-Cr	−64 ± 8	73 ± 6	2.50–50.0	1.2	0.4	0.9979	23
2,4-DMP	−136 ± 28	24 ± 1	12.5–250	11.6	3.5	0.9990	28

[a] Mean values ± the standard deviation, $n=3$, except for the lower concentration with $n=6$.
[b] Enrichment factor.

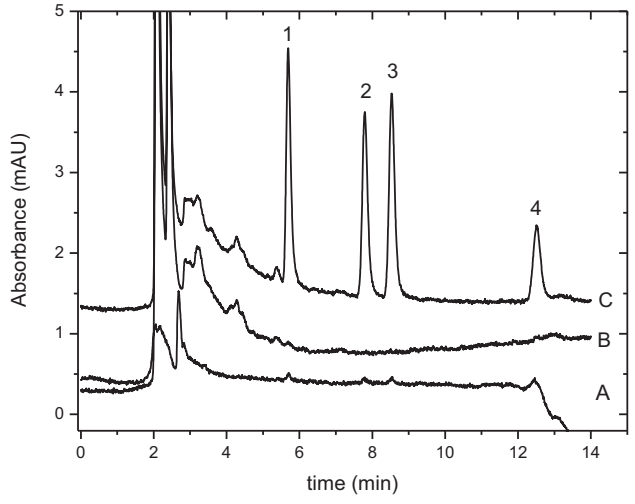

Fig. 9. Chromatograms of the direct determination of the soil extract spiked with 40.0 μg L^{-1} of each analyte without the use of LPME (A), chromatogram after the LPME of the soil extract without the spike (B), and chromatogram of the soil extract spiked with 40.0 μg L^{-1} of each analyte using the LPME (C). Peak identification: Ph (1), *m*-Cr+*p*-Cr (2), *o*-Cr (3) and 2,4-DMP (4).

water and forces them to solubilize in non-polar solvents or to interact on the surface of appropriate sorbents [33]. In order to study the effect of the ionic strength, NaCl was employed and the results are shown in Fig. 8.

Sodium chloride, in concentrations higher than 2.50%, increased the peak areas considerably, except for 2,4-DMP. Higher NaCl concentrations than 20% were not studied, due to the difficulty in dissolving the salt. In general, the impact of the ionic strength on LPME procedures has been a matter of controversy. Some authors have reported that the addition of salts in the donor phase did not affect the extraction process [8,12,24–27], and no control for ionic strength was made; although in some of this research low salt concentrations were used and some analytes had more apolar characteristics. In contrast, other authors showed significantly improved LPME performance when they used NaCl concentrations of 10% [9,29] and 20% [13,34] as well as saturated NaCl [5,35] in the donor phase, which suggested that higher salt concentrations were necessary to achieve a more efficient extraction. In the present work, although the peak areas obtained for 2,4-DMP decreased slightly at 20% NaCl, this concentration improved the extraction of the other target compounds significantly, and, hence, 20% NaCl was used hereafter.

3.9. Analytical performance

The optimal LPME conditions for the target analytes were as follows: 900 μL of *n*-octanol as solvent, NaOH at 0.60 mol L^{-1} in the acceptor phase, extraction time of 5.0 min, HCl at 0.01 mol L^{-1} in the donor phase, extraction temperature of 20.0 °C, and 20.0% (w/v) NaCl in the donor phase. Using these optimal conditions, the next step was to validate the LPME method on the basis of analytical curves constructed with five different concentrations of the analytes, between 2.50 and 50.0 μg L^{-1} for Ph, *m*-Cr+*p*-Cr, and *o*-Cr, and between 12.5 and 250.0 μg L^{-1} for 2,4-DMP. The concentrations of 125 and 250 μg L^{-1} were also tested for Ph, *m*-Cr+*p*-Cr, and *o*-Cr, but the best results were achieved for concentrations as high as 50.0 μg L^{-1}. For 2,4-DMP, the integration of the chromatographic peaks was not possible at concentrations lower than 12.5 μg L^{-1}. Table 1 summarizes the results.

For Ph, *m*-Cr+*p*-Cr, and *o*-Cr, analyte concentrations varying from 2.50 to 50.0 μg L^{-1} yielded coefficients of determination (r^2) higher than 0.9979, while for 2,4-DMP, the linear range was between 12.5 and 250 μg L^{-1}. These results suggested adequate linearity for all of the analytes. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated by the expressions: $LOD = 3 \times (s/S)$ and $LOQ = 10 \times (s/S)$, where *s* and *S* denote the standard deviation of the intercept and the average value of the slope from the analytical curves, respectively. The slopes demonstrated better sensitivity of the method for Ph and *o*-Cr and lower sensitivity for 2,4-DMP. Ph, *m*-Cr+*p*-Cr, and *o*-Cr displayed relatively low LOD (0.3–0.4 μg L^{-1}) and LOQ (1.2–1.4 μg L^{-1}), whereas the LOD and LOQ for 2,4-DMP were higher: 3.5 and 11.6 μg L^{-1}, respectively. To calculate LOD and LOQ, the enrichment factors (*EF*) between 15 and 70 were considered. *EF* was calculated by comparing the concentrations obtained with and without the use of the LPME procedure, and the resulting *EF* values were satisfactory, especially if one considers the high polarity of the analytes. Replicate determinations ($n=6$) for the lowest concentrations (12.5 μg L^{-1} for 2,4-DMP and 2.50 μg L^{-1} for the other analytes) helped to evaluate the precision of the method. The highest relative standard deviation (RSD) was 9.0% for 2,4-DMP. The other compounds presented RSD values lower than 8.0%, which is adequate considering the complexity of the method.

3.10. Analysis of real samples

When applied to samples of tap water, river water, seawater, groundwater, and soil extract, the proposed method did not detect the target compounds. Taking into account the phenolic analytes Ph, *m*-Cr, *p*-Cr, *o*-Cr, and 2,4-DMP, few results are available in the literature for comparison purposes. For different water samples,

Table 2
Recovery percent and the relative standard deviation (RSD) of the phenolic compounds for real water samples and a soil extract.

Analyte	Spike ($\mu g\ L^{-1}$)	Tap water		Sea water		Groundwater		River water		Soil extract	
		R[a] (%)	RSD[b] (%)	R[a] (%)	RSD[b] (%)	R[a] (%)	RSD[b] (%)	R[a] (%)	RSD[b] (%)	R[a] (%)	RSD[b] (%)
Ph	3.00	104.3	1.5	107.1	9.0	97.3	6.5	118.7	7.3	86.1	7.1
	15.0	108.2	1.7	72.5	2.4	89.0	3.4	99.4	2.6	95.0	2.2
	40.0	103.5	2.6	86.8	5.1	89.6	1.5	94.6	2.9	93.3	2.2
m-Cr+*p*-Cr	3.00	109.6	6.2	118.5	4.6	126.0	3.5	121.3	6.1	90.3	3.8
	15.0	104.8	2.3	102.8	4.7	111.2	7.9	103.1	1.1	106.8	2.4
	40.0	98.3	2.5	103.8	5.1	108.2	1.9	103.3	3.4	99.0	0.3
o-Cr	3.00	94.4	5.2	123.8	5.4	120.9	3.4	117.9	2.7	104.6	4.6
	15.0	99.6	3.3	91.0	5.0	100.3	3.4	98.3	2.0	108.9	3.3
	40.0	91.1	2.9	93.4	4.8	95.2	1.1	96.0	3.7	88.1	0.4
2,4-DMP	15.0	118.1	5.5	96.8	4.7	123.8	3.1	103.6	6.2	92.0	8.0
	40.0	107.6	5.2	92.2	2.8	116.2	2.8	90.3	5.5	86.9	11.5

[a] Average value of recovery
[b] Relative standard deviation, $n=3$.

Table 3
Comparison of the proposed method with other LPME methods for extraction and quantification of phenolic compounds in aqueous samples by LC–UV.

Analytes	Method	Matrix	Extraction time (min)	Linear range ($\mu g\ L^{-1}$)	*EF*[a]	LOD ($\mu g\ L^{-1}$)	RSD (%)	Recovery (%)	Reference
2,4-DMP, Ph, chlorophenols, nitrophenols	DLLME	Wastewater	15	0.1–200 5–500	30–373	0.01–1.3	2.6–16.6	65.5–108.3	8
Ph, nitrophenols, chlorophenols	[b]SM-LLLME	Tap water Well water River water	45	0.1–0.5	42.0–98.7	0.08–0.45	1.3–8.0	86–120	9
Nitrophenols	[c]SMS-LLME	Wastewater River water Pond water	30	2.0–1000	160–166	0.26–0.58	6.5–10.7	90.6–98.2	10
Ph, nitrophenols, *p*-chlorophenol	Two-step LPME	Tap water Lake water	10	1–500 10–1000	296–954	0.3–3.0	2.3–7.1	93.0–102.7	12
Cresols, chlorophenols	LPME-BE[d]	Tap water Reservoir water	30	1–1000 5–1000	> 100	0.5–2.5	5.4 –11.5	73.7–105.3	13
Alkylphenols, nitrophenols, chlorophenols	[e]HF-LPME	Wastewater River Mineral water	20	0.45–60 0.95–60	30–308	0.14–0.29	1.1–4.3	67.1–104.4	24
2,4-DMP, nitrophenol, bisphenol, naphthol	[f]IL-DLLME	Tap water River water Wastewater	2	4–400 20–400	ND[g]	0.68–10	1.9–4.8	94.9–108.2	25
Ph, benzenediols	DLLME	Tap water Lake water Effluents	0.5	0.05–100 0.1–100	ND[g]	7.0–29	2.1–13.1	83.2–117.8	26
Chlorophenols	DLLME-SDME	Rain water Tap water Lake water	12	0.2–250 1.0–250	67–309	0.016–0.084	2.5–7.0	90.1–104.7	27
4-nitrophenol, 2-naphthol, bisphenol	DLLME-SFO[h]	Tap water River water Spring water	1	5–1000	ND[g]	0.1–1.5	3.2–5.3	85.0–110.0	28
Ph, 2,4-DMP, chlorophenols	LPME	Tap water Mineral water	15	0.3–870 5–300	ND[g]	0.05–3.0	4.5–10.3	84.6–102.4	35
Ph, *o*-Cr, *m*-Cr+*p*-Cr, 2,4-DMP	LPME	Tap water Groundwater River water Sea water Soil extract	5	2.50–50.0 12.5–250	15–70	0.3–3.5	0.3–11.5	72.5–126.0	This work

[a] EF: enrichment factor.
[b] SM: stir membrane.
[c] SMS: supramolecular solvent.
[d] BE: back extraction
[e] HF: hollow fiber.
[f] IL: ionic liquid.
[g] ND: not divulged.
[h] SFO: solidified floating organic droplets.

such as tap water, river water and mineral water, phenol and some of these compounds were not detected [9,12,13,25,26,35]. On the other hand, between 12.9 and 15.5 $\mu g\ L^{-1}$ (Ph) and 3.3 $\mu g\ L^{-1}$ (2,4-DMP) have been detected in wastewater samples [8]. Also, relatively high phenol concentrations of 35.0 $\mu g\ L^{-1}$ [12] and 360 $\mu g\ L^{-1}$ [26] have been detected in lake water samples, and 980 $\mu g\ L^{-1}$ in hospital effluent [26]. These results and the LOQ values previously presented, suggest that the method could be applied for other aqueous samples.

A recovery study was carried out to evaluate the accuracy and precision of the method. Two or three different concentrations of the analytes were employed, in triplicate. Fig. 9 shows a

chromatogram recorded for the soil extract.

Direct determination of the phenolic compounds in the soil extract without LPME (chromatogram A) gave practically no signal, even after spiking of the sample with the target analytes at 40.0 $\mu g\,L^{-1}$. In chromatogram B, the sample submitted to LPME and not spiked with the analytes did not display any peaks relative to the target phenolic compounds, which indicated that the developed LPME method acted as a cleanup process. Chromatogram C exhibited well-defined peaks, which suggested that the method was suitable for the determination of the target compounds in real samples. The chromatograms obtained for the other water samples (not shown) presented a similar behavior. Table 2 lists the results of the recovery study.

Recoveries varied between 72.5% and 126.0%. Approximately 70% of the results ranged between 90.0% and 110% and were acceptable in terms of accuracy. It is noteworthy that lower accuracy did not occur for the different spikes, independent of the sample origin. All the RSD values were lower than 11.5%, and around 70% of the results presented RSD values lower than 5.0%, indicating adequate repeatability of the LPME method.

On the basis of these results and the figures of merit, the method proposed herein is a feasible strategy to pretreat aqueous samples for quantification of phenol and cresols.

3.11. Comparison with other LPME methods

Table 3 shows the comparison of the proposed method with other LPME methods described in the literature by means of LC–UV.

Most of the literature results presented in Table 3, concern the more apolar chlorophenols and nitrophenols in water matrices. The present work aimed to quantify the polar compounds, phenol and cresols in water samples and soil extracts. The figures of merit obtained herein after only 5 min of extraction from the donor phase were comparable with figures of merit reported in other works on LPME (Table 3). The EF values were relatively low compared with the EF values achieved with other methods, probably owing to the high polarity of the compounds targeted herein. Nevertheless, the LPME method developed in this work afforded an appropriate linear range and LOD, even for detection at a less sensitive wavelength of 270 nm (which was chosen to minimize possible interferences, especially in the case of the soil extract). These two figures of merit agreed well with the figures of merit obtained by authors who used similar wavelengths (between 274 and 290 nm) [10,12,25,28]. Also, the recovery study demonstrated adequate performance, with recoveries ranging between 72.5% and 126.0%, and RSD values between 0.3% and 11.5%, suggesting appropriate accuracy and precision in comparison with the literature values.

4. Conclusion

Careful study of the variables related to the LPME procedure enabled microextraction of phenolic compounds using small solvent volumes and short extraction times while employing simple and inexpensive laboratory glassware and reagents. Although determining highly polar analytes, such as phenol and cresols, is challenging, the method proposed herein presented attractive figures of merit. The characteristics of the method made it a successful sample pretreatment strategy to determine the target analytes in matrices like tap water, river water, groundwater, seawater, and aqueous soil extracts. The recovery and RSD data attested to the appropriate accuracy and precision of the method, and could constitute a selective cleanup procedure involving two extraction stages.

Acknowledgments

The authors acknowledge the financial and logistic support provided by Embrapa Clima Temperado (Grant no. 21400.10/0186-7, from Projeto Xisto Agrícola), Pelotas, RS, Brazil; PETROBRAS/SIX, São Mateus do Sul, PR, Brazil and CAPES/REUNI program, for the fellowship.

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