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Application of three phase hollow fiber based liquid phase microextraction (HF-LPME) for the simultaneous HPLC determination of phenol substituting compounds (alkyl-, chloro- and nitrophenols)

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ABSTRACT

This work proposes for the first time the use of a three phase hollow fiber liquid phase microextraction (HF-LPME) procedure for the simultaneous extraction, and the later HPLC determination, of some phenol substituting compounds (alkyl-, chloro- and nitrophenols) that are considered as highly toxic compounds and/or endocrine disrupting ones. The substances studied include four chlorophenols (CPs): 2,4-dichlorophenol (2,4-DCP), 2,5-dichlorophenol (2,5-DCP), 2,6-dichlorophenol (2,6-DCP) and pentachlorophenol (PCP), three nitrophenols (NPs): 2,4-dinitrophenol (2,4-DNP), 2,5-dinitrophenol (2,5-DNP) and 2,6-dinitriphenol (2,6-DNP) and two alkylphenols (APs): tert butylphenol (TBP) and sec butylphenol (SBP). The extraction was carried out through a dihexyl ether liquid membrane supported on an Accurel® Q3/2 polypropylene hollow fiber. Optimum pH for donor and acceptor phases and extraction time were established. The enrichment (preconcentration) factors obtained were between 30 and 700 that allows detection limits between 140 and 290 pg mL^{-1}. The method was successfully applied to the determination of the compounds in environmental water samples, including urban wastewaters.

1. Introduction

Phenolic compounds (PCs) are a wide range of substances that can be found in environmental samples due to their use in several industrial processes, phytosanitary applications and anthropogenic emissions. Alkylphenols (APs), chlorophenols (CPs) and nitrophenols (NPs) were considered, in general, as highly toxic compounds [1–4] and some of them have been described, additionally, as endocrine disrupting compounds (EDCs) [5–8] which have various adverse health effects on human, animals and microorganisms. EDCs can provoke mimicking or inhibiting the natural action of the endocrine system (synthesis, secretion, transport and binding).The adverse effects of EDCs have become an important issue and have received important attention in the last decade and so there are numerous published studies related to their toxicity [9,10].

The United States Environmental Protection Agency (EPA) classifies APs, CPs and NPs as priority pollutants [11] and the European Union has included some of them into a list of substances with maximum allowable concentrations in inland and surface waters [12,13]. So, the development of rapid and sensitive analytical procedures for the determination of these compounds in environmental water samples is essential taken into account both their low levels and possible matrix effects.

Several extraction procedures have been applied to PCs from aqueous samples. Solid phase extraction (SPE) is nowadays, as in other analytical fields, the most popular extraction procedure [14–16]. However, other extraction alternatives have been also used ranging from modifications on the classical liquid–liquid extraction (LLE) [17] to more recent techniques like solid phase microextraction (SPME) [18–20] or stir bar sorptive extraction (SBSE) [21]. Different configurations of liquid phase microextraction (LPME) have also been used: dispersive liquid–liquid microextraction (DLLME) [22], single drop liquid phase microextraction (SDLPME) [23,24] or hollow fiber liquid phase microextraction (HF-LPME) [3,25–34]. HF-LPME in two phase configuration has been used for the extraction of some CPs [3,25–28]; three phase HF-LPME have been used as extraction procedure for the analysis of some CPs using ionic liquid as supported liquid membrane (SLM) [29] and some NPs were extracted using other organic solvents as supported liquid membrane (SLM) [30–34].

Gas chromatography (GC) and high performance liquid chromatography (HPLC) coupled to mass spectrometry are the main techniques used for the CPs analysis. Due to the nature of the analytes, a pre-column derivatization step must be used if the analysis is carried out by GC [14,35,36].

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In this study, we outline for the first time the use of a three phase HF-LPME procedure for the simultaneous extraction, and later HPLC determination, of some APs, CPs and NPs. The compounds selected for this study were: four CPs, 2,4-dichlorophenol (2,4-DCP), 2,5-dichlorophenol (2,5-DCP), 2,6-dichlorophenol (2,6-DCP) and pentachlorophenol (PCP); three NPs: 2,4-dinitrophenol (2,4-DNP), 2,5-dinitrophenol (2,5-DNP) and 2,6-dinitriphenol (2,6-DNP); and two APs: tert butylphenol (TBP) and sec butylphenol (SBP). Three phase HF-LPME provides a simple, low-cost and disposable pre-treatment sample method that allows null carry over and high preconcentration and selectivity. The extracts obtained can be directly injected into liquid chromatographic systems providing, in general, excellent baselines even analyzing very complex samples. The HF-LPME experimental conditions and the chromatographic separation were studied and the proposed analytical method was successfully applied to the determination of the compounds in environmental water samples, including urban wastewaters.

2. Experimental

2.1. Chemicals and reagents

All chemicals were of analytical-reagent grade or better. All solutions and dilutions were prepared using ultrapure water form a Milli-Q Plus (Millipore, Billerica, MA, USA) water purification system. 2,4-DNP, 2,5-DNP, 2,6-DNP, 2,4-DCP, 2,5-DCP, 2,6-DCP, PCP, TBP, SBP, dihexyl ether, 1-octanol and peppermint oil were purchased from Fluka-Sigma–Aldrich (Madrid, Spain) and the rest of products were obtained from Merck (Darmstadt, Germany).

Aqueous working solutions of the compounds studied, were daily prepared by adequate dilutions from aqueous 100 μg mL^{-1} stock solutions. Accurel® Q3/2 polypropylene hollow fiber (600 μm i.d., 200 μm wall thickness and 0.2 μm pore size) was purchased from Membrana (Wuppertal, Germany).

2.2. Chromatographic conditions

The chromatographic separation was performed at 20 °C using an Agilent 1100 series liquid chromatography system (Palo Alto, CA, USA), with a quaternary pump, a vacuum degasser and a thermostated column compartment. For detection, HPLC was equipped with a diode array detector (DAD) and fluorescence detector (FLD). The injector was a Rheodyne manual injection valve Model 7725i, fitted with a 10 μL sample loop. Separations were carried out using a Lichropshere® C-18, 5 μm particle size column (250 mm × 4.6 mm i.d.) (VWR, Darmstadt, Germany) preceed by a guard column Kromasil® 100 Å, C18, 5 μm, (15 × 4.6 mm i.d.) (Scharlab S.L., Barcelona, Spain).

The mobile phase consisted of methanol (component A) and 0.1% formic acid (pH 2.6) (component B) and at a flow rate of 1.0 mL min^{-1}. An initial 45% component A was used in isocratic mode for 2 min and then a linear elution gradient was programmed from 45% to 70% A for 8 min and another linear gradient from 70% to 100% A for 10 min. Five minutes were waited between injections which allowed re-equilibration of the column to the initial conditions.

Table 1 shows the monitoring wavelengths for DAD and FLD detection and the retention times for the analyzed compounds. Fig. 1 shows a representative chromatogram for the proposed separation.

2.3. Supported liquid membrane preparation and extraction procedure

Hollow fibers were cut into 13 cm pieces, washed with acetone in an ultrasonic bath and dried. The fiber was soaked with dihexyl ether during 5 s to impregnate the pores and rinsed with water on the outside by placing it into an ultrasonic bath for 25 s in order to remove the excess of organic solvent. The lumen of the prepared fiber piece was filled with 30 μL of aqueous pH 13 acceptor phase using a HPLC syringe. Both open ends of the fiber were closed by means of a hot soldering tool and adhesive tape. During extraction the membrane portion that contains the acceptor phase was immersed in the 50 mL sample solution (pH 2, adjusted with HCl) contained into a 50 mL glass beaker. The sample was stirred 20 min for by means of a magnetic stirrer (ANS-00/1 Science Basic Solutions (Rubí, Barcelona, SPAIN) at 300 rpm. After extraction, the fiber was taken out, one of the ends was cut and the acceptor phase was extracted using a HPLC syringe and injected into the HPLC system.

Table 1
Monitoring wavelengths and retention times.

DAD			FLD		
	λ_{max} (nm)	t_R (min)	λ_{exc} (nm)	λ_{em} (nm)	t_R (min)
2,6-DNP	250	7.59	–	–	–
2,4-DNP	260	9.05	–	–	–
2,5-DNP	260	9.37	–	–	–
2,6-DCP	280	11.92	–	–	–
2,5-DCP	285	13.43	–	–	–
2,4-DCP	285	13.88	–	–	–
TBP	280	14.62	220	305	14.65
SBP	280	15.25	220	305	15.28
PCP	250	20.66	–	–	–

2.4. Preparation of water samples

Wastewater samples were obtained from E.D.A.R. San Juan del Puerto-AQUALIA wastewater Treatment Plant which is located in San Juan del Puerto, Huelva, SPAIN. The WWTP essentially receives urban wastewaters. The capacity of this WWTP is 119069 inhabitants and the discharged flow is 3000 m^3/day. Samples from the influent (raw water, WWR), after the physico-chemical treatment (WW1) and the effluent (after sedimentation, WWT) were analyzed.

Two samples from Guadalquivir River were analyzed. One (RIVER1) from Seville and other sample (RIVER2) was taken at the mouth of Guadalquivir River (Sanlúcar de Barrameda, Cádiz) where water has a high seawater proportion.

Drinking mineral water (MIN) Albarcin® (Eden Springs España, Almería, Spain) was from Sierra Nevada, Spain. Tap water sample (TAP) was obtained directly from the laboratory tap.

All samples, except drinking mineral and tap waters, were filtered through a GDU1 glass fiber filter bed (10 μm) (Whatman, Mainstone, UK) and then through a Pall Nylaflo™ 0.45 μm nylon membrane filter (Pall Corporation, Ann Arbor, MI, USA) and adjusted to pH 2 with HCl. Filtered samples were stored in the dark at 4 °C. Prior to HF-LPME extraction nitrogen was bubbled for 10 min into the water samples at pH 2 to eliminate dissolved carbonate.

3. Results and discussion

3.1. Selection of organic solvent for the supported liquid membrane

In some preliminary experiments, the attention was focused on the selection of the more adequate organic solvent to the liquid membrane for the impregnation of the polypropylene hollow fiber because this is one of the critical steps in LPME. According to our previous experience we have checked 1-octanol,

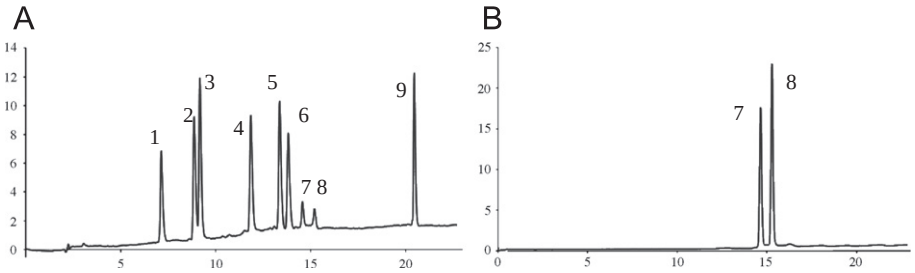

Fig. 1. DAD (a) and FLD (b) chromatograms from standard (1 μg mL^{-1}) aqueous solutions. ((1) 2,6-DNP; (2) 2,4-DNP; (3) 2,5-DNP; (4) 2,6-DCP; (5) 2,5-DCP; (6) 2,4-DCP; (7) TBP; (8) SBP; (9) PCP).

Table 2
Enrichment factors (E_f) with different solvents supported into the pores of the hollow fiber (*for details see text*).

	Dihexyl ether	1-octanol	Peppermint oil
2,6-DNP	54	32	29
2,4-DNP	55	23	19
2,5-DNP	115	115	115
2,6-DCP	308	210	51
2,5-DCP	281	189	40
2,4-DCP	253	143	34
TBP	30	15	–
SBP	33	22	–
PCP	308	210	51

– Not extracted.

Table 3
CAS registry number and pKa for the substituting phenols studied.

	CAS	pKa
2,4-DNP	[51–28–5]	4.04 ± 0.22
2,5-DNP	[329–71–5]	5.35 ± 0.19
2,6-DNP	[573–56–8]	3.49 ± 0.10
2,4-DCP	[120–83–2]	8.05 ± 0.18
2,5-DCP	[583–78–8]	7.53 ± 0.10
2,6-DCP	[87–65–0]	7.02 ± 0.10
PCP	[87–86–5]	4.68 ± 0.33
TBP	[98–54–4]	10.13 ± 0.13
SBP	[89–72–5]	10.36 ± 0.35

dihexyl ether and peppermint oil in order to check solvents that exhibit adequate analytes solubility and to maintain a stable layer of organic solvent during pre-extraction handling and during the extraction process.

Aqueous pH 2 solutions containing all compounds in concentration 100 ng mL^{-1} each were submitted to HF-LPME for 20 min using the different organic solvents as supported liquid membrane (SLM) and aqueous pH 12 solutions as acceptor phase. Table 2 shows the corresponding enrichment factors (E_f) obtained for each substance, defined as the relation between the concentrations in the acceptor and donor phases, and, as can be seen, the best results were obtained using dihexyl ether as SLM and it was used for the further experiments.

3.2. Effect of the donor phase pH

It is widely known that donor and acceptor pH optimizations are usually the more critical steps to establish the optimal experimental conditions for a three phase HF-LPME. Substances to be extracted must be in non-ionized form in the donor phase to cross the organic liquid membrane. Although the substances studied have phenolic character, the different nature of the ring substituents conditions their pK_a values (Table 3); thus, dichlorophenols ones have values in the 7.0–8.0 range, pentachlorophenol and dinitrophenols show lower pK_a values (between 4.0 and 5.3), and the alkylphenols show the higher values (about 10).

In order to check the LPME behavior with the donor phase pH, the pH of aqueous samples containing 25 ng mL^{-1} of the studied compounds were adjusted with HCl to values in the 0.5–6 range and submitted to LPME for 10 min using a pH 12 aqueous acceptor phase. Fig. 2 shows the evolution of the enrichment factor (E_f) versus the pH of the donor phase and, as can be seen, in general, the behavior through the interval of pH tested is a little significant. DCPs, TBP and SBP show light increases in the 0.5–1 range, more marked for 2,4 and 2,5-DCP. E_f decreases, in general, for pH values upper 3 (more marked from pH 2 for 2,4 and 2,5-DCP). It is clear that optimum donor phase pH for all analyzed substituting phenols is in the 1.0–2.0 range. Additionally, it is

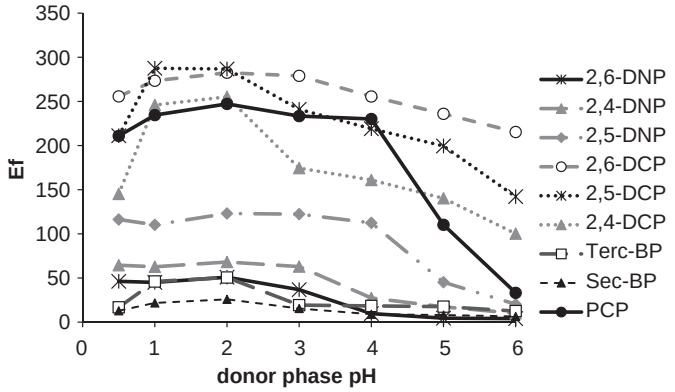

Fig. 2. Influence of the donor phase pH on the HF-LPME.

remarkable the wide E_f obtained for the different analyzed compounds, from lower than about 15 for SBP and TBP to values in the range 200–300 for all analyzed chlorophenols.

3.3. Effect of the acceptor phase pH

The effect of the acceptor phase pH was checked using pH 2 aqueous samples containing 25 ng mL^{-1} of the studied compounds. The samples were extracted for 10 min. As acceptor phase aqueous NaOH solutions with pH values ranged between 8 and 13 were tested. Results obtained were shown in Fig. 3. In general, E_f increases with the pH increase but showing different tendencies between the analyzed phenol substituting compounds. Thus, E_f values increase from pH 8 to 11 in a more or less constant way for all the compounds except for the alkylphenols. E_f values dramatically increase for PCP and all the DCP in the pH 11–12 range. From pH 12 the behavior ranges between slight increases or decreases. Alkylphenols show a radically different behavior: E_f strongly increases from pH 12 for SBP and TBP. Thus, a pH value about 13 is the best option in order to obtain the best sensitivity for all the analyzed compounds.

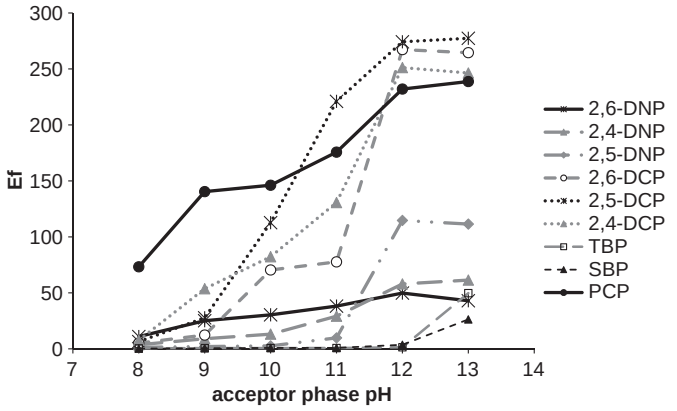

Fig. 3. Influence of the acceptor phase pH on the HF-LPME.

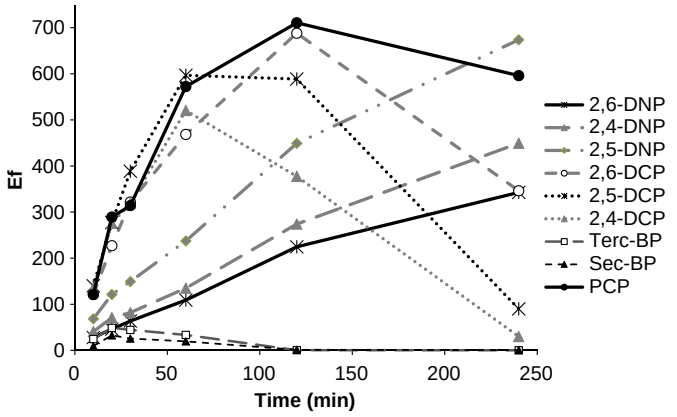

Fig. 4. Influence of the stirring time (300 rpm) on the HF-LPME.

3.4. Effect of the saline concentration in the extraction efficiency

Donor phase solutions containing additionally NaCl (2–6 M) or Na_2SO_4 (0.5 M to saturation) were submitted to the HF-LPME procedures in order to check the possible influence of the salting out on the extraction efficiency. No significant effects were observed with this experimental parameter so salting out was not considered for further experiences.

3.5. Effect of the extraction time

Donor phase solutions were magnetically stirred using the maximum stirring speed that do not produces a vortex preventing contact within donor solution and the SLM avoiding extraction efficiency decreases. Extraction time has proven to be a very critical parameter. Fig. 4 shows the E_f versus the extraction time for aqueous pH 2 solutions containing 25 ng mL^{-1} of each analyte using aqueous pH 13 as acceptor phase. As it can be seen, the behavior of the compounds is structurally dependent.

Thus all DNP compounds show an almost constant E_f increase when extraction time increases. SBP and TBP show their better (but very low) enrichment factors at 20 min. that decrease practically to null enrichment values after 120 min. PCP and 2,4-, 2,5- and 2,6-DCP show a similar behavior, a high initial increase (up to 60 min for 2,4 and 2,5-DCP, and up to 120 min for 2,6-DCP and PCP) and later sharp decreases.

In order to propose an optimal extraction procedure for all the analyzed compounds, we have selected 20 min as optimum extraction time because higher extraction times allow to poor extraction efficiencies for alkylphenols.

3.6. Linearity, sensitivity and precision

Linearity of the response function was studied from external calibration. A 10-point (in triplicate) calibration curve was constructed using a least-square linear regression analysis of standards mixtures of the analytes at different concentrations. Using the selected HF-LPME conditions standard aqueous pH 2 solutions containing different PCs concentrations were extracted through a Q3/2 polypropylene hollow fiber supporting dihexyl ether as supported liquid membrane. As acceptor phase aqueous pH 13 solutions were used. The extracts obtained were analyzed according to the proposed HPLC procedure. The corresponding peak areas were proportional to concentrations in the donor phase. A linear relationship was obtained with correlation coefficients $r \geq 0.999$ and the calibration curves obtained showed no changes over the course of two weeks.

Detection and quantitation limits were calculated as the minimum concentration of an analyte giving peaks whose signal to noise ratios are 3 and 10, respectively. Table 4 shows the detection (LOD) and quantitation limits (LOQ) obtained using the diode array detector for nitrophenols and chlorophenols and the fluorescence detector for the alkylphenols due to the low sensitivity obtained in the diode array detector for these compounds. As can be seen, the detection limits were in the 140–300 pg mL^{-1} range. According to the study of the extraction time, if necessary, nitrophenols can be analyzed with better sensitivities (detection limits about 50–70 pg mL^{-1}) using an extraction time of 240 min.

To evaluate the repeatability and the intermediate precision, spiked samples (validation standards) at three concentrations levels 2, 20 and 50 ngmL^{-1} of each compound, in triplicate, were subjected to the entire analytical procedure and measured in one single day and one day per week during one month, respectively. Intermediate precision was performed using the prediction of actual concentrations from the validation standards selected for the analytical assay in the $m \times p \times n$ design (m=analytical levels, p=days and n=replications). From the corresponding ANOVA, the intermediate precision was computed [37]. The repeatability, expressed as relative standard deviation, was in the range 1.1%–4.3%. Intermediate precision also expressed as relative standard deviation, was in the range 2.1%–6.0%.

3.7. Extraction from water samples

Using the proposed extraction procedure, water samples from different provenances, selected taking into account the maximum

Table 4
Detection and quantitation limits, and linear ranges obtained using the proposed HF-LPME procedure at different extraction times.

	LOD	LOQ	Linear range[a]
2,6-DNP[b]	0.16	0.52	0.52–60
2,4-DNP[b]	0.18	0.60	0.60–60
2,5-DNP[b]	0.20	0.68	0,68–60
2,6-DCP[b]	0.14	0.45	0.45–60
2,5-DCP[b]	0.19	0.64	0.64–60
2,4-DCP[b]	0.25	0.82	0.82–60
TBP[c]	0.24	0.81	0.81–75
SBP[c]	0.25	0.83	0.83–75
PCP[c]	0.29	0.95	0.95–60

LOD: detection limit (ng mL^{-1}).
LOQ: quantitation limit (ng mL^{-1}).
[a] (ng mL^{-1}).
[b] Diode array detector.
[c] Fluorescence detector.

Table 5
Recoveries (average of three determinations ± standard deviation) from spiked water samples submitted to the HF-LPME procedure.

	Spiked level [a]	Water sample						
		WWR	WW1	WWT	River1	River2	MIN	TAP
2,6-DNP	2	65.6 ± 2.2	75.0 ± 2.3	72.8 ± 1.9	97.0 ± 1.2	99.8 ± 1.4	96.6 ± 1.7	96.6 ± 0.9
	20	67.8 ± 1.9	77.2 ± 2.4	71.9 ± 2.0	96.5 ± 0.9	101.4 ± 0.8	98.7 ± 1.0	99.8 ± 1.1
	50	67.3 ± 1.9	76.9 ± 2.0	76.3 ± 2.3	98.8 ± 1.2	102.8 ± 0.8	98.5 ± 0.9	99.0 ± 0.8
2,4-DNP	2	69.9 ± 2.7	72.7 ± 2.3	74.1 ± 1.9	94.6 ± 1.2	93.5 ± 2.1	89.1 ± 2.3	87.8 ± 2.4
	20	70.0 ± 2.6	73.6 ± 1.8	74,7 ± 0.9	99.0 ± 1.2	98.2 ± 1.6	88,3 ± 1.9	88.4 ± 2.2
	50	71.1 ± 2.7	72.5 ± 2.1	77.0 ± 0.9	97.9 ± 1.0	98.9 ± 1.8	92.5 ± 1.9	89.0 ± 2.0
2,5-DNP	2	61.1 ± 2.6	62.0 ± 2.5	60.1 ± 2.6	80.0 ± 0.8	80.0 ± 1.8	78.7 ± 1.9	84.4 ± 0.9
	20	64.2 ± 2.3	66.4 ± 2.3	63,5 ± 2.6	81.4 ± 1.2	85.9 ± 1.4	82.0 ± 1.2	88.5 ± 1.2
	50	66.2 ± 1.9	70.0 ± 2.1	68.0 ± 2.3	86.1 ± 0.9	87.8 ± 1.3	84.9 ± 1.2	88.1 ± 1.4
2,6-DCP	2	***	***	***	***	***	3.7 ± 0.4	7.0 ± 0.8
	20	***	***	2.9 ± 0.8	4.7 ± 0.3	6,34 ± 0.3	3.6 ± 0.7	6.2 ± 0.8
	50	***	***	3.2 ± 0.3	5.0 ± 0.4	6.78 ± 0.8	4.0 ± 0.5	7.0 ± 0.4
2,5-DCP	2	–	–	***	–	–	***	***
	20	***	***	***	–	***	***	***
	50	***	***	***	***	***	***	***
2,4-DCP	2	–	–	–	–	–	–	–
	20	–	–	***	***	***	***	***
	50	***	***	***	***	***	***	***
TBP	2	–	–	–	–	–	–	–
	20	–	–	–	–	–	–	–
	50	–	–	–	–	–	–	–
SBP	2	–	–	–	–	–	–	–
	20	–	–	–	–	–	–	–
	50	–	–	–	–	–	–	–
PCP	2	–	–	–	***	***	***	***
	20	***	***	***	***	***	***	***
	50	***	***	***	***	***	***	***

–Not detected;
[a] (ng mL^{-1}).
*** Detected.

variability with respect to provenance and matrix composition, were submitted to the proposed HF-LPME and none of the compounds studied were detected.

The water samples were spiked at three concentration levels to obtain 2, 20 and 50 ng mL^{-1} solutions containing all the substituting phenols studied and the resulting samples were submitted to proposed HF-LPME procedure. Surprisingly poor recoveries were obtained for most of the analyzed compounds, even from drinking mineral water. Table 5 shows the recoveries obtained from external calibrations that took into account the corresponding enrichment factors. Only dinitrophenols compounds showed high recoveries percentages (80%–102%) somewhat lower (about 70%) in the case of more complex matrices as wastewater samples. Chlorophenols, in general, were only detected and recoveries in the 2%–7% range were obtained for 2,6-DCP. Alkylphenols were not detected to any spiked concentration level.

It was evident that the analyzed water samples contained some interference that decreased the extraction efficiency for most of the studied compounds. The fact that, even recoveries obtained for the "clean" mineral drinking water were very low, suggested that the interfering substances should be common specie in natural waters. The influence of several common ions (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Fe^{3+}, Al^{3+}, Cl^{-}, F^{-}, SO_4^{2-}, NO_3^{-}, CO_3^{2-} and PO_4^{3-}) at 100 μg mL^{-1} level was checked; additionally, the effect of 500 μg mL^{-1} sodium dodecyl sulfate (SDS) was also tested according to our previous studies on wastewater samples [38]. Only CO_3^{2-} provoked interference on the HF-LPME proposed procedures at the levels tested, so a more detailed interference study was carried out for this ion.

Table 6
Recoveries from aqueous 20 ng mL^{-1} solutions containing different amounts of CO_3^{2-} submitted to the HF-LPME procedure.

	CO_3^{2-} concentration (μg mL^{-1})				
	1	5	10	50	100
2,6-DNP	100.0 ± 1.8	97.9 ± 2.1	100.2 ± 1.2	97.7 ± 2.0	100.6 ± 2.1
2,4-DNP	94.9 ± 0.9	93.5 ± 0.8	98.0 ± 1.4	97.9 ± 1.8	96.8 ± 1.8
2,5-DNP	96.8 ± 1.2	95.8 ± 1.8	97.9 ± 0.9	97.3 ± 1.1	93.2 ± 1.9
2,6-DCP	90.1 ± 2.0	75.1 ± 1.9	37.1 ± 1.7	7.3 ± 0.8	5.1 ± 0.7
2,5-DCP	90.0 ± 1.8	58.3 ± 1.0	35.1 ± 0.6	***	–
2,4-DCP	91.1 ± 1.8	57.0 ± 0.8	36.5 ± 0.6	***	–
TBP	90.0 ± 1.3	29.7 ± 0.8	14.5 ± 0.6	–	–
SBP	86.5 ± 1.1	25.5 ± 1.1	14.7 ± 0.9	–	–
PCP	88.9 ± 1.2	73.4 ± 1.2	39.7 ± 1.0	***	***

–Not detected.
*** Detected.

Aqueous solutions containing 20 ng mL^{-1} of each analyte and amounts between 1 and 100 μg mL^{-1} of CO_3^{2-} were submitted to the above described HF-LPME procedure and the obtained recoveries are depicted in the Table 6. It can be seen that chloro- and alkylphenols are extremely sensitive to the CO_3^{2-} presence, even at low concentrations. This fact confirmed the results obtained for some of the spiked water samples, thus mineral drinking water has a CO_3^{2-} label content of 155 μg mL^{-1} and tap water has a monthly average CO_3^{2} content about 95 μg mL^{-1}, so the low recoveries obtained were according to these contents.

Table 7
Recoveries (average of three determinations ± standard deviation) from spiked water samples submitted to the elimination of dissolved carbonate prior the HF-LPME procedure.

	Spiked level[a]	Water sample						
		WWR	WW1	WWT	River1	River2	MIN	TAP
2,6-DNP	2	89.9 ± 1.7	90.0 ± 1.3	89.1 ± 2.4	97.0 ± 1.3	98.1 ± 1.9	99.0 ± 2.3	94.6 ± 0.9
	20	98.6 ± 0.9	91.1 ± 2.1	104.2 ± 2.6	96.8 ± 0.9	103.1 ± 2.1	101.1 ± 1.8	97.9 ± 1.1
	50	95.7 ± 1.7	93.2 ± 1.8	102.1 ± 2.6	98.1 ± 1.2	100.1 ± 1.1	103.4 ± 2.2	99.8 ± 0.7
2,4-DNP	2	76.9 ± 3.4	84.6 ± 3.0	98.7 ± 1.9	90.9 ± 1.2	90.0 ± 2.1	94.8 ± 1.7	89.0 ± 1.6
	20	80.4 ± 3.1	84.1 ± 2.3	102.5 ± 2.1	90.0 ± 1.9	92.9 ± 2.1	98.7 ± 1.1	96.9 ± 1.6
	50	82.0 ± 2.8	86.0 ± 2.1	99.6 ± 1.1	91.7 ± 1.2	94.2 ± 1.8	97.0 ± 0.9	101.3 ± 2.0
2,5-DNP	2	71.2 ± 1.9	76.1 ± 1.8	87.0 ± 2.1	87.7 ± 3.1	83.4 ± 2.0	96.7 ± 3.0	98.1 ± 2.0
	20	75.2 ± 1.6	79.9 ± 1.8	85.3 ± 1.9	93.4 ± 2.5	86.2 ± 1.8	101.3 ± 1.9	97.9 ± 1.8
	50	77.1 ± 1.6	81.1 ± 1.6	90.0 ± 2.0	91.1 ± 2.5	89.0 ± 2.1	103.6 ± 2.2	100.1 ± 1.2
2,6-DCP	2	79.9 ± 3.0	88.2 ± 1.8	88.8 ± 1.1	88.0 ± 3.5	94.6 ± 2.8	89.1 ± 2.6	93 ± 0.9
	20	84.6 ± 3.0	79.0 ± 1.6	98.7 ± 1.9	90.01 ± 2.3	93.0 ± 2.3	99.8 ± 2.3	95.4 ± 1.1
	50	83.2 ± 2.8	90.1 ± 1.2	97.9 ± 2.1	93.1 ± 2.4	95.0 ± 2.1	91.0 ± 1.8	92.1 ± 0.8
2,5-DCP	2	80.1 ± 3.1	80.0 ± 2.7	83.9 ± 3.0	96.8 ± 1.9	93.4 ± 1.8	88.8 ± 1.7	90.0 ± 2.8
	20	84.0 ± 3.3	83.2 ± 2.6	88.0 ± 3.1	100.8 ± 2.0	97.0 ± 1.2	88.9 ± 1.0	92.2 ± 2.1
	50	88.0 ± 2.8	80.9 ± 2.7	93.5 ± 2.5	101.3 ± 1.2	95.7 ± 1.2	93.2 ± 1.6	90.0 ± 1.7
2,4-DCP	2	76.9 ± 2.6	79.0 ± 2.0	81.7 ± 2.7	90.1 ± 1.3	93.7 ± 1.1	97.7 ± 1.1	99.1 ± 1.9
	20	79.1 ± 2.1	76.5 ± 2.2	88.7 ± 2.3	91.1 ± 1,7	91.7 ± 0.8	91.6 ± 1.8	100.5 ± 2.1
	50	79.0 ± 2.3	80.1 ± 2.1	92.6 ± 1.9	90.1 ± 0.9	101.5 ± 1.4	96.2 ± 1.1	100.9 ± 2.1
TBP	2	69.6 ± 2.1	70.0 ± 2.5	89.9 ± 1.3	80.0 ± 3.0	87.6 ± 2.8	91.0 ± 1.8	90.0 ± 1.3
	20	73.3 ± 2.2	76.9 ± 2.1	102.3 ± 1.9	87.6 ± 2.7	93.0 ± 2.1	91.2 ± 1.2	102.6 ± 1.0
	50	77.0 ± 2.2	77.1 ± 2.0	94.4 ± 1.8	88.9 ± 2.1	91.9 ± 1.8	91.0 ± 1.2	97.0 ± 0.9
SBP	2	78.8 ± 3.2	77.1 ± 2.8	81.0 ± 3.1	80.9 ± 2.6	91.0 ± 2.1	101.3 ± 2.8	101.6 ± 2.1
	20	80.3 ± 3.0	78.6 ± 2.2	104.4 ± 2.4	79.2 ± 2.1	93.5 ± 1.9	97.9 ± 2.0	99.8 ± 1.8
	50	82.4 ± 2.8	85.4 ± 2.2	100.1 ± 2.0	83.5 ± 2.4	95.8 ± 2.2	99.1 ± 2.4	98.1 ± 1.2
PCP	2	67.1 ± 2.7	79.9 ± 2.0	86.2 ± 1.8	76.9 ± 3.3	83.8 ± 2.8	81.9 ± 1.7	90.0 ± 2.1
	20	68.5 ± 2.3	79.5 ± 1.9	83.3 ± 1.8	79.9 ± 3.2	86.1 ± 2.5	80.8 ± 1.1	88.0 ± 1.9
	50	67.9 ± 2.4	81.3 ± 1.6	86.8 ± 1.2	79.2 ± 2.4	88.9 ± 1.8	83.1 ± 1.6	91.1 ± 1.2

[a] (ng mL^{-1}).

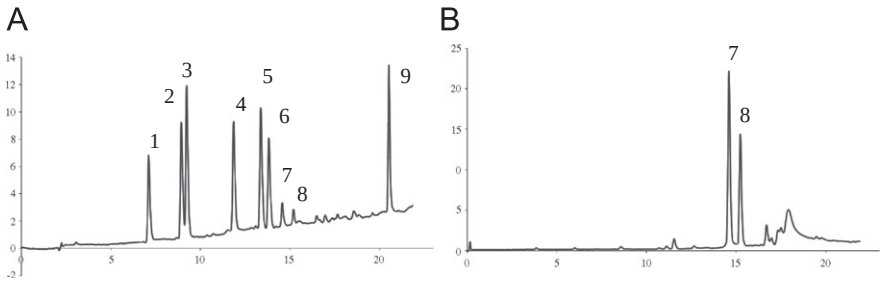

Fig. 5. DAD (a) and FLD (b) chromatograms from spiked (20 ng mL^{-1}) raw urban wastewater (WWR) sample using the HF-LPME procedure. ((1) 2,6-DNP; (2) 2,4-DNP; (3) 2,5-DNP; (4) 2,6-DCP; (5) 2,5-DCP; (6) 2,4-DCP; (7) TBP; (8) SBP; (9) PCP).

In order to eliminate the CO_3^{2-} interference some tests were carried out and it was found that 10 min of nitrogen bubbling into the sample at pH 2 prior its HF-LPME was enough to eliminate the interference. So, this pretreatment procedure was applied to the same spiked water samples above described. The results obtained are depicted in Table 7, showing that, after their previous CO_3^{2-} elimination, the recoveries were in most of the cases upper 90%, showing values in the 60%–100% range only for the "more complex" wastewater samples. Fig. 5 shows representative chromatograms obtained for the raw urban wastewater sample (WWR) and it is remarkable the excellent baselines and well defined peaks obtained even analyzing this complex matrix.

4. Conclusions

The present work has demonstrated that LPME allows the extraction of some substituting phenol compounds (alkyl-, nitro- and chlorophenols) prior to their HPLC analysis using simple, inexpensive and disposable extraction devices based on a porous polypropylene hollow fiber. The experimental conditions for a three phase HF-LPME were studied. The best selection for the supported liquid membrane was dihexyl ether. The optimal pH for the donor phase was pH 2 and an optimal pH 13 for acceptor phase was found. Optimum extraction of 20 min was selected; however, extraction time conditions the extraction efficiency and

could be selected according to the expected levels in the studied compounds. Enrichment (preconcentration) factors were in the 30–700 range that allows detection limits between 140 and 300 pg mL^{-1}. We have observed that carbonate is a serious interference in the extraction for most of the phenolic compounds analyzed, but it can be easily removed by nitrogen bubbling. Additionally, excellent baselines were obtained even in the HPLC analysis of urban wastewater samples.

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