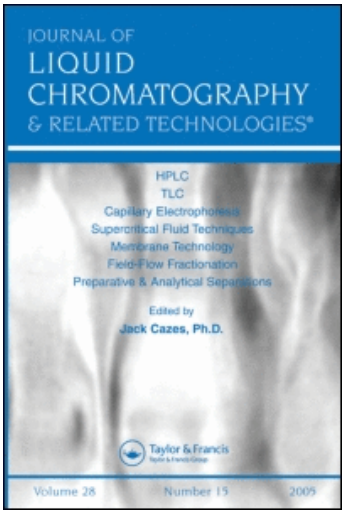

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COMPARATIVE STUDY OF C_{18}- AND STYRENE-DIVINYLBENZENE-BASED SORBENTS FOR THE ENRICHMENT OF PHENOLS FROM WATER

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ABSTRACT

The potential of solid-phase extraction with C_{18}- and styrene divinylbenzene-based sorbents for the preconcentration of phenols from water samples has been evaluated for a variety of phenols of different polarities: phenol, *o*-, *m*- and *p*-cresol, 2-chlorophenol, and 4-chloro-3-methylphenol. The extraction efficiencies have been calculated for different volumes of samples containing the analytes at different concentration levels. The UV limits of detection were of 1-5 ng/mL, for the method using Bond Elut C_{18} cartridges and sample volumes of 25 mL, and 0.05-0.1 ng/mL (except for 4-chloro-3-methylphenol) for the method using the polymeric sorbent Bond Elut PPL and 1000 mL of the samples. Possible applications of each method are discussed in view of the enrichment factors that can be reached.

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INTRODUCTION

Phenolic compounds are chemical substances that are present in aquatic environment as a result of contamination from a variety of sources. Due to their toxicity, their tendency to bioaccumulation, and their solubility in water, phenols are considered priority pollutants in the aquatic medium by the US Environmental Protection Agency (EPA) and by many European countries. Moreover, many phenols, particularly chlorophenols, are especially toxic and potentially carcinogenic. Consequently, there is a great interest in analytical methods allowing the detection and quantification of very low concentrations of these compounds.

Although several techniques are currently employed, chromatographic methods are usually preferred for the analysis of phenols. Gas chromatography (GC) has been traditionally recommended due to the high sensitivity and resolving power achieved. However, GC is unsuitable for the separation of most polar phenols in aqueous samples, thus making necessary a previous derivatization. High performance liquid chromatography (HPLC) is a good alternative to GC and it overcomes the above-mentioned limitation. In any case, the need to determine phenols at low ppb levels entails preconcentration (and/or chemical derivatization) of the analytes prior to the chromatographic separation.

For a number of well-known reasons, solid-phase extraction on cartridges and membranes is gradually superseding liquid-liquid extraction as the technique of choice for the enrichment of organic compounds at trace levels. In this sense, a variety of sorbents have been tested for the preconcentration of phenols from water. However, since contradictory results have been reported, it is rather difficult to unambiguously conclude which is the most useful type of sorbent. For example, C_{18}-based sorbents are used for the enrichment of phenols in many chromatographic procedures, but the success of the enrichment procedure depends on the polarity of the compound to be considered, thus limiting the degree of concentration possible, particularly for the most polar phenols.[1,2]

Some authors have found that styrene-divinylbenzene copolymers are a good alternative for the preconcentration of polar phenols,[3-5] but these results differ from those obtained by other workers.[6] Graphitized carbon black are sorbents of more recent use for preconcentration of phenols, and nearly quantitative recoveries have been reported when processing sample volumes as large as 1 L.[4,7] However, these sorbents have not yet gained widespread acceptance, probably due to the problems associated with the high affinity of these sorbents for some phenols.

In spite of the sorbent type and properties, most published procedures proposed for the preconcentration of phenols involve re-extraction, consecutive extractions in different sorbents, solvent evaporation (with the risk of loosing volatile phenols), or even derivatization of the analytes to convert them into more

retainable compounds.[8] As a result, the overall analytical process can be very tedious and prone to errors.

In this work, we have evaluated the possibility performing enrichment of phenols with either C_{18}- or styrene-divinyl benzene-based sorbents, without any reextraction or evaporation step. Different cartridges have been evaluated for phenols of different polarities (phenol, *o*-, *m*- and *p*-cresol, 2-chlorophenol, and 4-chloro-3-methylphenol). Possible applications are discussed in view of the enrichment factors that can be reached.

EXPERIMENTAL

Apparatus

The chromatographic system used consisted of a quaternary pump (Hewlett-Packard, 1050 Series, Palo Alto, CA, USA), and a automatic sample injector (Hewlett-Packard, 1050 Series). For detection, a UV detector (Hewlett-Packard, 1100 series) or a fluorescence detector (Hewlett-Packard, 1046 series) was used. The detectors were linked to a data system (Hewlett-Packard HPLC Chem Station) for data acquisition and storage. The fluorescence detector operated at 230 nm for excitation and 305 nm for emission, whereas the UV signal was monitored at 220 nm.

Reagents

All the reagents were of analytical grade. Acetonitrile (J. T. Baker, Deventer, Netherlands) and methanol (Scharlau, Barcelona, Spain) were of HPLC grade. Phenol, *o*-cresol, *m*-cresol, and *p*-cresol were obtained from Merck (Darmstadt, Germany), whereas 2-chlorophenol and 3-methyl-4-chlorophenol were purchased from Aldrich (Steinheim, Germany). Sodium hydroxide and sodium chloride (Panreac, Barcelona, Spain), phosphoric acid (Probus, Badalona, Spain), and sodium dihydrogen phosphate monohydrate (Merck) were also used. Water was distilled, deionized, and filtered in 0.45 μm nylon membranes (Teknokroma, Barcelona, Spain).

Preparation of Solutions

Stock standard solutions of phenols (1000 μg/mL) were prepared in water. Working solutions of the phenols were prepared by dilution of the stock solutions with a NaCl solution (at a concentration of 35 g/L) acidified to pH 3 with H_3PO_4.

The 0.1 M phosphate buffer was prepared daily by dissolving sodium dihydrogen phosphate monohydrate in water. Next, the pH was adjusted to 7 with 10% NaOH (w/v). All solutions were stored in the dark at 2°C.

Columns and Mobile Phases

A LiChrospher 100 RP_{18}, 5 μm, 125 mm x 4 mm I. D., (Merck) column was used for separation of phenols. For chromatography of samples processed with C_{18} cartridges, a 0.1 M phosphate buffer (pH 7)/acetonitrile mixture was used as mobile-phase, at a flow rate of 0.75 mL/min. The acetonitrile content was increased from 20% at zero time to 30% at 10 min, and to 50% at 15 min, and after 15 min the acetonitrile content was kept constant. For samples processed with the styrene divinylbenzene cartridges, the mobile-phase was an acetonitrile-water mixture (40:60, v/v) at a flow-rate of 1.0 mL/min. The volume of sample injected was 20 μL.

All solvents were filtered with nylon membranes, 0.45 μm, (Teknokroma) and degassed with helium before use.

Solid-Phase Extraction

Various C_{18} solid-phase extraction cartridges were evaluated for the retention of phenols: Bond Elut C_{18}, 100 mg/mL (Varian, Harbor City, CA, USA), ExtraSep C_{18} 100 mg/mL, (Teknokroma), and 3M Empore C_{18} SPE disks, 10 mm × 6 mL (Varian). The cartridges were conditioned previously by drawing with 2.0 mL of methanol, followed by 5.0 mL of water (acidified to pH 3 with H_3PO_4). According with previous studies, samples were acidified to pH 3 with H_3PO_4 and then NaCl was added to the samples (at a concentration of 35 g/L).

Variable volumes of the samples were then drawn through the cartridges under reduced pressure, by using the Vac Master-10 sample processing Station (International Solvent Technology, Hengoed, England) at a flow rate of about 5 mL/min. Next, the cartridges were dried, first by flushing with air (by means of a 10 mL syringe), and then under vacuum for 5 min (at about 0.4 bar). After drying, phenols were eluted from the cartridges with 1.0 mL of 50:50 water (acidified to pH 3)-acetonitrile (unless otherwise stated), and collected into 2 mL glass vials. Finally, 20 μL aliquots of the collected extracts were injected into the chromatographic system.

Cartridges containing styrene-divinylbenzene sorbent (Bond Elut PPL, 6 mL/500 mg, Varian) were also evaluated. The cartridges were conditioned as described for the C_{18} cartridges. After sample loading, the cartridges were washed with 1 mL of a mixture of acetonitrile-water (25:75, v/v), and the cartridges were dryed as decribed above. Finally, the analytes were desorbed from

the cartridges with 3 mL of acetonitrile-water (50:50, v/v) and injected into the chromatographic column.

The extraction efficiency for the tested phenols was evaluated by comparing the peak areas obtained for a sample processed in the solid-phase extraction cartridges, with those obtained for a standard solution of the compounds of interest directly injected into the chromatographic system (for an equivalent amount of the analytes injected).

Limits of Detection

The limits of detection were estimated by analysis of standard solutions of decreasing concentration of each phenol. 25 mL or 1000 mL of each prepared solution were drawn through C_{18} or styrene-divinylbenzene cartridges, respectively, and processed as indicated in the solid-phase extraction procedure. They were established as the concentration required to generate a signal-to-noise of 3. The obtained values were confirmed by analysis of water samples spiked with the appropriate amount of phenols to produce a concentration equivalent to the estimated limits of detection and processed as standard solutions.

RESULTS AND DISCUSSION

In preliminary experiments, we tested different elution conditions (mobile-phase compositions, gradients and flow-rates) for the resolution of the phenols used in this study. In all conditions tested, we observed a severe overlapping between *m*- and *p*-cresol. Although HPLC is well suited for the separation of phenols in aqueous samples, coelution of cresols is a well documented problem in methods operating under reversed-phase conditions.[1,6] However, the successful resolution of *m*- and *p*-cresol by mathematical methods has been reported.[1,9] Therefore, and since this study was focused on the enrichment of phenols, further studies on the resolution of cresols under a different chromatographic mode (normal-phase, for example) or by mathematical methods were not undertaken.

The working conditions were, thus, optimized in order to achieve a suitable resolution for the other compounds in the minimum time of analysis. Shown in Figure 1, is a chromatogram obtained for a mixture of the phenols tested under the conditions finally selected.

Enrichment into C_{18} Sorbents

We tested different cartridges for retention of the phenols: Extra Sep C_{18} and Bond Elut C_{18} column cartridges, and 3M Empore C_{18} disk cartridges. The

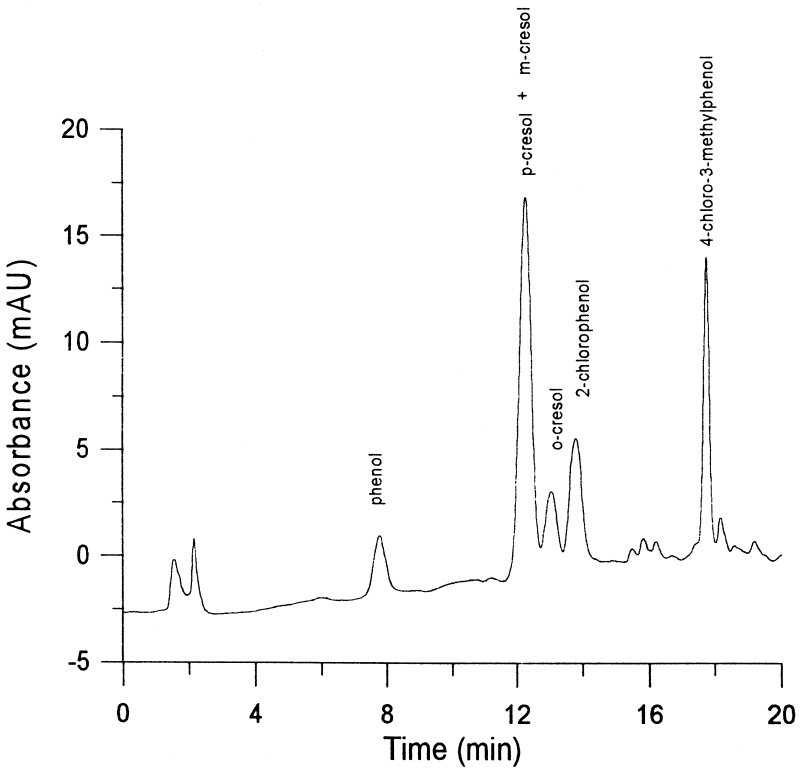

Figure 1. Chromatogram obtained for a mixture of phenols. Conditions: UV detection at 220 nm; concentration of each compound, 4.8 μg/mL. For other experimental details, see text.

efficiency of each cartridge was evaluated by obtaining the percentages of phenols recovered from different volumes of water (5, 25, 50, 75, and 100 mL). In all instances, the final concentration of the phenols in the concentrated extracts (for a theoretical recovery of 100%) was 4.8 μg/mL.

Preconcentration on the C_{18} disk cartridges led to very poor recoveries of all the analytes (with values lower than 20 %), even for a sample volume as low as 25 mL. This means that the enrichment factors that can be reached under the described conditions were of 1.3-4.5. Recoveries lower than 40% were observed for a sample volume of 5 mL. Therefore, the C_{18} disk cartridges were considered to be unsuitable for the preconcentration of phenols. Although recoveries of about 60% (for a sample volume of 25 mL) were observed for 4-chloro-3-methylphenol (the most apolar and thus the most retained compound), the per-

centages of the other phenols recovered when using the Extra Sep C_{18} cartridges were also low.

Preconcentration over the Bond Elut C_{18} cartridges lead to much better recoveries. Results suggest, that nearly complete retention is achieved with this type of cartridges for the analytes, except for phenol (which was significantly less retained). In Figure 2, the effect of the sample volume on the percentages of analytes retained in the Bond Elut C_{18} cartridges can be observed. Quantitative recoveries are obtained for most phenols within the sample volume interval 5-50 mL, except for phenol. For this compound incomplete retention is observed, even for the lowest sample volume assayed. The low retention of phenol is a problem

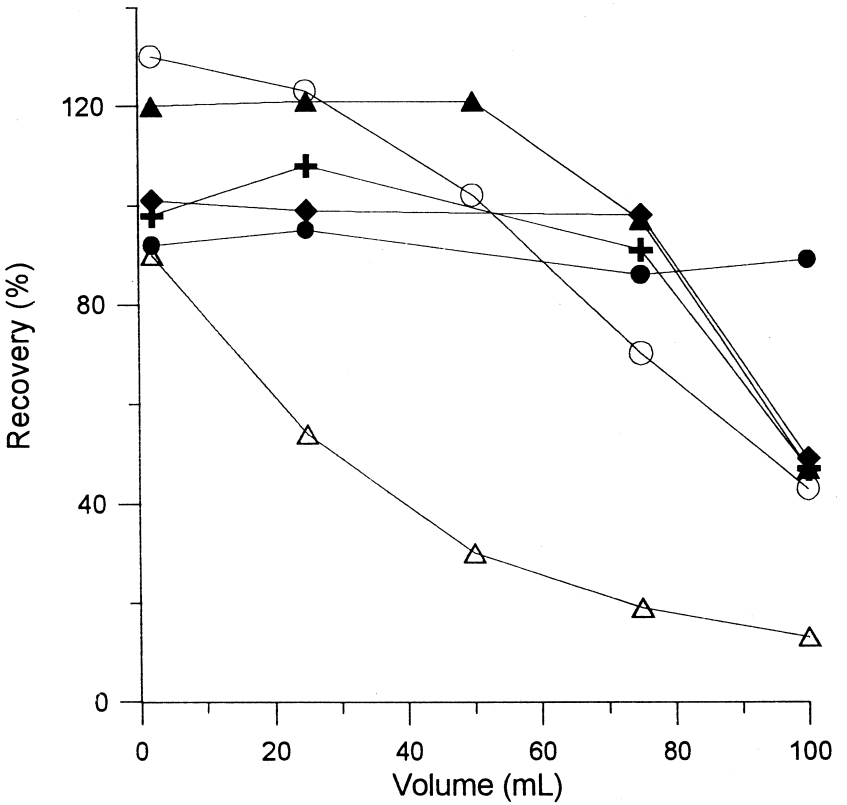

Figure 2. Effect of the sample volume on the recoveries of phenols: (○) phenol, (+) *m*-cresol, (▲) *p*-cresol, (△) *o*-cresol, (◆) 2-chloromethylphenol and (●) 4-chloro-3-methylphenol. For experimental details, see text.

encountered in a majority of the methods proposed for the enrichment of phenols, even when using other type of packings.

In principle, the analytical responses can be increased for most phenols by increasing the sample volume. However, maximum analyte responses for phenol were observed when using sample volumes of 25-50 mL. The reason for this is that the employment of larger volumes of sample does not compensate the losses of phenol due to breakthrough. Therefore, a volume of sample of 25 mL was chosen as the best compromise between sensitivity and the time of extraction, when working with this type of cartridges. Under these conditions, no breakthrough was observed for any of the compounds assayed (except for phenol), and the enrichment factors for phenols were in the 14-25 interval. The recoveries observed for most analytes ranged from 95% to 121%. The relatively poor recovery obtained for phenol (54 %) can be considered acceptable, taking into account the values reported by other solid-phase extraction procedures.[10]

On the other hand, the application of the ANOVA method demonstrated that the recoveries obtained at the different concentrations assayed (10.0 - 20.0 ng/mL) can be considered statistically similar. Moreover, the extraction is quite reproducible, with coefficients of variation ranging from 4 to 8 % (n=5). The method provided adequate linearity for both UV and fluorescence detection, for samples containing phenols in the 5-20 ng/mL concentration interval. The results of this study are summarized in Table 1.

Similar sensitivities were observed for the UV and fluorescence methods, except for 4-chloro-3-methylphenol; for the later compound, better sensitivity was clearly achieved with the fluorescence detector. The UV limits of detection (LODs) calculated as the concentration required to generate a signal to noise of 3,

Table 1. Linearity of the Method Proposed for the Analysis of Phenols (n =8)

Compound	UV	Fluorescence
Phenol	$y = -1.251 + 0.357\ x$ $r^2 = 0.97$	$y = -0.032 + 8.90\ 10^{-3}\ x$ $r^2 = 0.97$
m-Cresol	$y = 0.014 + 0.112\ x$ $r^2 = 0.98$	$y = 7.54\ 10^{-3} + 7.03\ 10^{-3}\ x$ $r^2 = 0.96$
p-Cresol	$y = -0.261 + 0.124\ x$ $r^2 = 0.990$	$y = -0.022 + 8.49\ 10^{-3}\ x$ $r^2 = 0.991$
o-Cresol	$y = -0.401 + 0.104\ x$ $r^2 = 0.98$	$y = -9.095 + 3.124\ 10^{-3}\ x$ $r^2 = 0.97$
2-Chlorophenol	$y = 0.337 + 0.061\ x$ $r^2 = 0.98$	—
4-Chloro-3-methylphenol	$y = 0.524 + 0.088\ x$ $r^2 = 0.992$	$y = -0.061 + 15.1\ 10^{-3}\ x$ $r^2 = 0.992$

were 2 ng/mL for phenol and *o*-cresol, and 3 ng/mL for *m*-cresol, *p*-cresol, and 2-chlorophenol, and 5 ng/mL for 4-chloro-3-methylphenol. The LODs found with the fluorescence detector were 2 ng/mL for phenol and the cresols, and 1 ng/mL for 4-chloro-3-methylphenol; 2-chlorophenol was not detected.

In Table 2 are compared the LODs obtained under the described conditions, with those reported by other chromatographic assays recently reported. This table also shows other analytical properties of interest. As can be deduced from this table, the LODs reported by other HPLC methods with either UV or fluorescence detectors are of about one order of magnitude lower than those obtained by the present procedure.[3,7,11] The sensitivity is comparable to that reported in references 1 and 2. The described procedure is clearly worse in sensitivity than that which is obtained by Lanzettel et al.[13] This latter method involved precolumn derivatization and fluorescence detection (the authors did not consider previous sample treatment). On the other hand, the present procedure provides LODs similar, or slightly higher, than those reported by GC methods.[4,10]

Figure 3 shows the chromatogram obtained from one of the samples analyzed. Phenol was the only compound detected in the samples assayed. The estimated concentration of phenol in such a sample was (6.11 ± 0.07) ng/mL. In accordance with the retention times, we did not find any other phenol, which means that their concentrations (if present) were lower than 1-2 ng/mL.

Enrichment into Styrene-Divinylbenzene-Based Sorbents

Since C_{18} materials were clearly unsuitable for the trace analysis of phenol, the usefulness of polymeric sorbents based on styrene-divinylbenzene (Bond Elut PPL cartridges) were evaluated for the enrichment of this compound. The percentages of phenol recovered from different volumes of samples within the 6 - 1000 mL interval can be observed in Figure 4. As can be deduced from this figure, this sorbent exhibits a high affinity for phenol, and nearly quantitative retention was achieved even for a sample volume of 1000 mL. Moreover, the percentage of phenol recovered was not dependent on the sample volume, the mean recovery of phenol being 89 ±10 % (n=39) in the tested interval. As for the Bond Elut C_{18} cartridges, the recovery was found to be independent on the concentration of phenol in the samples (see Figure 5), and good linearity was observed in the tested concentration range. The reproducibility was also comparable to that provided by the Bond Elut C_{18} cartridges.

Phenols recoveries were also assessed using sea water (collected at different points from Valencia harbour) instead of distilled water. According to previous studies,[9] the samples were filtered in Nylon membranes, 0.45 μm, and then acidified to pH 3 with phosphoric acid. The recovery dependence on the sample volume and on the concentration of phenol, were found to be similar to those

Table 2. Analytical Properties of Different Methods Recently Proposed for the Analysis of Phenols

Sample Type	Sample Volume (mL)	Sample Treatment	Recovery (%)	Chromatography and Detection	LOD (ng/mL)	Amount of Analyte Detected (ng)	Reference
Drinking water	200	SPE in styrene-divinylbenzene and C_{18} phases followed by solvent evaporation and redisolution	7 - 103	LC - UV -FLD	<0.1	≈ 0.36 ≈ 0.014	11
Natural water	100	SPE in C_{18} phase	30 - 100	LC - UV	1	3	1
Waste and industrial effluents	700 - 1000	SPE in polymeric sorbents (Isolute, LiChrolut and Prorapack) followed by solvent evaporation	70 -100	LC - UV	< 0.1	—	3
Natural and drinking water	1000 - 4000	SPE in graphitized carbon black	> 90	LC - UV	< 0.2		7
Waste water	2 × 250	SPE in polymeric and C_{18} phases followed by combination of the extracts, solvent evaporation, redisolution and post-column derivatization	—	LC - UV	—	1 - 20	2
Waste water	0.1	Derivatization	—	LC - FLD	—	0.009 - 0.011	12
Drinking water	200 - 2000	Derivatization followed by SPE in graphitized carbon black and solvent evaporation or SPE in styrene divinylbenzene followed by solvent evaporation	> 80	GC - microwave induced plasma-atomic emission	< 0.5	—	4
Natural water	100	SPE in styrene divinylbenzene, activated carbon or C_{18}	21 - 99 %	GC - FID	0.8 - 32.0	—	10
Natural and waste water	25	SPE in C_{18}	54 -121	LC - UV - FLD	2 - 5 1 - 2	2.5 - 6.26 1.25 - 2.5	This work
	1000	SPE in styrene divinylbenzene	45-79	- UV	0.05 -0.5*	2.5 - 6.25	

* 4-Chloro-3-methylphenol was not detected.

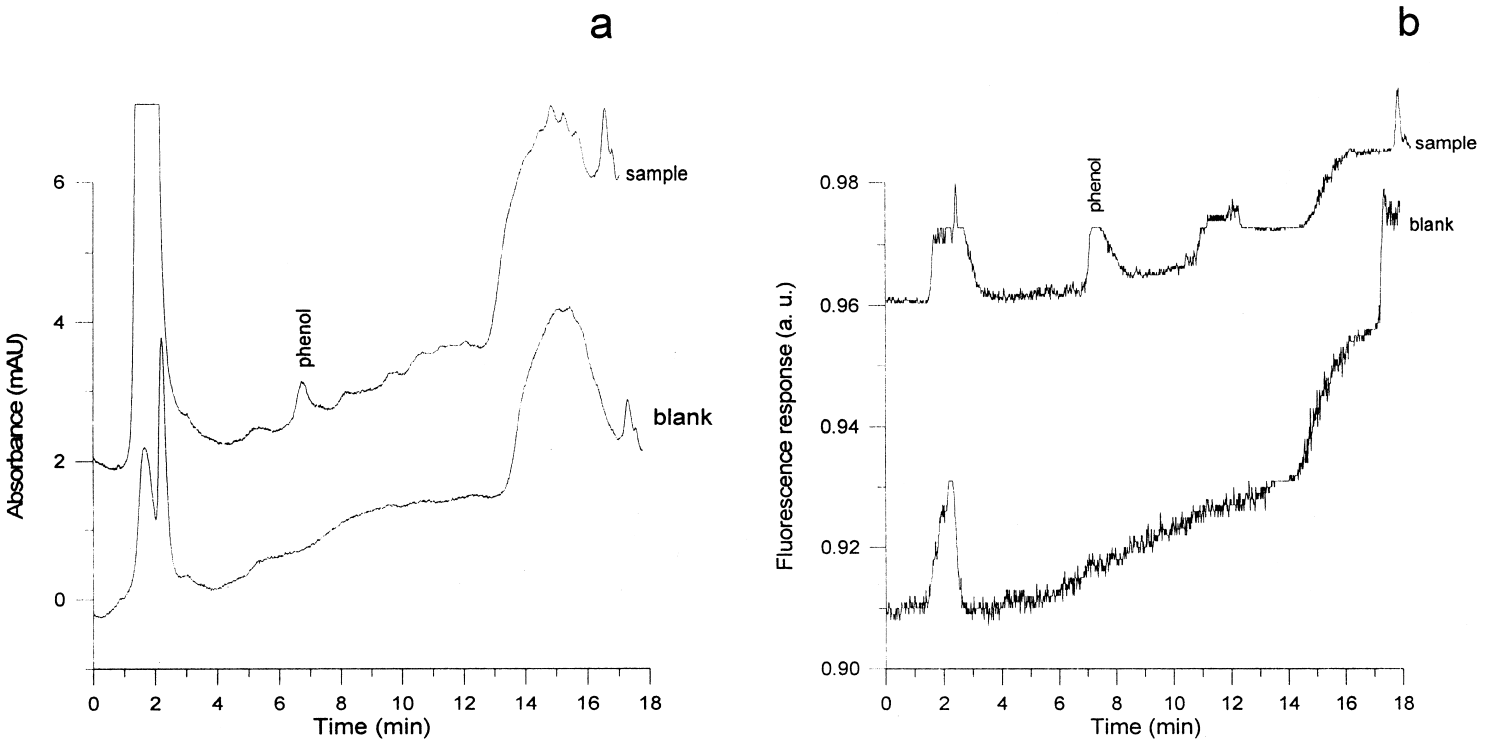

Figure 3. UV (a) and fluorescence (b) chromatograms obtained for a blank (HPLC water) and for a sample collected in the Valencia Harbour. For experimental details, see text.

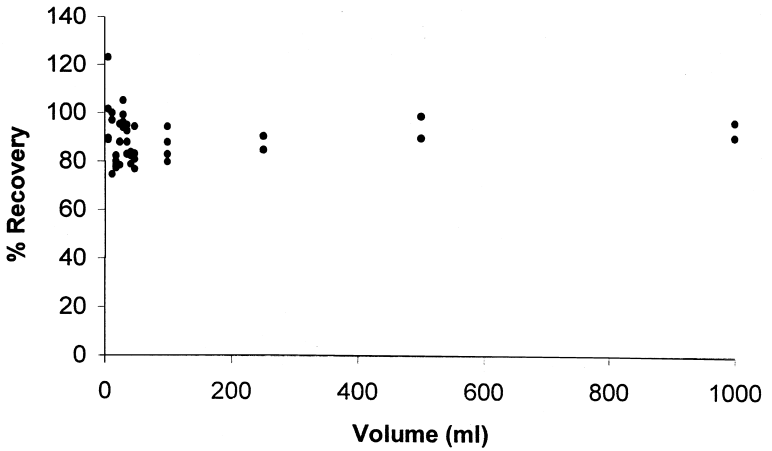

Figure 4. Found recoveries of phenol in function of the volume of solution processed.

observed for standard solutions of phenol in distilled water. However, the mean recovery of phenol in the sea water spiked between 0.3 and 0.075 1g/mL for sample volumes between 100 and 1000 mL was slightly lower, 74 ± 15% (n=6). This behaviour is in concordance with previously reported results for other sorbents.[3]

The styrene-divinylbenzene cartridges were also evaluated for the enrichment of the other phenols of interest. The recoveries obtained were satisfactory

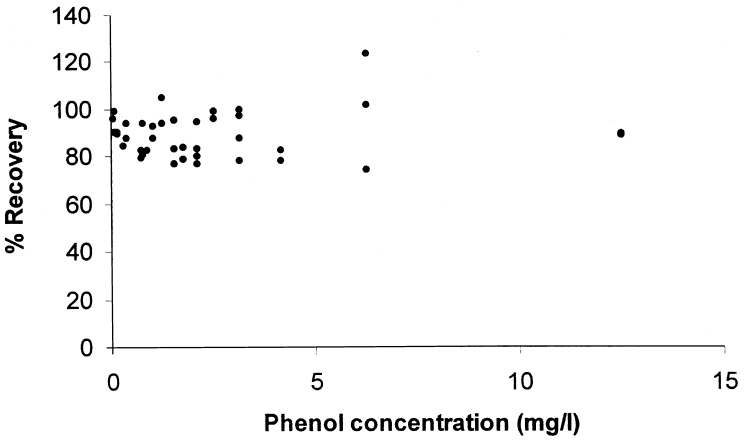

Figure 5. Found recoveries of phenol in function of the analytical concentration of phenol.

for the most polar analytes, such as the cresols and 2-chlorophenol phenol. However, 4-chloro-3-methylphenol was insufficiently retained. Since the latter compound was not detected even when using a volume of sample as low as 250 mL, the volume of water finally selected was 1000 mL. Under the proposed conditions, the recoveries found for spiked sea water samples were: 80% for phenol, 48% for *m*-cresol, 47% for *p*-cresol, 56% for *o*-cresol, and 45% for 4-chlorophenol. Therefore, the enrichment factors attainable with the described method varied from 150 to 267.

The estimated LODs (for a signal-to-noise ratio of 3) in sea water with UV detection were 0.05 ng/mL for phenol, and 0.1 ng/mL for the other phenols; 4-chloro-3-methyl phenol could not be detected. The found concentrations of the tested compounds in the sea water samples were below or near to the detection limits; the spiking of the samples with phenols confirmed the above findings. As it can be see in Table 1, the styrene-divinylbenzene sorbents provide excellent results in the enrichment of phenol. The LOD obtained is comparable or even better than those reported by other LC methods, which involve evaporation to dryness of the collected extracts and subsequent redissolution in a small volume. Although less sensitive, the method can also be applied for the other phenols of high-medium polarity. For these compounds, the LODs are also lower than those achieved with the C_{18} cartridges, and of about the same order as those reported by most LC previously described procedures with a variety of sorbents (see Table 1). Obviously, the proposed conditions is inadequate for 4-chloro-3-methylphenol and, presumably, for other apolar phenols.

CONCLUSIONS

In spite of the difficulties of retaining phenol, Bond Elut C_{18} solid phase extraction cartridges can be used for the enrichment of phenolic compounds from water when speed and simplicity, rather than sensitivity, are required. For example, with the described procedure, preconcentration of sample volumes of 25 mL was found to be adequate for the analysis of these compounds in waste water or in industrial effluents. Moreover, under the described conditions, the analysis is very simple and rapid, since no reextraction, dryness, redisolution of the analytes is effected.

Since, for the analysis of phenols in drinking water, methods allowing the analysis of phenols at sub ppb levels are required, the tested styrene-divinyl benzene sorbent is a good alternative, and sample volumes up to 1000 mL can be processed with recoveries varying from 45 to 80% for most compounds. The main limitation of the described procedure, compared with the method using C_{18} cartridges, is that it cannot be applied to the enrichment of very apolar phenols.

The successive extraction into the Bond Elut C_{18} and PPL cartidges would be an alternative for the simultaneous analysis of polar and apolar phenols in this type of samples. Another disadvantage is the relatively long time required to pass the samples though the cartridges. However, the proposed method is still rapid in comparison with most previously reported procedures, which involve derivatization, reextraction, and/or evaporation steps.

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