DETERMINATION OF ELEVEN PRIORITY EPA PHENOLICS AT ng L^{-1} LEVELS BY ON-LINE SOLID-PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY WITH UV AND ELECTROCHEMICAL DETECTION

ABSTRACT

The eleven priority, EPA phenolic pollutants were determined by liquid chromatography followed by two detectors in series; UV and electrochemical. Three different sorbents, Envi-Carb (a carbon black) and two functionalized polymeric resins, Bond Elut PPL and another synthesized in our laboratory with an *o*-carboxybenzoyl moiety, were compared for solid-phase extraction (SPE) to detect lower concentrations of the eleven phenolics in natural waters. Higher recoveries were obtained using the functionalized polymeric sorbents compared with Envi-Carb. When real samples were analysed, the synthetic sorbent gave lower interferences than Bond Elut PPL and phenol was determined at low levels with no humic and fulvic acid interference when Na_2SO_3 was added. The linear range for most compounds in tap water was between 0.05-20 μg l^{-1} and the limits of detection were <35 ng l^{-1}. Repeatibility and reproducibility between days for real samples spiked at 0.1 μg l^{-1}, expressed as relative standard deviation, were <8% and 10%, respectively.

Keywords: Column liquid chromatography; Electrochemical detection; Phenolics in water; On-line preconcentration.

INTRODUCTION

Phenolic compounds are common by-products of many industrial processes (manufacture of dyes, plastics, drugs, antioxidants and paper and the petroleum industry), the degradation of some pesticides and the biodegradation of humic substances, tannins and lignins. The European Community Directive 80/778/EEC states that the maximum admissible concentration of phenolic compounds in drinking water should be 0.5 μg l^{-1} for the total content and 0.1 μg l^{-1} for the individual content [1] and Directive 75/440/EEC states that; in surface water for drinking purposes, it should be in the 1-10 μg l^{-1} range depending on how it is treated.

Phenolic compounds are usually determined by reversed-phase liquid chromatography (RPLC) with UV or diode-array detection (DAD) [2-8] or electrochemical detection (EC) [5-11]. Amperometric detection is more sensitive than UV detection and the detection limits decrease quite considerably when EC, and not UV, detection is used [6]. This increase in sensitivity is particularly significant when the compounds are eluted isocratically but the isocratic elution of phenolic compounds means that the analysis time is long and that the last-eluted compounds give broad peaks leading to decreased sensitivity [11].

It should be mentioned that the nitrophenolic compounds give a better response with a UV detector than with an electrochemical one. So, in order to obtain good sensitivity a common set-up is to connect a UV detector in series to an electrochemical one so that the nitrophenolic compounds can be detected with the UV detector and the rest of the compounds with the electrochemical detector [11]. Gradient elution is preferable to isocratic elution because the analysis time is shorter and the last-eluted peaks are not so broad.
To achieve the low levels required by the directives an enrichment step is needed before chromatographic analysis in both detection systems [8]. Nowadays, SPE is the most common technique for sample enrichment, because of its advantages over liquid-liquid extraction (LLE) [12]. Several sorbents have been tested for determining phenolic compounds in water. Those which have been studied most

are C_{18} chemically bonded to silica [4,6,13], carbon black [14-18] and polymeric sorbents such as PLRP-S [3-5,7,11]. Carbon blacks have been used to analyse phenols in water but elution in the backflush mode is necessary due to excessive retention on these solids [15,16].

The main problem for determining phenolic compounds is their high polarity and that of some nitrophenolic compounds which means the breakthrough volume is small and they elute on a hump of the chromatogram due to the humic and fulvic acids. This makes determination difficult [2,10,22].

Recently, several highly crosslinked polymers (such as Lichrolut EN [2,3,19,20], Styrosorb [21], Isolute ENV [2] and HYSphere-1 [18,22]) have been applied in the SPE of these analytes. They have an open structure (highly porous material) which maximises the active surface compared with the previously mentioned sorbents, leading to better breakthrough volumes for the polar compounds.

Chemically-modified polymeric resins with a polar functional group such as acetyl [3,23-25], hydroxymethyl [23,25], benzoyl [26] and *o*-carboxybenzoyl [27] have been developed for use in the SPE of polar compounds. In most cases, the breakthrough volumes of polar compounds with these sorbents are larger than volumes obtained with their unmodified analogues [3]. Lightly sulphonated resins have also been used [23,28].
Nowadays on-line SPE is preferred to off-line SPE because automation is easier, reproducibility is better and the limits of detection are lower. When SPE is on-line coupled to LC, the incompatibility between the sorbent of the precolumn and the stationary phase of the analytical column can cause peak broadening because the mobile phase is not polar enough to elute the compounds trapped in the precolumn. This problem may be solved by slightly modifying the LC instrument and eluting the compounds retained in the precolumn only with the organic component of the mobile phase [4].

The aim of this study was to compare three different sorbents (a carbon black, Envi-Carb, and two functionalized polymeric resins, Bond Elut PPL and another sorbent which was synthesized in our laboratory with an *o*-carboxybenzoyl moiety [27]), for SPE of the eleven priority EPA phenolic pollutants from water. The SPE was on-line coupled to a liquid chromatograph with UV detection, to determine the nitrophenols, and electrochemical detection, to determine the rest of the analytes, connected in series.

The method was applied to detect these compounds in several surface waters. Na_2SO_3 was added to eliminate the free chlorine in tap water, which may react with phenols and produce chlorophenols [6]. Previously [27], we observed that when Na_2SO_3 was added the band which appears at the beginning of the chromatogram due to the presence of humic and fulvic acids decreased. For this reason, addition of sulphite was made to the tap and river water samples to decrease the effect of these acids and so determine most polar compounds more accurately.

EXPERIMENTAL

Equipment

Experiments were performed using two Shimadzu (Tokyo, Japan) LC-10AD pumps, with a Shimadzu SPD-10A UV spectrophotometric detector and an HP-1049A electrochemical detector (Hewlett-Packard, Palo Alto, CA, USA) connected in series. The mobile phase was degassed with a Shimadzu DGU-4A degasser. The temperature of the column was controlled by a Shimadzu CTO-10A oven and chromatographic data were collected and recorded using an HP-3365 Series II Chemstation controlled by Windows 3.11 (Microsoft). The compounds were

separated using a 25 x 0.46 cm I.D. Kromasil 100 C_{18} column, particle size 5 µm, supplied by Teknokroma (Barcelona, Spain).

To check the response of the instrument, standard solutions were injected through a Rheodyne (Cotati, CA, USA) valve with a 20 µl loop, and an automatic Must column-switching device (Spark Holland, Emmen, The Netherlands) was used with on-line SPE. The on-line trace enrichment process was carried out using steel precolumns of 10 x 3 mm I.D. laboratory-packed with the different sorbents studied. A Waters (Milford, MA, USA) M45 pump was used to deliver the sample and condition the precolumn.

Reagents and Standards

The compounds studied were the eleven priority EPA phenolic pollutants: phenol (Ph), 4-nitrophenol (4-NP), 2,4-dinitrophenol (2,4-DNP), 2-chlorophenol (2-CP), 2-nitrophenol (2-NP), 2,4-dimethylphenol (2,4-DMP), 2-methyl-4,6-dinitrophenol (2-M-4,6-DNP), 4-chloro-3-methylphenol (4-C-3-MP), 2,4-dichlorophenol (2,4-DCP), 2,4,6-trichlorophenol (2,4,6-TCP) and pentachlorophenol (PCP). They were all supplied by Aldrich Chemie (Steinheim, Germany), except PCP which was from Jansen Chemie (Geel, Belgium). Standard solutions of 2000 mg l^{-1} of each compound were prepared in methanol. Working standard solutions were prepared weekly by diluting the stock standard solutions with Milli-Q water (Millipore, Bedford, MA, USA), and more diluted working solutions were prepared daily by diluting with Milli-Q, tap or river water. All solutions were stored at 4ºC.

HPLC-quality acetonitrile (Fisher Scientific, Leicestershire, UK) and Milli-Q water with 1% acetic acid (Probus, Badalona, Spain) were used to prepare of the mobile phase. To adjust the ionic strength of the eluent, 0.05 g l^{-1} of potassium chloride (BDH, Poole, UK) was added.

Hydrochloric acid (Probus, Badalona, Spain) was added to adjust the pH of the sample to 2.5 before the SPE step in order to prevent the analytes from assuming in ionic form. 500 or 1000 μl of 10% solution of Na_2SO_3 (Panreac, Barcelona, Spain), for each 100 ml of sample, was added to tap and river water samples, respectively, to eliminate the free chlorine in tap water, which may react with phenols and produce chlorophenols, and to reduce the peak that appears at the beginning of the chromatogram because of the presence of humic and fulvic acids [27].

Tap and river water samples were filtered through a 0.45 μm nylon membrane (Whatman, Maidstone, UK) before the SPE to eliminate particulate matter.

Chromatographic Conditions

The eluents for chromatographic separation were a solution of acetic acid (1%) containing 0.05 g l^{-1} of KCl as solvent A and acetonitrile as solvent B. The flow rate was 1 ml min^{-1} and the temperature of the column oven was set at 65ºC. The gradient profile was: initially 40% B, 75% B after 25 min, 100% B at 27 min, isocratic for 2 min, and the mobile phase returned to initial conditions in 2 min for a subsequent analysis run.

UV detection was at 316 nm for 4-NP and 280 nm for the other nitrophenols (2,4-DNP, 2-NP and 2-M-4,6-DNP). The electrochemical detector worked in the amperometric mode with a glassy carbon electrode at a potential of 1.0 V, between the working and the reference electrodes, to determine the rest of the phenolic compounds studied. A solid-state Ag-AgCl reference electrode was used, so the eluent had to contain KCl (0.05 g l^{-1}). Electrochemical cleaning was used every 10

injections to correct the electrodeposition on the electrode surface, by applying a cyclic treatment with alternate potentials. The working electrode was polished in the conventional way about every 40 injections.

On-line Trace Enrichment Procedure

The on-line solid-phase extraction process was performed using three different sorbents: a carbon black Envi-Carb (Supelco, Bellefonte, PA, USA), a functionalized polymeric resin Bond Elut PPL (Varian, Harbor City, CA, USA) and a chemically modified polystyrene-divinylbenzene sorbent, with an *o*-carboxybenzoyl moiety, obtained from porous, crosslinked, polystyrene-divinylbenzene described previously [27]. These sorbents were laboratory-packed in a 10 x 3 mm I.D. stainless steel precolumn. The program for the Must automatic, column-switching device used in sample preconcentration is shown in Table 1.

Table 1
Sample preconcentration program in on-line SPE process

Step	Time (min)	Flow rate (mL min^{-1})	Event	Solution
1	0	2	Washing tubes	acetonitrile
2	5	2	Conditioning precolumn	acetonitrile
3	6	2	Washing tubes	Milli-Q water at pH 2.5
4	11	2	Activating precolumn	Milli-Q water at pH 2.5
5	12	2	Washing tubes	sample
6	17	4	Sample preconcentration	
7	*		Analyte desorption	acetonitrile of mobile phase

* depends on sample volume

Analytes were desorbed in the backflush mode, using only by the organic solvent (acetronitrile) of the mobile phase, so as to prevent peaks from broadening due to the different nature of the analytical column and the precolumn sorbent [4], and then both solvents were mixed before reaching the analytical column.

RESULTS AND DISCUSSION

Chromatographic separation

Fig. 1 shows chromatograms, obtained from both detectors, of a standard solution of 0.5 mg l^{-1} of analytes. Separation was in less than 25 min. Some peaks of the compounds detected by EC detection eluted very close to others which appeared in the UV chromatogram, but they did not interfere with each other. For EC detection to respond better to nitrophenolic compounds it is necessary to work at higher potentials [11], and this involves a higher background, especially when gradient elution is applied. For this reason, UV detection was used with these compounds working at their maximum absorbance wavelength. For other compounds, EC detection at 1.0 V was selected because they have a higher response to this detector.

Good linearity was found for all nitro compounds at 0.015-20 mg l^{-1} using the UV detector, and 0.01-5 mg l^{-1} for 2,4,6-TCP and PCP, and 0.01-1 mg l^{-1} for the remaining compounds using EC detection. In both instances the regression coefficients (r^2) were >0.9990.

On-line Solid Phase Extraction

Breakthrough curves were recorded for phenol in order to establish the capacity of the sorbents used. These curves were recorded in the same way as in previous work [3,26], with 10 mg l^{-1} phenol dissolved in Milli-Q water pH 2.5 at 1.0 ml min^{-1}, and the signal was measured at 280 nm. If the breakthrough volume is that at which the detector reaches 10% of its total value, the breakthrough volumes of phenol with these sorbents were 2 ml for Envi-Carb, 14 ml for Bond Elut PPL and 14.2 ml for the synthesized sorbent. Volumes obtained with Bond Elut PPL and the synthesized sorbent were higher than those with other commercial polymers such as PLRP-S, Envi-Chrom P and Amberchrom (4.5, 6.8 and 7.5 ml respectively) [3,26]. The breakthrough volume on Envi-Carb is similar to that obtained with another carbon black, Carbopack B, used previously [18]. The slopes of the breakthrough curves for Bond Elut PPL and the synthesized sorbent were similar. However the slope of the curve for Envi-Carb was sharper, as already seen for Carbopack [18]. This reflects the different interactions between analytes and the carbon black or polymeric sorbents.

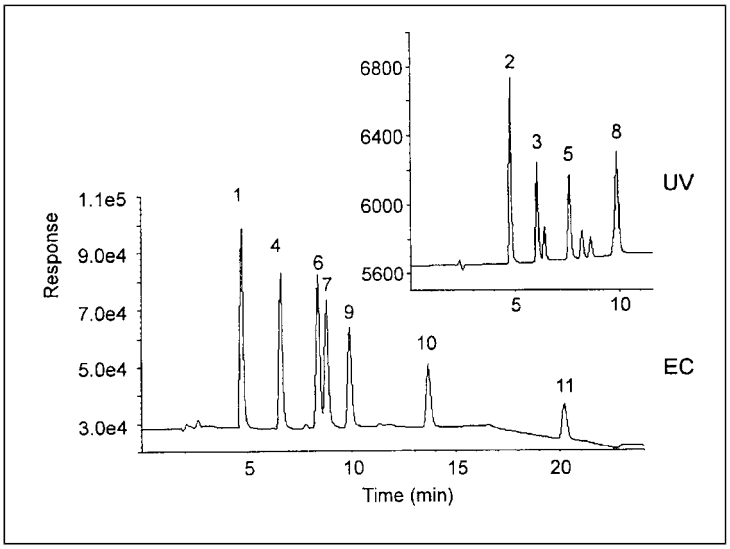

Fig.1. Chromatograms of standard solution of 0.5 mg l^{-1} analytes by direct injection UV and EC detection. Conditions, see text. Peak assignations: (1) Ph, (2) 4-NP, (3) 2,4-DNP, (4) 2-CP, (5) 2-NP, (6) 2,4-DMP, (7) 4-C-3-MP, (8) 2-M-4,6-DNP, (9) 2,4-DCP, (10) 2,4,6-TCP, (11) PCP.

Application to Real Samples

The performance of the three sorbents was checked with tap water. As in previous experiments, 500 µl of 10% Na_2SO_3 solution, for each 100 ml of sample, was added to all tap water samples to remove residual chlorine and reduce the matrix effect due to humic and fulvic acids [27].

Two sample volumes, 10 and 25 ml, were preconcentrated with these sorbents. When 25 ml of sample spiked with 0.1 µg l^{-1} of the eleven analytes was preconcentrated with Bond Elut PPL and the synthesized sorbent, recoveries were similar in both cases and >70% for all compounds, except for PCP which was nearly 45%. However, with Envi-Carb, the recoveries with the same sample volume and concentration were lower, mainly for the most polar phenolic compounds such

as Ph, 4-NP and 2,4-DNP which had recoveries of 35, 55, and 25% respectively, and for PCP which had a recovery of nearly 20%. Although the two functionalized polymeric sorbents had similar recoveries, the band that appeared at the beginning of the chromatogram was narrower with the synthesized sorbent than the band with Bond Elut PPL, mainly in the EC detector. For these reasons, the synthesized sorbent was selected for further analyses. Figs. 2 (electrochemical detection) and 3 (UV detection) show chromatograms obtained when preconcentrating 25 ml tap water with (b and d) and without (a and c) addition of 0.1 μg l^{-1} of the eleven phenolic compounds using Bond Elut PPL (a and b) and the synthesized sorbent (c and d).

The linearity of response for the total analytical system, including the preconcentration step with the sorbent with the *o*-carboxybenzoyl moiety, was initially checked for both detectors for 25 ml tap water spiked at different concentrations. Blank experiments showed that phenols were undetectable in this water. Linear ranges and the detection limits, calculated by the statistical program ULC (Univariate Linear Calibration) with *k* equal to 6 [29] are shown in Table 2. In some samples of tap water two peaks appeared at the same retention time as 4-NP and 2-NP in the UV chromatogram (see Fig. 3) but the concentration was near the limit of detection.

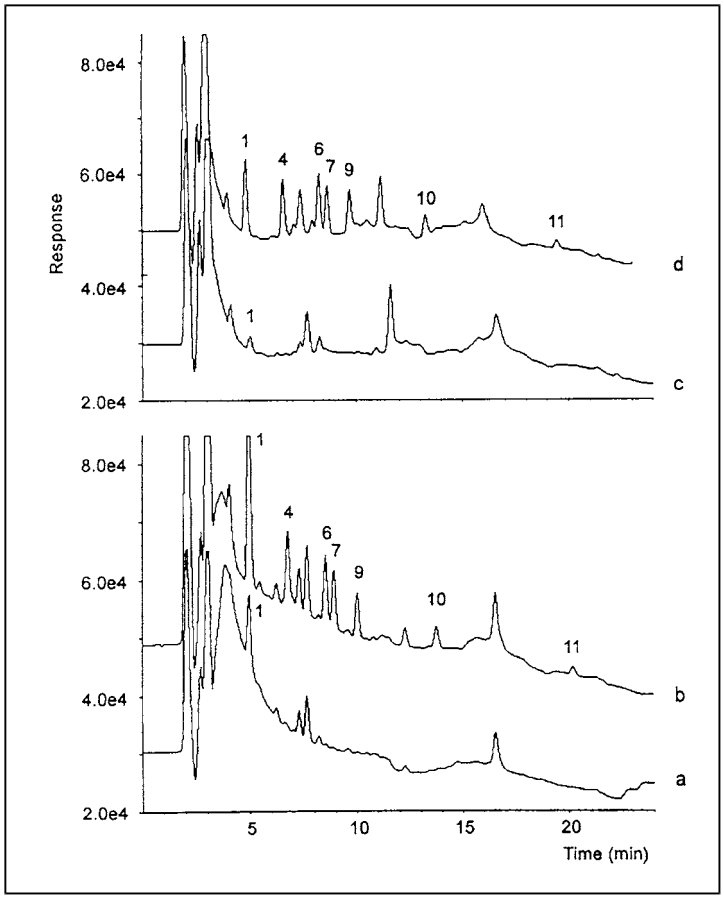

Fig. 2. Chromatograms obtained with EC detector when preconcentrating 25 ml tap water (pH 2.5) with (b,d) and without (a,c) addition of 0.1 µg l^{-1} of all analytes using Bond Elut PPL (a,b) and synthethic sorbent (c,d). Peaks as Fig. 1.

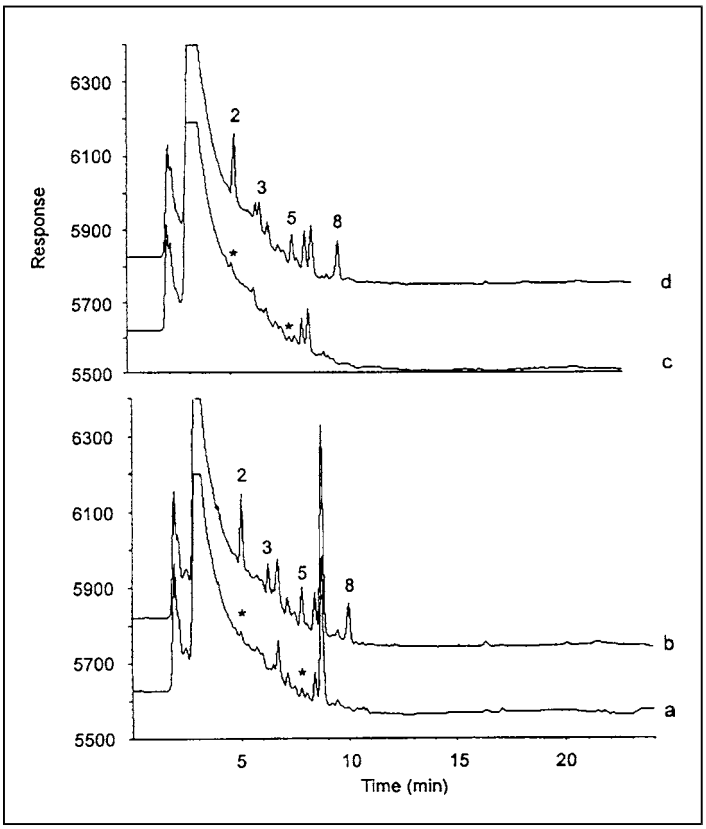

Fig. 3. Chromatograms obtained with UV detector when preconcentrating 25 ml tap water (pH 2.5) with (b,d) and without (a,c) addition of 0.1 µg l^{-1} of all analytes using Bond Elut PPL (a,b) and the synthethic sorbent (c,d). Peaks as Fig. 1.

In the EC chromatogram one peak appeared at the same retention time as phenol (see Fig. 2) and was identified from the electrochemical ratios of areas obtained by repeatedly injecting the same sample at different potentials (0.8, 0.9, 1.0 and 1.1 V) [6]. Table 3 gives the electrochemical ratios of two 25 ml standard solutions (one of Milli-Q waterand the other of tap water) of 0.1 µg l^{-1} phenol. Phenol was present in tap water at 50 ng L^{-1} level. The repeatibility and the reproducibility between days of

the method, expressed as the relative standard deviation of 5 analyses of tap water samples spiked at 0.1 μg l^{-1}, were <8% and 10%, respectively, for all compounds.

Table 2
Linear range and detection limits in on-line preconcentration of real samples with synthetic sorbent

Compound	Tap water[a)]			River water[b)]		
	Linear range (μg l^{-1})	r^2	LOD (ng l^{-1})	Linear range (μg l^{-1})	r^2	LOD (ng l^{-1})
Ph	0.05-2	0.9984	25	0.25-5	0.9998	77
4-NP	0.05-20	0.9998	13	0.1-20	0.9992	25
2,4-DNP	0.1-20	0.9998	25	0.1-20	0.9994	30
2-CP	0.05-2	0.9985	12	0.1-5	0.9999	22
2-NP	0.075-20	0.9998	30	0.1-20	0.9993	36
2,4-DMP	0.05-2	0.9998	25	0.1-2	0.9999	30
4-C-3-MP	0.05-2	0.9977	32	0.1-2	0.9994	28
2-M-4,6-DNP	0.05-20	0.9998	24	0.1-20	0.9993	34
2,4-DCP	0.05-2	0.9993	15	0.1-2	0.9990	24
2,4,6-TCP	0.075-5	0.9998	35	0.1-5	0.9983	32
PCP	0.1-2	0.9975	27	0.1-5	0.9995	30

[a)]Analysis of 25 ml tap water at pH 2.5 (with 500 μl sulphite for each 100 ml sample).
[b)]Analysis of 10 ml Ebro river water at pH 2.5 (with 1000 μl sulphite for each 100 ml sample).

Table 3
Electrochemical ratios of peak areas with same retention time as phenol at different voltages

Sample	Ratio of voltages					
	0.8/0.9 V	0.8/1.0 V	0.8/1.1 V	0.9/1.0 V	0.9/1.1 V	1.0/1.1 V
Standard solution of phenol	0.19	0.13	0.13	0.70	0.66	0.94
Tap water	0.16	0.12	0.11	0.72	0.63	0.90
Ebro river water	0.15	0.14	0.10	0.76	0.69	0.91
Llobregat river water	0.23	0.12	0.07	0.52	0.32	0.61
Ter river water	0.14	0.06	0.05	0.46	0.38	0.82

Ten and 25 ml samples of Ebro river water were preconcentrated with the synthetic sorbent; 1000 µl of 10% Na_2SO_3 solution was added for each 100 ml of river water. Even when sulphite was added to preconcentrate 25 ml of Ebro river water, the matrix peak at the beginning of the chromatogram masked the phenol peak and prevented it from being identified and quantified. For this reason 10 ml was selected to check the linearity with this sample. The results of the linearity study and the detection limits, calculated by the ULC program [29], are shown in Table 2. No peaks corresponding to phenols were observed in blank chromatograms. Recovery values were similar to those obtained with tap water. Fig. 4 shows chromatograms obtained in the preconcentration of 10 ml of Ebro river water from Amposta (Tarragona, Spain; June 1997) with and without standard addition of 0.5 µg l^{-1} of each phenolic compound with both detectors. Two peaks appeared at the same retention time as Ph and 2,4-DCP in the EC chromatogram. The electrochemical ratios of the peak areas at different voltages for both analytes were determined, but 2,4-DCP was not identified. Phenol was identified and its concentration was 3 µg l^{-1} (see Table 3). Two peaks were tentatively identified as 4-NP and 2-NP in the UV chromatogram but their presence was not confirmed by an additional technique. The repeatibility and the reproducibility between days, with Ebro river water, were similar to those obtained with tap water.

It is pointed out that phenol may be determined in river water without interference using the *o*-carboxybenzoyl sorbent with an addition of Na_2SO_3. However, when other sorbents are used, phenol appears on the hump of the humic and fulvic acids and it cannot be determined in river water [2,10,22].

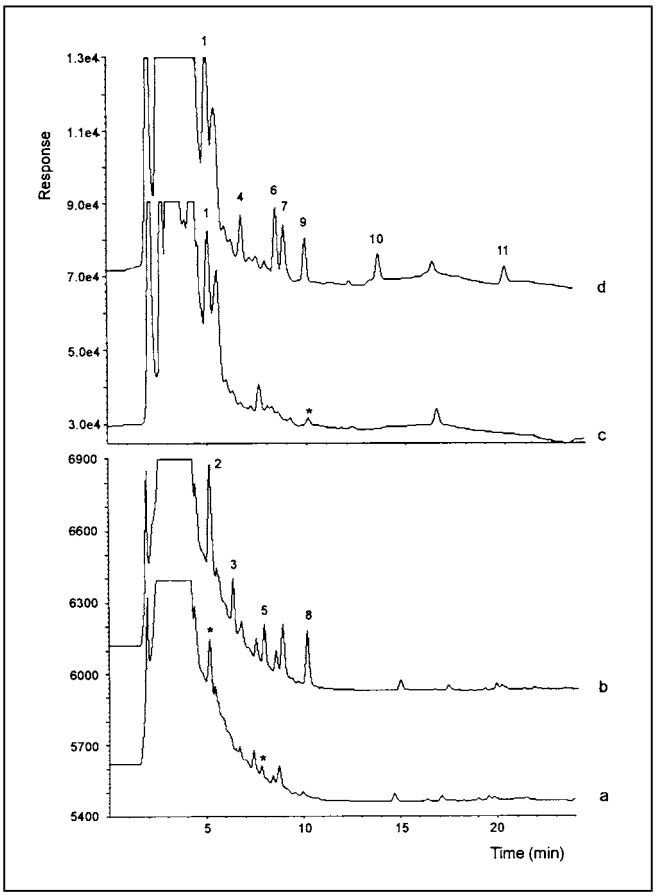

Fig. 4. Chromatograms with UV (a,b) and EC (c,d) detectors when preconcentrating 10 ml Ebro river water (pH 2.5) with (b,d) and without (a,c) addition of 0.5 μg l^{-1} each phenolic compound using the synthethic sorbent. Peaks as Fig. 1.

Water samples from the Llobregat and Ter rivers (near Barcelona city) were also analysed; 10 ml from each river was preconcentrated. In both samples a peak appeared at the same retention time as phenol but, from the electrochemical ratios (see Table 3) it was not assigned to this compound. The rest of the phenolic compounds did not appear in these samples.

CONCLUSIONS

UV and electrochemical detectors coupled in series enabled nitrophenolic compounds which give low responses with electrochemical detection in liquid chromatography and gradient elution, to be determined in less than 25 min.

It has been shown that the *o*-carboxybenzoyl sorbent is more suitable for determining the eleven priority EPA phenolic pollutants in environmental waters than other commercially available sorbents, such as Envi-Carb and Bond Elut PPL, because it gives higher recoveries than Envi-Carb and similar ones to Bond Elut PPL but it had a narrower band at the beginning of the chromatogram, due to humic and fulvic acids present in natural waters. Addition of Na_2SO_3 to the samples enabled phenol to be detected without interference.

This method enabled the eleven phenolic compounds to be determined at levels <0.1 μg l^{-1} in tap and river water, the maximum concentration allowed in water for human consumption.

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