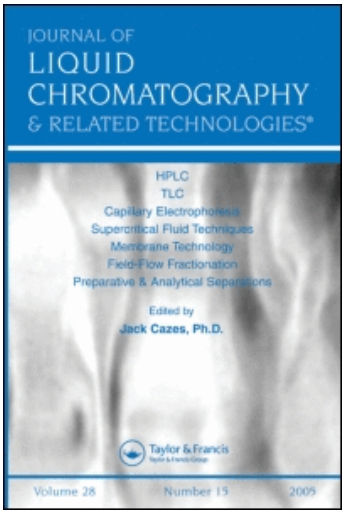


**Journal of Liquid Chromatography & Related Technologies**
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# HPLC-UV-EC Determination of Brominated Organic Compounds in Water

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**To cite this Article** Quintana, M. C. , Iglesias, V. , Silva, M. P. da , Hernández, M. and Hernández, L.(2006) 'HPLC-UV-EC Determination of Brominated Organic Compounds in Water', Journal of Liquid Chromatography & Related Technologies, 29: 1, 87 — 98
**To link to this Article: DOI:** 10.1080/10826070500362995
**URL:** http://dx.doi.org/10.1080/10826070500362995

*Journal of Liquid Chromatography & Related Technologies*®, 29: 87–98, 2006

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070500362995

# HPLC-UV-EC Determination of Brominated Organic Compounds in Water

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**Abstract:** A simple, fast, and sensitive high performance liquid chromatography with electrochemical and UV-Vis detection method for the determination of bromophenols, BPs, (2-bromophenol, 3-bromophenol, and 4-bromophenol), and bromobiphenyls, BBPs, (2-bromobiphenyl, 3-bromobiphenyl and 4-bromobiphenyl) has been developed. The detection limits ranged from 18.2 up to 65.3 μg/L. The optimized method was successfully applied to river water samples after the development of a simple and fast solid phase extraction (SPE) method allowing the preconcentration and clean up of the analytes. The performance of the complete procedure was satisfactory irrespective of the spiking level with recoveries higher than 65%, and repeatability evaluated as the relative standard deviation, better than 12%.

**Keywords:** Column liquid chromatography, Solid phase extraction, Spectrophotometrical and electrochemical detection, Bromophenols, Bromobiphenyls, River water

## INTRODUCTION

The characteristic toxicity and the great variety of sources of halogenated organic compounds in the environment explain the need for developing new analytical methodologies for the selective detection and sensitive quantitation of these compounds in different matrices.

Many investigations have dealt with the toxicities of halophenols and their adverse effects on human health and the ecosystem. Based on these

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studies, the US-EPA has assigned eleven phenolic compounds as major priority pollutants.[1,2] Most halogenated organic compounds have moderate to high toxicity if inhaled. The brominated materials, widely used as antiseptic germicides (as bromophenols) and flame retardants (as polybromobiphenyls PBBs and polybromodiphenylethers PBDEs)[3] tend to be particularly toxic, and much of their toxicity is due to the fact that these substances are not metabolized. Also, they have many properties in common with chlorinated organic compounds,[4] which make them long-lasting, bioaccumulating, and environmental pollutants. Moreover, like chlorophenols, brominated compounds are known to be precursors in the formation of dibenzo-p-dioxins and furans via the formation of ether bonds during the industrial processes at high temperatures.[1,3]

The interest in the study of brominated compounds has grown since a large number of persons were accidentally poisoned in Michigan in the 70's. Pentahexa and hepta-brominated biphenyl components in serum samples from farming families and from Michigan Chemical Corporation employees, were analysed by chromatography-mass spectrometry. Quantities of $\mu g/g$ of several PBBs were detected in at least 298 persons.[5,6]

Anthropogenic sources of contamination by brominated organic compounds are well known. However, biotics, although less recognized, are also widespread sources of haloaromatics. Some sediment dwelling marine species (through the action of haloperidases) produce high levels of volatile brominated secondary malodorous and toxic metabolites, such as bromophenols, bromopyrroles, and bromoindoles.[7] Among these compounds, 2-bromophenol, 4-bromophenol, 2,4-dibromophenol, 2,6-dibromophenol, and 2,4,6-tribromophenol have been widely found distributed in marine fish and seafoods, and have been recognized as important contributors to sea- and iodinelike flavours in these products.[8–10] Each compound has a threshold concentration (FTC).[11] However, bromophenols contribute recognisable marine or ocean-like flavours to seafood even below their FTC.[12]

Gas chromatography-mass spectrometry (CG-MS) techniques are typically used for the analysis of bromophenols and bromobiphenyls residues in samples such as sediment-dwelling marine species,[7,13] seafood,[9,14] ocean fish,[10] and marine algae.[15,16] CG-MS is also used to analyse environmental samples such as water,[1,17] air,[1] and sediments.[1,4] Nevertheless, some methodologies involving the use of pressurised hot water extraction coupled on-line with liquid chromatography-gas chromatography (PHWE-LC-GC) have also been described.[4] Chromatographic separation of the brominated biphenyls in polymeric materials has typically been carried out by high performance liquid chromatography with UV-Vis detectors (HPLC-UV).[18,19] Methods based on electrochemical techniques are less extended in spite of being sensitive and rapid techniques,[20,21] and despite the knowledge of the easy electrochemical oxidation of phenolic compounds.

This study tests the feasibility of developing a new and fast chromatographic method based on the on-line coupling of UV-EC detectors for the simultaneous analysis of brominated phenol and biphenyl compounds.

## EXPERIMENTAL

### Reagents

Pure standards of the target compounds 2-bromophenol (98%), 3-bromophenol (98%), 4-bromophenol (98%), 2-bromobiphenyl (98%), 3-bromobiphenyl (98%), and 4-bromobiphenyl (98%), were purchased from Sigma Chemical Co. (USA).

Working stock solutions of the individual compounds were prepared in methanol at a concentration level of 1000 μg/mL and used for further dilution and spiking of the samples. The standard solutions were conserved at 4°C and protected from light.

All reagents used were of analytical reagent grade. Methanol was HPLC grade (Scharlau, Spain) and Milli-Q Milli Ro water was purified with a Millipore system (Bedford, USA). Extraction cartridges of Oasis HLB (60 mg, Waters, Milford, USA) and $C_{18}$(500 mg, Waters, Milford, USA) were used for sample enrichement.

Water samples were obtained from Jarama River (Madrid, Spain).



### Instrumentation

The LC system consisted of a Jasco Analytica (Madrid, Spain) PU-1580 high pressure pumping system equipped with a Rheodyne 7125 injection valve provided with a 20 μL loop and a stainless steel prepacked $C_{18}$ reversed-phase column (150 × 4.0 mm i.d.; 5 μm particle size) from Kromasil (Spain). Spectrophotometric detection was performed by using a LC-785A Perkin Elmer Hispania (Madrid, Spain) UV-Vis detector. Electrochemical detection was performed by using a LC-4C (BAS) (USA) EC detector equipped with a thin layer flow cell with a glassy carbon, Ag/AgCl/KCl 3M, and a gold one as working, reference, and auxiliary electrodes, respectively. Data were acquired with the Borwin software (from JMBS Software for scientists, Jasco Analytica, Madrid, Spain).

The UV-Vis absorption spectra of the analytes were recorded with a U-2000 UV-Vis Spectrophotometer (HITACHI).

Measurements with a rotating glassy carbon electrode were performed with a CV 27 (BAS) (USA) potenciometer. A Pt counter electrode and Ag/AgCl/KCl 3M reference electrode were coupled to a X-Y Recorder (BAS) (USA).

The extraction and purification of the water samples with the SPE cartridges, were carried out in a Vac Elut system from Varian Ibérica, S.L.

(Madrid, Spain) and employing a sample concentrator Techne DRIBLOCK DB 20, equipped with temperature control and $N_2$ flow (Genesys Instrumentation, S.L., Madrid, Spain).

### Procedure

In all experiments, the SPE cartridges were placed in a Vac Elut system apparatus from Varian Ibérica, S.L. (Madrid, Spain), attached to a water aspirator via a pressure-metering valve and conditioned with 2 mL of methanol and 2 mL of ultrapure water before being used.

After optimization of the different parameters affecting the HPLC-UV-EC method, a typical experiment for mixtures of monobrominated phenols and biphenyls determination was performed in the acidification of river water sample (2.0 mL) with $H_3PO_4$ to pH = 2.0. The mixture was directly applied to the Oasis SPE cartridge at a flow rate of c.a. 0.25 mL/min. After washing the cartridge with 1 mL of 2% methanol, it was dried for 5 min under suction. The analytes were eluted from the SPE cartridge with 5.0 mL of methanol. Extracts were concentrated under a gentle nitrogen current, reconstituted in 1.0 mL of the selected mobile phase and analysed, without any additional treatment, by 20 μL injection in the HPLC-UV-EC system. The development of the chromatogram began with the use of methanol-borate buffer $10^{-3}$ M pH = 9.5 (60 : 40) (v/v), as the mobile phase. After 6 min of analysis, during which separation, identification, and electrochemical determination (E = + 0.8V) of bromophenols were carried out, the mobile phase was changed to methanol-borate buffer $10^{-3}$ M pH = 9.5, (80 : 20) (v/v). In these new conditions, elution and UV ($\lambda = 250$ nm) detection of the bromobiphenyls family are possible.

Mobile phases and solutions were filtered through a Millipore Durapore filter (0.45 μm pore size, Millipore Ibérica, Madrid, Spain) and deaereated by agitation under vacuum for 10 min before injection into the chromatographic system.

## RESULTS AND DISCUSSION

### Optimization of the Experimental Conditions

Preliminary experiments were carried out to optimize the experimental parameters affecting both the chromatographic separation and the detection with UV-Vis and electrochemical techniques.

According to the UV spectra of the analytes investigated, wavelengths of 250 nm and 230 nm were selected for detection of bromobiphenyls and bromophenols, respectively. The feasibility of different mixtures of solvents for chromatographic resolution of all six analytes was tested. Mixtures of

methanol-water containing 70% of the organic modifier at different pH values were studied using different salts in concentration of $10^{-2}$ M. In all cases, the flow rate was kept at 1.0 mL/min. Retention times decreased slowly when the pH of the mobile phase was increased. On the other hand, a significant influence of pH on the value of the peak areas was not observed. In all the conditions tested, the high difference in polarity between the two groups of compounds led to great differences in the retention times recorded. Therefore, while the three isomers of bromophenol were eluted from the column during the first 4 min, the retention times for bromobiphenyls ranged from 25 to more than 50 min.

Some preliminary experiments were carried out to study the electrochemical response of the analytes under investigation. Threrefore, cyclic voltammograms of solutions containing a concentration of 5 μg/mL of each analyte at different pH values and using a rotating glassy carbon electrode were recorded. The use of this type of electrode allows the reproduction of the hydrodynamic conditions of the mass transport to the electrode surface that will occur in the chromatographic system. Figure 1 shows the cyclic voltammograms obtained when the potential was varied between $E_1 = -0.6$ V and $E_2 = +1.4$ V at v = 100 mV/s. As was expected, an oxidation wave (due to OH group oxidation) that was getting more cathodic with increasing pH value, according with the equation $E_p = E_o - 0.059/n \cdot pH$, was obtained when the bromophenols were studied. No electrochemical response under tested conditions was obtained for Br-biphenyls family.

These preliminary results showed the possibility of detecting the six analytes under investigation by using a UV technique. However, an amperometric technique can only be used in the detection of the bromophenols that will greatly improve selectivity and sensitivity. Therefore, it was decided to optimize the chromatographic conditions in an HPLC-EC system for the analysis of brominated phenols. Afterwards, a UV-Vis detector will be connected on line with the electrochemical one, in order to allow the

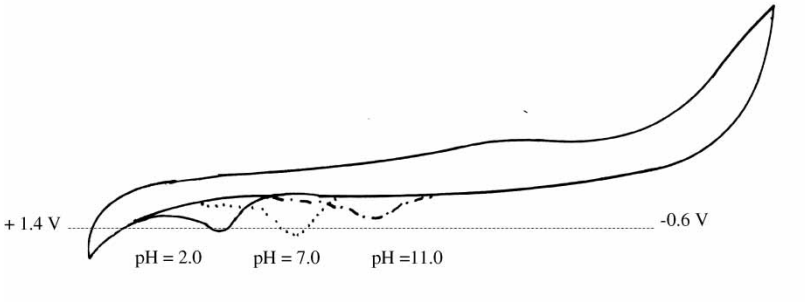


***Figure 1.*** Voltammograms of 5 μg/mL 2-BP obtained with a rotating glassy carbon electrode in 0.1 M phosphoric buffer. $\omega = 2000$ rpm, $V_b = 100$ mV/s, (s = 5 nA/cm).

simultaneous detection of both groups of compounds. This approach will require the adjustment of the chromatographic conditions to achieve their simultaneous analysis.

## Optimization of HPLC-EC Parameters

Prior to any experiment, the working electrode was activated for 5 s by holding the potential at +1.5 V in the mobile phase and then at −1.0 V for 3 s. In order to select the potential applied to the working electrode, several i-E hydrodynamic curves were recorded by using different supporting electrolytes in the mobile phase.

Figure 2 shows that the signal decreased when the pH of the mobile phase was increasing. This evidence would lead to the work with buffer solutions of alkaline pH, in order to increase the selectivity of detection conditions. The recommended working conditions for the stationary phase were between pH = 2 and pH = 11, however, it was decided to work at pH = 9.5 (borate buffer) as a less extreme condition (a small loss of selectiviy without a big loss of sensitivity). Under these experimental conditions, the potential applied to the working electrode was + 0.8 V.

The effect of electrolyte concentration on the analytical signal was studied for values ranging between $5 \cdot 10^{-4}$ M and $1 \cdot 10^{-3}$ M. Experiments were carried out in a mobile phase methanol-borate buffer pH = 9.5, (50 : 50) (v/v) (bromophenols in concentration 0.2 μg/mL). A better signal-to-noise ratio was obtained when a $10^{-3}$ M buffer concentration was used.

Afterwards, the effect of the percentage of organic modifier in the mobile phase was studied in the 50–70% range. Other chromatographic conditions

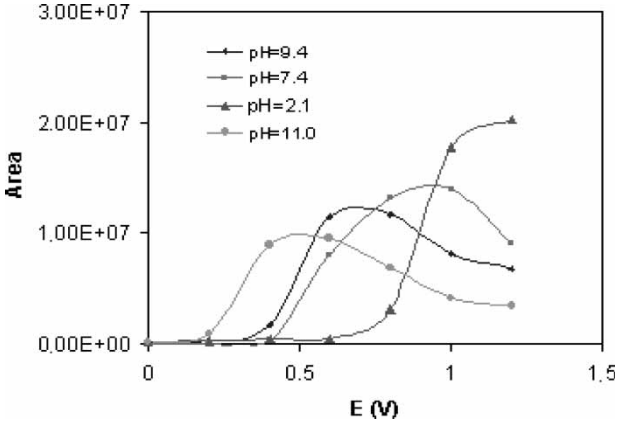


***Figure 2.*** Hydrodynamic curves i/E. Mobile phase: methanol-water 50 : 50 (v/v), q = 1.0 mL/min, loop = 20 μL.

were kept at the values indicated above. The results show that the best resolution was found when 60% methanol was used, therefore, this percentage was used for the following studies.

## Analysis of Brominated Phenols and Biphenyls with HPLC-UV-EC

Once the experimental conditions for the analysis of bromophenols in the HPLC-EC system were optimized, this detector was connected to a UV-Vis detector in order to carry out the determination of the brominated biphenyls. A double channel solvent delivery system was used to allow the eluent composition to be change during the development of the chromatogram. An improvement of the chromatographic conditions was required for the separation of mixtures of the two groups of compounds to carry out their simultaneous analysis. When the conditions of the separation of bromobiphenyls were studied, it was necessary to consider that the stability of the chromatographic system was altered due to the change of the mobile phase composition. For this reason, several methanol/water mixtures (70–90% v/v) and flow rates were assayed.

Figure 3 shows the results when 20 μL of a mixture of the 6 brominated organic compounds were injected in the chromatographic system under the best conditions chosen for their simultaneous determination. The sample was injected in the mobile phase methanol-borate buffer $10^{-3}$ M pH = 9.5, (60 : 40) (v/v). After 6 min of analysis, during which separation, identification, and electrochemical determination (E = + 0.8 V) of bromophenols were carried out, the mobile phase was changed to methanol-borate buffer $10^{-3}$ M pH = 9.5, (80 : 20) (v/v). It is possible to observe the complete separation of all the analytes in excellent analysis time (a decrease from 50 to 15 min) with better sensitivity results, when working with amperometric detection of bromophenols than when the spectrophotometric detection is used.

The statistical data for the calibration graphs for all the compounds and both techniques studied are compared in Table 1. The linearities of the calibration graphs are excellent, as shown by the correlation coefficients, and the linear dynamic range covers two orders of magnitude of concentration (20–1000 μg/L). The detection limits obtained for the brominated phenols and byphenyls with the two techniques are also summarized in Table 1; lower detection limits are obtained using electrochemical detection for bromophenols.

## Application of the Proposed Method to River Water Samples

Some preliminary experiments were carried out to optimize the experimental parameters affecting the SPE procedure with both $C_{18}$ and Oasis sorbents; these included flow rate during the application of the sample, type and

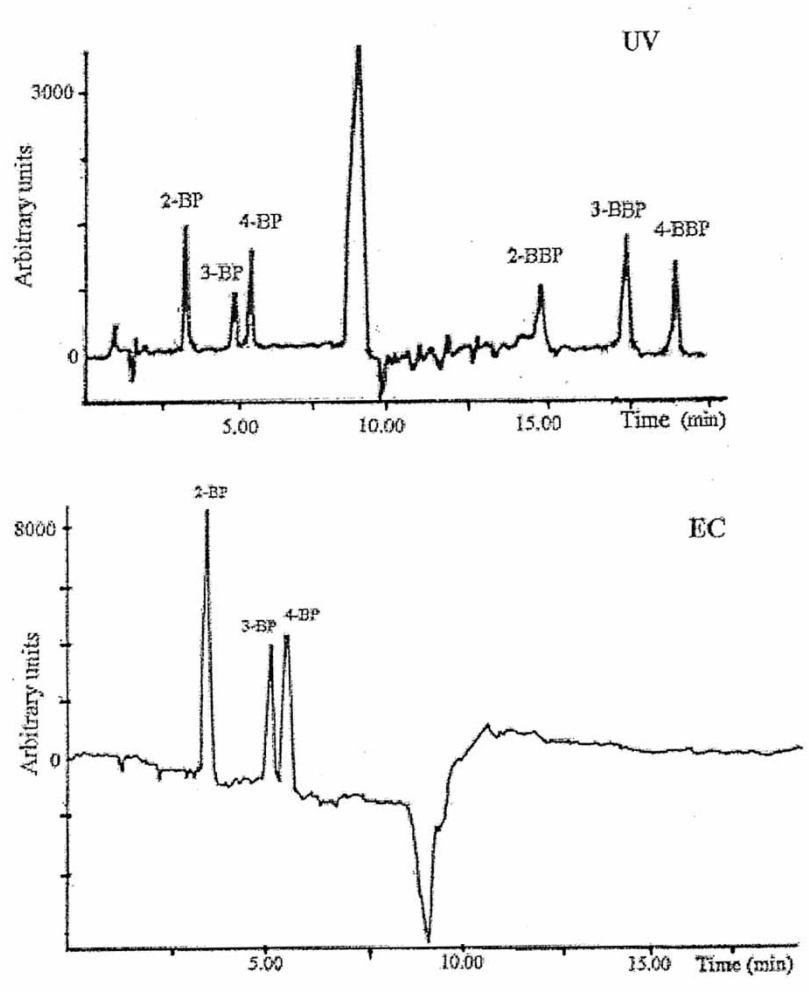


***Figure 3.*** Chromatogram of the six brominated compounds with the HPLC-UV-EC system. [Analytes] = 0.4 μg/mL, loop = 20 μL, mobile phase methanol-water 60 : 40 (v/v), borate buffer $10^{-3}$ M pH 9.5, and methanol-water 80 : 20 (v/v), borate buffer $10^{-3}$ M pH 9.5, $\lambda = 250$ nm, E = + 0.8 V, q = 1.0 mL/min.

volume of washing solvent, drying conditions, and selective elution of the two types of compounds investigated. In the first part of the study, sub-samples of 1.0 mL of water, spiked with either the bromophenols and bromobiphenyls studied at the 100 μg/L level and treated as previously described in Procedure Section, were applied to the corresponding SPE cartridge at a flow rate of about 0.25 mL/min. In all cases, cartridges were washed with 1 mL of Milli-Q water with 2% methanol and dried for 5 min under suction. The feasibility of methanol-water mixtures (containing 60–100% (v/v)

***Table 1.*** Statistical treatment of calibration graphs and limits of detection (signal-to-noise ratio = 3 : 1). Injection volume = 20 μL

| Analyte | Calibration curve (μg/mL) | LOD (μg/L) | LOQ (μg/L) | Recovery (%) | RSD (%) |
|---|---|---|---|---|---|
| 2-$BP_{EC}$ | $A = 2.19 \cdot 10^3$ (4.1%) + $1 \cdot 10^6$ (0.9%) C, r = 0.994 | 18.2 | 39.8 | ≤11.1 | ≤3.4 |
| 3-$BP_{EC}$ | $A = 4.16 \cdot 10^3$ (3.1%) + $5.25 \cdot 10^5$ (1.1%) C, r = 0,992 | 16.8 | 26.8 | ≤9.9 | ≤3.4 |
| 4-$BP_{EC}$ | $A = 4.12 \cdot 10^3$ (3.9%) + $5.2 \cdot 10^5$ (2.3%) C, r = 0.9991 | 19.3 | 34.3 | ≤12.2 | ≤4.2 |
| 2-$BP_{UV}$ | A = 678 (2.8%) + $2.04 \cdot 10^4$ (2.1%) C, r = 0.997 | 53.1 | 61.3 | ≤7.8 | ≤3.1 |
| 3-$BP_{UV}$ | A = 257 (2.4%) + $1.18 \cdot 10^4$ (1.2%) C, r = 0.997 | 48.3 | 56.3 | ≤8.4 | ≤2.4 |
| 4-$BP_{UV}$ | A = 846 (1.2%) + $2.70 \cdot 10^4$ (0.8%) C, r = 0.9990 | 51.2 | 60.2 | ≤6.4 | ≤3.6 |
| 2-BBP | A = −15.2 (3.4%) + 20.7 (2.8%) C, r = 0.992 | 61.0 | 79.6 | ≤5.2 | ≤5.7 |
| 3-BBP | A = −2.5 (2.8%) + 76.6 (2.7%) C, r = 0.992 | 59.2 | 76.3 | ≤5.1 | ≤4.7 |
| 4-BBP | A = −1.7 (4.7%) + 88.2 (3.9%) C, r = 0.996 | 65.3 | 83.2 | ≤4.6 | ≤5.4 |



organic modifier) for elution of the two groups of analytes studied was tested by collecting 1 mL fractions, which were independently analyzed. The elution of all analytes from both sorbents was obtained when 5.0 mL of pure methanol were used. After evaporation to dryness and the dissolution of the residue in 1.0 mL of mobile phase, the extract was analyzed by HPLC-UV-EC without any additional clean-up. These experiments revealed that the Oasis sorbent provided higher recoveries of the analytes (in the range of 60–95%, n = 3) than did $C_{18}$ (36–63%). Once the performance of the SPE method was tried for academic solutions of water, river water samples (Jarama River, Madrid, Spain) were used to further proceed with method validation using Oasis SPE cartridges. Unspiked and spiked subsamples of 2.0 mL of river water at three different levels of concentration (50, 100, and 150 μg/L), were analysed following the procedure previously described (Figure 4). Three separate analyses of all the subsamples were carried out. Relevant analytical data are summarized in Table 2.

## CONCLUSIONS

A simple, rapid, and sensitive HPLC-UV-EC method for the determination of brominated organic compounds has been developed. The method allowed the

SPE preconcentration and clean-up of river water samples. The extracts obtained can be directly analysed by HPLC-UV-EC, showing that the proposed method is suitable for monitoring purposes with sufficient repeatability and accuracy. The performance of the procedure was satisfactory at concentration levels similar to those seen in previously published studies using similar techniques (LC).[18] Although detection limits offered by methods based on GC-MS techniques (using different samples such as water, air and soil) are in some cases lower,[1,4] the proposed procedure presents enough sensitivity to allow the analysis of these kinds of compounds, in a variety of samples in which the total concentration of

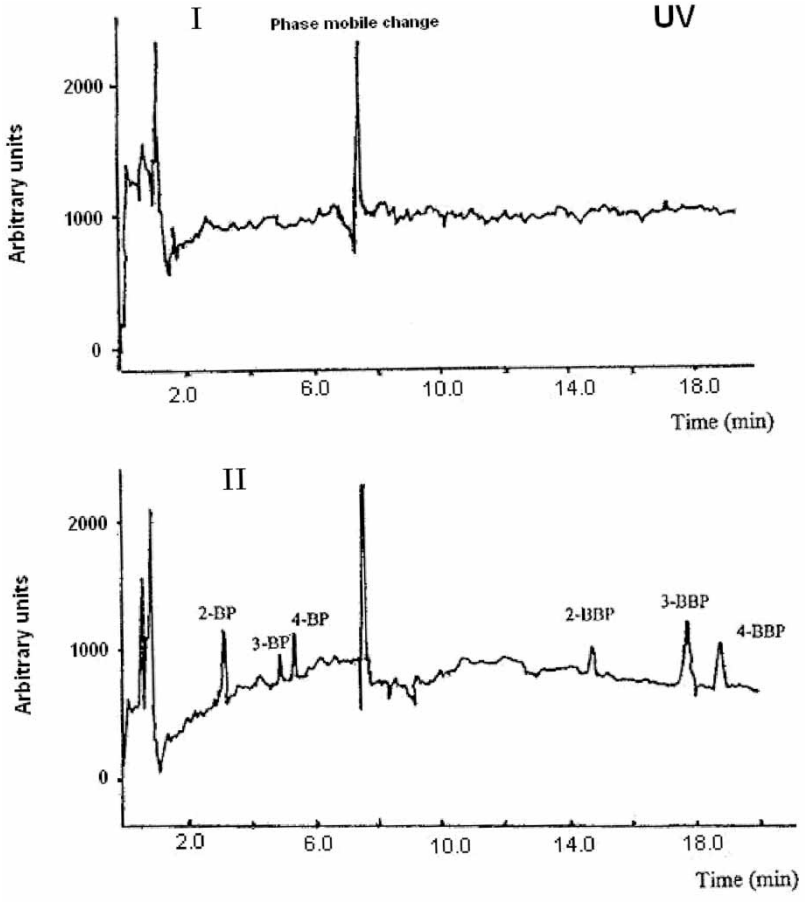


***Figure 4.*** Chromatogram of an extract from river water sample. **(I)** water sample (2.0 mL) **(II)** water sample (2.0 mL) + 100 ng/mL of each compound. Loop = 20 μL. (*continued*)

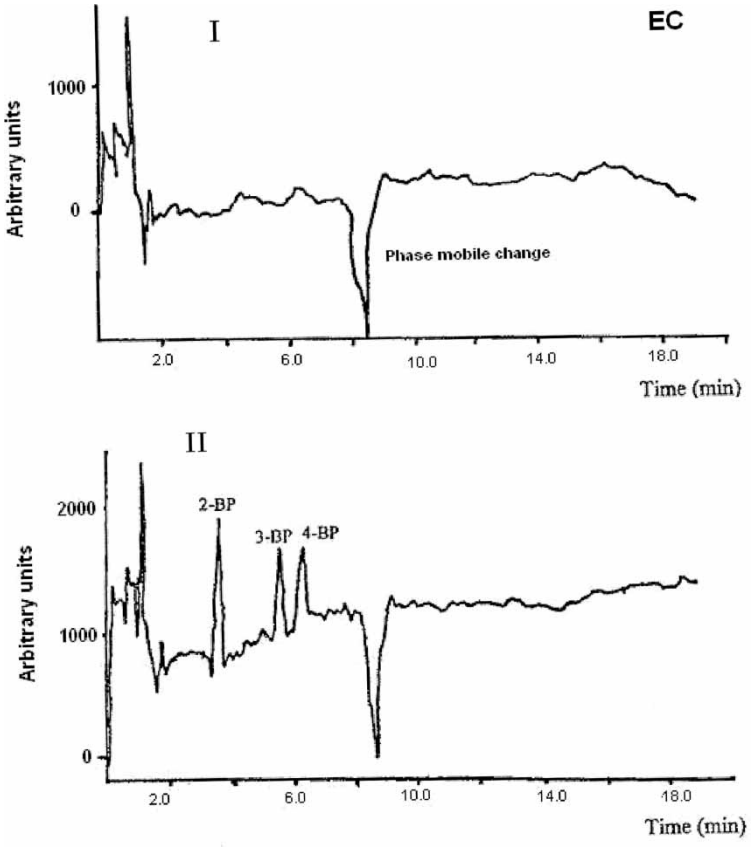


***Figure 4.*** Continued.

***Table 2.*** Relevant analytical data related to the complete SPE plus HPLC-UV-EC proposed method for water samples

| Analyte | LOD[a] (μg/L) | Recovery[b] (%) | Repeatibility, CV (%) n = 3 (μg/L) | | |
|---|---|---|---|---|---|
| | | | 50 | 100 | 150 |
| 2-Bromophenol | 35.2 | 81.2 | 7.4 | 6.0 | 8.0 |
| 3-Bromophenol | 31.4 | 89.7 | 12.0 | 8.9 | 7.4 |
| 4-Bromophenol | 40.2 | 87.3 | 8.5 | 5.1 | 6.6 |
| 2-Bromobiphenyl | 67.5 | 79.7 | 8.2 | 12.8 | 8.3 |
| 3-Bromobiphenyl | 71.9 | 67.2 | 7.6 | 10.2 | 6.8 |
| 4-Bromobiphenyl | 73.7 | 65.0 | 9.6 | 12.5 | 9.8 |

[a]As calculated for real water samples.
[b]Average from data obtained at the three spiking levels mentioned.

bromophenols and related compounds (TBC) is a relevant control parameter.[9,10] The present method shows the advantage of using instrumentation that is cheaper and easier to use than GC-MS.

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Received July 12, 2005
Accepted August 1, 2005
Manuscript 6685