Evaluation of a New Reagent: Anthraquinone-2-Sulfonyl Chloride for the Determination of Phenol in Water by Liquid Chromatography Using Precolumn Phase-Transfer Catalyzed Derivatization

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Abstract

A new reagent, anthraquinone-2-sulfonyl chloride, is used for the derivatizaton of phenols. Several compounds with different polarities are selected to evaluate the new reagent and derivatives of these phenols that are prepared via a facile pathway. The optimal conditions for analytical derivatization and mechanism of the derivatization reaction are discussed. The derivatization procedure involves an ion-pair extraction of the deprotonated phenols with a tetrabutylammonium counter ion in the organic phase. At the interface of two phases, the derivatization reaction occurs quantitatively at room temperature within 3 min. The derivatives are stable and readily amenable to analysis by normal-phase (NP) and reversed-phase (RP) high-performance liquid chromatography (HPLC). Excellent linearity response was demonstrated over the concentration range of 0.2–200 µmol/L at 320 nm for NP-HPLC and at 256 nm for RP-HPLC. Combined with preconcentration using a Waters Sep-Pak Plus C_{18} cartridge, detection limits of phenols for water-sample analysis are as low as 1×10^{-9} mol/L (~ 0.1 µg/mL).

Introduction

Phenolic compounds are present in the environment as a result of their industrial applications (1). Because of their toxicity and unpleasant organoleptic properties (concentrations of as low as 3 µg/L of phenols affect the taste and odor of water and fish), phenols have been included in the list of priority pollutants of many countries. The European Union has classified several phenols as priority contaminants. The 80/778/EC directive states that a maximum allowable concentration for total phenols in drinking water is 0.5 µg/mL, and individual phenol concentrations should be less than 0.1 µg/mL.

The determination of phenols has been described by different chromatographic methods, with high-performance liquid chromatography (HPLC) being prefered because of its robustness. Direct analysis of trace phenols in their biologic or environmental matrix by a single chromatographic technique is usually difficulty because the concentrations are very low and phenols generally lack the attributes necessary for sensitive determination. HPLC and fluorescence detection or electrochemical detection after solid-phase extraction (SPE) were reported to be effective methods for water analysis (1–3). However, it is difficult to achieve high-breakthrough volumes when using SPE cartridges and disks that constrain the limit of detection (LOD) at the legislated level for phenols (4). Kwakman et al. (5) used dansyl chloride to derive phenols after preconcentration of the phenols with a C_{18} cartridge in order to achieve high detection sensitivity, but the procedure removal remained in excess of the derivatization reagent with an amino SPE column before HPLC analysis. In addition, dansyl chloride and its derivatives are usually sensitive to light, requiring precaution during the analysis procedure.

Our studies have focused on developing a new reagent for the derivatization of phenols and other compounds according to the following criteria: (*i*) rapid quantitative reaction under mild conditions; (*ii*) specificity for the target compounds without side reactions; (*iii*) separation of the excess reagent from the reaction mixture; (*iv*) high detection sensitivity for the reaction products caused by high extinction coefficients to visible or UV light, fluorescence, or electrochemical activity; and (*v*) high stability of the derivatization reagent and derivatives, which is essential for routine laboratory use.

A new reagent, anthraquinone-2-sulfonyl chloride (ASC), has been previously developed and validated in our laboratory to derivatize amines quickly and simply (6). In this paper, use of the reagent as model analytes is investigated using several phenols with low to relatively high polarity. After enrichment of the trace phenols in a water sample with Waters Sep-Pak Plus C_{18} car-

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tridges (Waters, Milford, MA), phenols were converted quantitatively into their ASC derivatives under mild conditions within 3 min. These derivatives were readily amenable to analysis by normal-phase (NP) and reverse-phase (RP) HPLC with UV detector and are detectable at nanomolar concentrations (~ 0.1 µg/L). In order to evaluate the new derivatization reagent, standard phenol derivatives were prepared and water samples were analyzed.

Experimental

Reagents

Tetra-*n*-butylammonium chloride (TBACl, 96%); tetrabutylammonium iodide (TBAI, 98%); tetrabutylammonium bromide (TBABr); tetrabutylammonium hydroxide (TBAOH); phenol; *p*-tert-butylphenol; 2,4-dichlorophenol; and 3-methoxy phenol were acquired from Nacalai Tesque (Kyoto, Japan). Chlorosulfonic acid (Chameleon reagent, 96% purity) was purchased from Canxita (Kyoto, Japan). Dichloromethane, benzene, toluene, methanol, acetonitrile, acetone, *n*-hexane, calcium chloride, and sodium sulfate were all analytical-reagent-grade and used as received. ASC was prepared in our laboratory according to the reported method (5). HPLC-grade methanol, acetonitrile, and water were filtered through a 4-µm filter and degassed under vacuum prior to use.

Equipment

Mass spectra were recorded at 70 eV on a JMS-SX 102A mass spectrometer (Data Company, Kyoto, Japan). IR spectra were obtained with a Nicolet Impact 410 spectrometer (Nicolet, San Diego, CA), NMR spectra data were acquired on INVOA-400 (Varian, Somerset, NJ). UV spectra were obtained using a Shimadzu UV–vis recording spectrophotometer (Shimadzu, Kyoto, Japan). Elemental analyses were accomplished on a CHN CORDER model MT-5 (Osaka, Japan). Commercially available precoated silica thin-layer plates (Kieselgel 60 F_{254}) were from Merck (Darmstadt, Germany). Sep-Pak Plus C_{18} environmental cartridges were from Waters. The separation and determination experiments of derivatives were performed with a Model 10A high-performance liquid chromatograph (Shimadzu) equipped with a UV–vis spectrophotometric detector and a Model 5J sample injector (Shimadzu). For NP-HPLC, a Waters 5 Si-π column (250-× 4.6-mm i.d.) (Cosmosil, Osaka, Japan) was used as the stationary phase with a mobile phase consisting of benzene–hexane (80:20) at a flow rate of 1.0 mL/min. The detection wavelength for the HPLC analysis was at 325 nm. The chromatographic analysis of RP-HPLC was achieved on a 5-µm C_8 column (250-× 4.6-mm i.d.) (Cosmosil) using a methanol–aqueous solution (containing 0.01M NaClO$_4$) 80:20 as the mobile phase. The flow rate was 0.7 mL/min. This column had a detection wavelength of 256 nm. All experiments were performed at room temperature.

Solutions preparation

ASC was prepared and stored in a desiccator at room temperature, according to the procedure reported in the literature (6). A solution of 0.05M ASC was prepared by dissolving 0.3 mg of ASC in 20 mL of dichloromethane (or toluene and benzene) and stored at room temperature in daylight.

For optimum analytical derivatization, the phenol-standard stock solutions were prepared by accurately weighing and dissolving the appropriate amounts of the four compounds in toluene. The required working solutions were obtained by further dilution with toluene. The standard solutions of phenols for fortification were prepared in methanol. The standard solutions of derivatives were prepared weekly in acetonitrile and refrigerated when not in use.

Solutions of tetrabutylammonium salts (0.1 mol/L) and sodium hydroxide (1–4 mol/L) were prepared in water. All of the mentioned solutions were stable for at least two weeks under daylight at room temperature.

Preparation and purification of standard phenol derivatives

Using aprotic solvents with phenols in excess (and the addition of potassium hydroxide as a base to neutralize the resulting HCl produced from sulfonate formation), standard phenol ASC derivatives were prepared using the following procedure: ASC (1 mmol) and phenols (5 mmol) were dissolved in 100 mL of benzene. The solution, which changed color immediately from yellow to red-orange (or deep red) when a grain of potassium hydroxide was added, was stirred for 30 min, washed with 20 mL of water three times, separated from the aqueous phase, and dried over anhydrous Na$_2$SO$_4$. Benzene was removed by rotary evaporation. Dry-column chromatography (silica gel) with benzene as the solvent was used to purify the products further and to remove ASC, which was the primary byproduct. Chromatographic fractions were collected and checked for product purity (> 97%) by silica-gel thin-layer chromatography. Removal of solvent yielded the derivatives.

The four derivatives [phenol-ASC (APH, pale-yellow solid), *p*-tert-butylphenol-ASC (ABP, yellow solid), 2,4-dichlorophenol-ASC (ACP, orange solid), and *m*-methoxyphenol-ASC (AMP, dark red)] were obtained through the mentioned process. The derivatives were characterized by NMR and mass spectrometry, and they had the structure shown in Figure 1.

Trace enrichment step

Before use, a C_{18} cartridge (Sep-Pak Plus, Waters) was activated by passing 2 mL of methanol through it, followed by distilled water (5 mL). The water sample (≤ 100 mL) was transferred into a glass beaker. After acidification to pH 2–3 by dropwise addition of sulfuric acid, the water sample was passed through the cartridge at a flow rate of 0.5 mL/min with the aid of a pump. The cartridge was eluted with acetone, and 2.0 mL of eluent were collected. The acetone extract was dried at room temperature using a gentle stream of nitrogen, and the residual was redissolved in 0.5 mL of toluene.
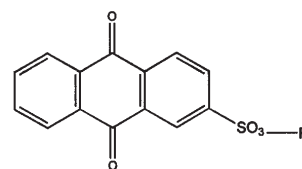

Figure 1. Structure of the four derivatives (APH, ABP, ACP, and AMP) characterized by NMR and mass spectrometry.

Precolumn derivatization procedure

The derivatization reaction of phenols was carried out in a 10-mL glass-stopped vial by mixing each of the following solutions: (*a*) 1.0 mL aqueous solution containing sodium hydroxide (1.0 mol/L) and TBACl (0.05 mol/L); (*b*) 0.5 mL ASC solution (0.05 mol/L in toluene); and (*c*) 0.5 mL phenols solution (2×10^{-3} mol/L for each phenol). The reaction mixture was shaken for 3 min at room temperature using a vortex mixer. From the organic layer, a 20-μL volume of the crude reaction mixture was injected directly onto the NP and RP chromatographic columns.

Analysis of water sample

Tap water and river water spiked with a number of selected phenols were adjusted to pH 2–3 with 1 mol/L sulfuric acid prior to preconcentration. Then the phenols were analyzed by enrichment, derivatization, and HPLC determination.

Results and Discussion

### Two-phase derivatization of phenolic compounds

ASC is a hydrophobic reagent. Derivatization in an aprotic solvent should be preferable in principle. Experiments carried out at room temperature using a single organic phase, toluene (or dichlormethane, benzene, or acetone) containing ASC (0.05 mol/L), phenol (as a model compound, 1×10^{-3} mol/L), and saturated potassium hydroxide (or other organic bases such as triethylamine, pyridine, or 4-dimethylaminopyridine) did not result in the formation of the corresponding phenolic derivatives. However, the addition of a 1.0-mL aqueous solution containing 1 mol/L of sodium hydroxide and 0.05 mol/L quaternary salt (i.e., TBACl to the organic phase), which created a two-phase system, resulted in the very rapid formation of a derivative. Similar results were obtained after the addition of water saturated with approximately 1 mol/L of sodium orthophosphate. However, the addition of pure water or water containing 1 mol/L potassium sulfate had no effect. These results suggest that a strong base must be present in the aqueous phase of the two-phase system for derivatization to occur.

TBAI, TBACl, TBABr, and TBAOH all gave satisfactory results in the present two-phase derivatization system. However, faster kinetics were observed with the more water-soluble TBACl, TBABr, or TBAOH. Because the use of TBAOH resulted in the formation of a larger amount of the side-product of the ASC ether (discussed later), and TBABr produced a derivatization yield lower than that obtained using TBACl, TBACl was selected for further experiments.

Figure 2 shows the influence of TBACl on the ASC derivatization of phenol. NP-HPLC was used for separation and analysis using benzene as the mobile phase at 1 mL/min. Figure 2A indicates that no derivatization occurred when TBACl was not present in the two-phase system. Figure 2B is the chromatograph of the derivatization mixture using alkali aqueous solution containing 0.05 mol/L TBACl. It is obvious that the existence of TBACl is essential for conversion of phenols to their derivatives. Quantitative derivatization of phenols resulted from the using of a 7.5× molar excess of TBACl (vs. total phenols content). Increasing the excess of reagent beyond this level had no significant effect on conversion. With as little as a 6× molar excess of TBACl, derivatization was carried out with a yield of greater than 85% with a turbid interface between the organic and aqueous phase. In order to guarantee the derivatization yield, the concentration of TBACl in the aqueous layer was set 0.05 mol/L.

The results suggest that a "phase-transfer catalyst" took place at the interface of the two phases. A proposed reaction mechanism for the two-phase derivatization of phenolic compounds is shown in Figure 3. The phenolic compounds present in the organic phase were deprotonated at the interface by means of a strong base that was present in the aqueous phase. The analyte anion was transferred into the organic phase as an ion pair with the tetrabutylammonium cation as the counter ion. The "naked" analyte anion reacts with ASC in the organic phase to form the ASC derivatives. A similar mechanism could account for the possible
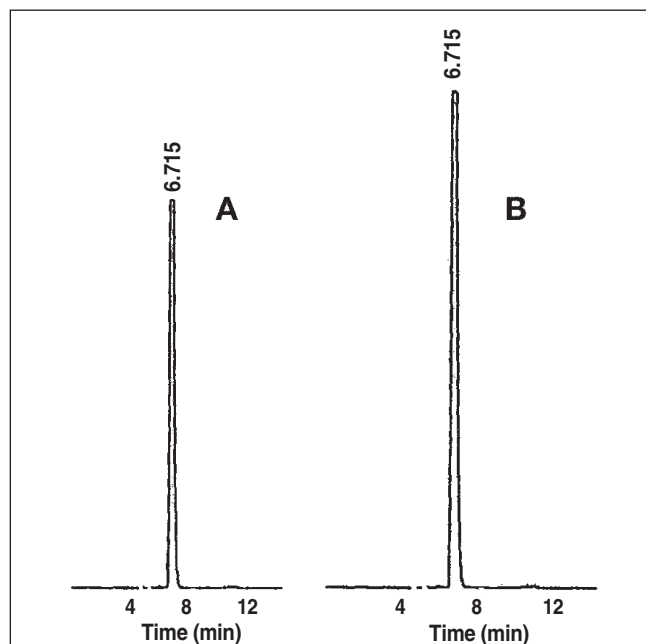
Figure 2. Chromatogram of reaction mixtures of derivatization (NP-HPLC). (A) Reaction without TBACl and (B) with TBACl.
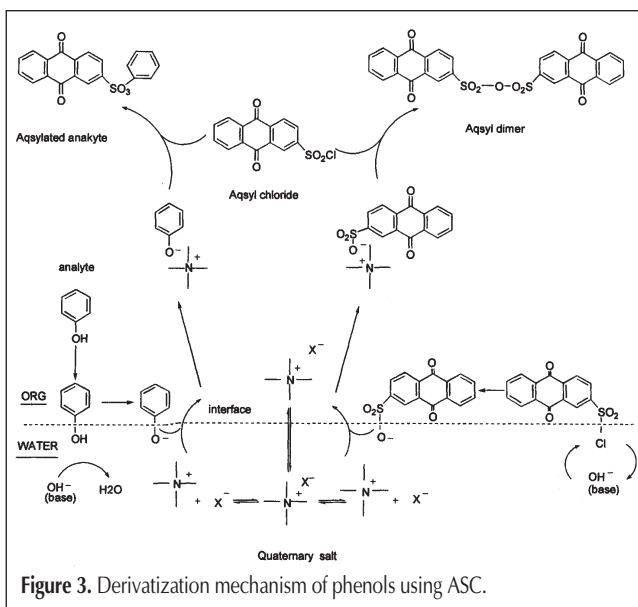
Figure 3. Derivatization mechanism of phenols using ASC.

formation of the ASC dimer, which only appeared when TBAOH was used as the phase transfer catalyst and the concentration of TBACl was high. Compared with the derivatization process, polymerization of ASC to form a dimer was slower under the reaction conditions described previously.

To support the derivatization mechanism, the influence of base concentrations and mixing time of the two phases were studied further. When the concentration of the base was varied from 0.05 to 2.0 mol/L and the mixing time was varied from 0 to 5 min, the derivatization yield was greater than 95%. If the base concentration was relatively low (i.e., 0.05 mol/L in aqueous solution), the mixing time became important to the quantitative conversion. Even though the reaction kinetics were much faster when the surface of the reaction interface was increased by vortex mixing (< 3 min) in a system containing a base with concentration of greater than 1.0 mol/L, a very low yield could still be obtained if the reaction interface was limited. When the two phases were kept together for 30 min without mixing, only a 58% yield could be realized, even if 2.0 mol/L of sodium hydroxide was present in the aqueous solution.

Solvent effect on derivatization

Benzene, dichloromethane, toluene, and other organic solvents were tested for their compatibility as reaction solvents for the derivatization procedures. The result indicated that toluene was the solvent of choice because it yielded complete derivatization.

Influence of ASC concentration on derivatization

Derivatization yields of phenols (1×10^{-3} mol/L, respectively)

were examined using different ASC concentrations from 1×10^{-2} mol/L to 4×10^{-2} mol/L (final solution concentrations in the organic layer). All reactions were performed in a two-phase system containing 1.0 mL aqueous solution and 1.0 mL organic solution. The concentrations of TBACl, sodium hydroxide in aqueous solution, were 0.05 and 1.0 mol/L, respectively. Yields, as a percentage of the peak area compared with that of standard derivatives recorded from the same chromatograph, demonstrated that there was little effect of ASC concentrations in the total organic phase in the range of 2×10^{-2} to 4×10^{-2} mol/L. Therefore, 0.05 mol/L ASC in 0.5 mL toluene was finally selected. It was also observed that if the reagent was insufficient to obtain maximum yields, the addition of more reagent could reproducibly increase the yield to a maximum.

Elution solvent for SPE

Acetone, methanol, and acetonitrile were chosen from the different solvents usually recommended for the desorption of phenols from SPE C_{18} adsorbents (4–6). Experiments showed that all of the three solvents were suitable for this purpose. Because it easily evaporates at room temperature, acetone was selected as the SPE eluent. To get a maximum enrichment factor, the elution curve (%recovery vs. volume of acetone) for each analyte (1×10^{-7} mol/L) was established. From these curves, the

Table I. Calibration Plot Equations and LODs for Four Derivatives

Compounds	Calibration plot*	R‡	LOD† ($\times 10^{-7}$ mol/L) Without SPE	After SPE
APH	A = 703.7 + 4937C	0.9999	1.03	0.01
ACP	A = 1904 + 4691C	0.9998	1.11	0.011
ABP	A = 1307 + 4879C	0.9997	0.97	0.01
AMP	A = 853.2 + 4753C	0.9999	0.98	0.01

* Peak area (y, relative units) versus standard concentrations (x, mg/L).
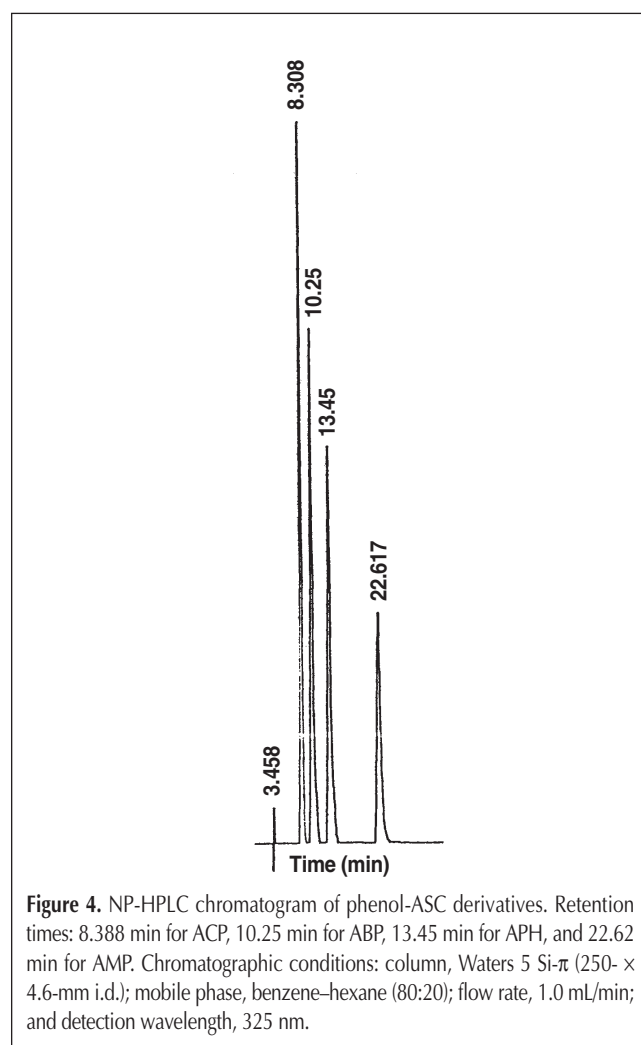† LOD: concentration corresponds to an area of s/n ~ 3.
‡ Correlation coefficients.

Table II. Variability of the Present Method

Derivative	Retention time ($n = 5$) Within day (%RSD)	Between day (%RSD)	Peak area ($n = 5$) Within day (%RSD)	Between day (%RSD)
APH	0.2	0.3	0.6	1.2
ACP	0.3	0.4	1.0	1.5
ABP	0.1	0.3	1.2	1.4
AMP	0.1	0.3	1.1	1.5

* %RSD, percent relative standard deviation.

Figure 4. NP-HPLC chromatogram of phenol-ASC derivatives. Retention times: 8.388 min for ACP, 10.25 min for ABP, 13.45 min for APH, and 22.62 min for AMP. Chromatographic conditions: column, Waters 5 Si-π (250- × 4.6-mm i.d.); mobile phase, benzene–hexane (80:20); flow rate, 1.0 mL/min; and detection wavelength, 325 nm.

minimum solvent volume needed to remove the absorbed analytes quantitatively was found to be 1.5 mL. A quantity of 2.0 mL of methanol was selected in the end to achieve the complete desorption of the analytes.

Figures of merit

Figures of merit of the present method were assessed by the following criteria: linearity, precision, stability, LOD, and limit of quantitation (LOQ).

The assays exhibited linearity between the response (Ai/As) and the corresponding concentration of derivatives (Ci) over the

Figure 5. RP-HPLC chromatogram of phenol-ASC derivatives. Retention times 6.8 min for APH, 9.083 min for AMP, 11.258 min for ACP, and 14.192 min for ABP. Chromatographic conditions: C_8 column (5 μm, 250- × 4.6-mm i.d.); mobile phase, methanol–aqueous solution (containing 0.01 mol/L NaClO$_4$) 80:20; flow rate, 0.7 mL/min; detection wavelength, 256 nm.

range of 2×10^{-7} to 2×10^{-3} mol/L using UV detection. The results are presented in Table I. In all instances, the linearity of the calibration was good ($n = 5$, $r > 0.9997$). In order to express the sensitivity into concentration units, in the chromatogram, an area that corresponds to a signal-to-noise ratio (s/n) of approximately 3 was identified and an LOD of approximately 1×10^{-7} mol/L could be achieved (Table I). To obtain LODs as low as 1×10^{-9} mol/L, the preconcentration of 100-mL water sample on a Waters Sep-Pak plus C_{18} precolumn was needed.

The LOQs were evaluated by the calibration plots to be the concentration that could easily be quantitated and have an s/n equal to 10. An LOQ of 2×10^{-7} mol/L was achieved under the nominal experimental settings (see the Experimental section). After the preconcentration of a 100-mL water sample on a Waters Sep-Pak plus C_{18} precolumn, an LOQ as low as 2×10^{-9} mol/L was obtained.

Reproducibility was studied both for peak area and retention time. The repeatability of the method was represented by within-day precision and reproducibility by between-day precision at a concentration of 2×10^{-4} mol/L (for each phenol). In Table II, the relative standard deviation values were in the range of 0.1–1.5%.

The stability of derivatives in the organic phase was investigated at a concentration of 2×10^{-4} mol/L (for each phenol). After derivatization, the mixtures of the reaction were stored under ambient temperature and in daylight over 24 h, and the organic phases were analyzed. There was virtually no change in the chromatograms and responses of the peaks. Therefore, the derivatives were stable enough to be analyzed.
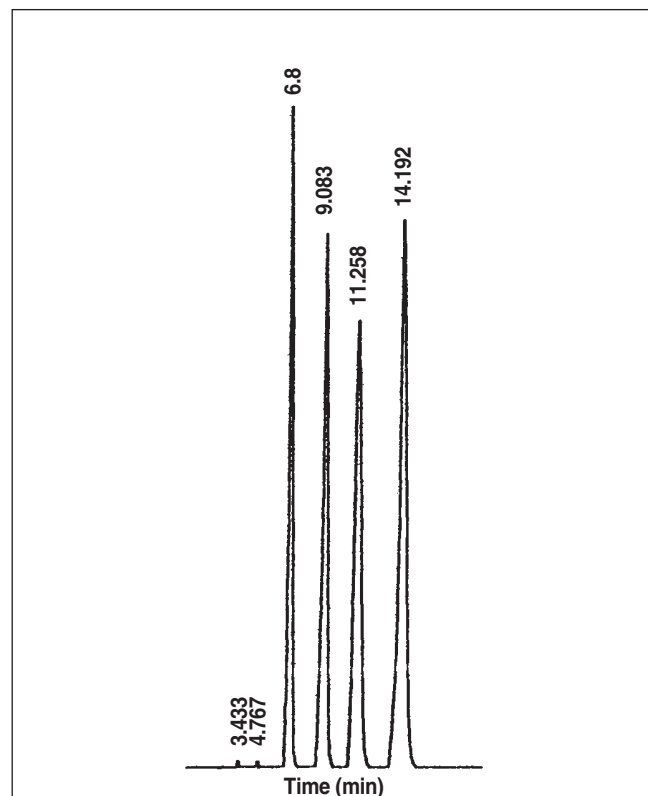
Tap and river water sample

Tap and river water were spiked with phenols at three concentration levels ranging from 2×10^{-9} to 2×10^{-3} mol/L. The samples were acidified, preconcentrated, precolumn derivatizatied, and analyzed by HPLC. Figures 4 and 5 show the chromatograms of a tap water sample (spiked with phenol; p-tert-butylphenol; 2,4-dichlorophenol; and m-methoxyphenol at the 2- × 10^{-6}-mol/L level, respectively).

The recoveries obtained from river water are given in Table III. Good recoveries for all compounds were obtained. The lower value of APH may have been caused by the larger polarity of phenol, which resulted in a low recovery of phenol when it was concentrated from large amount of water at low concentrations by SPE (4).

Table III. Mean Recoveries (R*) of Four Phenols Spiked in River Water

| Derivative | Concentrations (mol/L) | | | | | |
| | 2×10^{-9} | | 2×10^{-7} | | 2×10^{-5} | |
	%R	%RSD	%R	%RSD	%R	%RSD
APH	87.1	2.5	95.6	2.1	94.7	1.5
ACP	93.3	1.7	97.3	1.5	101.1	1.1
ABP	92.6	1.5	95.4	1.3	96.8	1.4
AMP	91.5	1.2	94.5	1.6	95.4	1.3

* $n = 3$.

Conclusion

A new derivatization reagent (ASC) was used to mark phenols for HPLC analysis. Combining the derivatization step with preconcentration, phenols (of concentrations as low as 1×10^{-9} mol/L) could be detected. It should be emphasized that the system described previously shows a nearly universal UV response for all the phenols of the molar concentrations used. The applicability of the present system for the trace analysis of other phenolic compounds (e.g., drugs and their metabolites) will be investigated in the future.

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