Evaluation of Anthraquinone-2-Sulfonyl Chloride for Determination of Phenol in Water by Liquid Chromatography Using Pre-Column Phase-Transfer Catalysed Derivatization

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Abstract A new regent anthraquinone-2-sulfonyl chloride ASC has been used for the derivatization of phenols. Several compounds with different polarity were selected to evaluate the new regent and derivatives of these phenols 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 to an 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 and reversed-phase high performance liquid chromatography HPLC. Excellent linearity response was demonstrated over the concentration range of 0.2 μmol L−1 to 200 μmol L−1 at 320 nm for normal-phase HPLC at 256 nm for reversed-phase HPLC. Combined with preconcentration using a Waters Sep-Pak Plus C18 cartridge detection limits of phenols for water sample analysis were as low as 1 × 10−9 mol L−1 or 0.1 μg L−1.

Key words anthraquinone-2-sulfonyl chloride derivatization solid phase extraction phenols water

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Phenolic compounds are present in the environment as a result of their industrial application. Because of their toxicity and unpleasant organoleptic properties phenols have been included in the list of priority pollutants of many countries. The European Union EU has classified several phenols as priority contaminants and the 80/778/EC directive states that a maximum allowable concentration for total phenols in drinking water is 0.5 μg L−1 while individual phenol concentration should be under 0.1 μg L−1.

The determination of phenols has been described by different chromatographic methods high performance liquid chromatography HPLC being the one preferred because of its robustness. Direct analysis of trace phenols in their biological or environmental matrix by a single chromatographic technique is usually difficult as the concentrations are very low and phenols lack in general the necessary attributes for the sensitive determination. HPLC in combination with fluorescence detection or electrochemical detection
after solid phase extraction were reported as effective methods for water analysis. However, it is difficult to achieve high breakthrough volumes when using solid phase extraction SPE cartridges and disks consistent with the limit of detection LOD at the legislated level for phenols. Kwakman et al. used dansyl chloride to derivatize phenols after preconcentration of the phenols with C18 cartridge to get high detection sensitivity but the procedure needed to remove the excess of derivatizing reagent with an amino SPE column before HPLC analysis. In addition, dansyl chloride and its derivatives are usually unstable to light requiring precautions during the analytical procedure.

Our studies have focused on developing a new reagent for derivatization of phenols and other compounds according to the following criteria: rapid quantitative reaction under mild conditions; specificity for the target compounds without side reactions; separation of the excess reagent from the reaction mixture; and high detection sensitivity for the reaction products due to high extinction coefficients to visible or UV light or fluorescence or by electrochemical activity. High stability of the derivatizing reagent 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. In this paper, use of the reagent was investigated using several phenols with low to relatively high polarity as model analytes. After enrichment of the trace phenols in water samples with Waters Sep-Pak Plus C18 cartridges phenols are converted quantitatively into ASC derivatives under mild conditions within 3 min. These derivatives are readily amenable to be analyzed by normal-phase NP and reversed-phase RP HPLC with UV detector and are detectable at nano-molar concentrations. In order to evaluate the new derivatizing reagent, standard phenol derivatives were prepared and water samples were analyzed.

1 Experimental

1.1 Reagents

Tetra-tert-butylammonium chloride TBACl 96% purity tetrabutylammonium iodide TBAI 98% purity tetrabutylammonium bromide TBABr tetra-tert-butylammonium hydroxide TBAOH phenol p-

tert-butylphenol 2 4-dichlorophenol 3-methoxyphenol were acquired from Nacalai Tesque Inc Kyoto Japan. Chlorosulphonic acid Chameleon reagent 96% purity was purchased from Canxita Inc Japan. Dichloromethane benzene toluene methanol acetonitrile acetone n-hexane calcium chloride sodium sulfate were all analytical reagent grade and used as received. ASC was made in our laboratory according to the reported method. HPLC-grade methanol acetonitrile and water were filtered through a 4 μm filter and degassed under vacuum prior to use.

1.2 Apparatus

Mass spectra were recorded at 70 eV on a JMS-SX 102A mass spectrometer Data Company Kyoto Japan. Infrared spectra were obtained with a Nicolet Impact 410 spectrometer Nicolet Madison WI USA nuclear magnetic resonance spectra data were acquired on INVQA-400 Varian Palo Alto CA USA. Ultraviolet spectra were obtained using a Shimadzu UV-Vis recording spectrophotometer UV-240. Elemental analysis was accomplished on a CHN model CORDER MT-1 Japan. Commercially available pre-coated silica thin layer plates Kieselgel 60 F254 were from Merck. Sep-Pak Plus C18 environmental cartridges Part No. wet 023635 were from Waters Palo Alto CA USA. The separation and determination experiments of derivatives were done with a Model 10A high performance liquid chromatograph equipped with a UV-Vis spectrophotometric detector and a model 5J sample injector Shimadzu Kyoto Japan. For NP-HPLC a Waters 5 Si II column 250 mm × 4.6 mm i.d. Cosmisorp Japan was used with a mobile phase consisting of benzene-hexane 80:20 volume ratio at a flow rate of 1.0 mL/min. Detection wavelength for the derivative analysis was 325 nm. The chromatographic analysis of RP-HPLC was achieved on a 5μ C8 column 250 mm × 4.6 mm i.d. Cosmisorp Japan using methanol-aqueous solution containing 0.01 mol/L NaClO4 80:20 volume ratio as mobile phase. Flow rate was 0.7 mL/min and detection wavelength was 256 nm. All the experiments were accomplished at room temperature.

1.3 Preparation of solutions

ASC was prepared and stored in a desiccator at room temperature according to the procedure reported. A solution of 0.05 mol/L ASC was prepared
by dissolving 0.3 mg of ASC in 20 mL of dichloromethane or toluene benzene and kept at room temperature and in the presence of daylight.

Phenol standard stock solutions for optimum of analytical derivatization 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 in acetonitrile weekly and refrigerated when not in use.

Solutions of tetrabutylammonium salts 0.1 mol L and sodium hydroxide 1 mol L were prepared with water. All the solutions above were stable for at least two weeks in daylight at room temperature.

1.4 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 by the following procedure. ASC 1 mmol and phenol 5 mmol were dissolved in 100 mL of benzene. The solution which changed color immediately from yellow to red-orange or deep red while a grain of potassium hydroxide added was stirred for 30 min washed with 20 mL of water for three times separated from aqueous phase and dried over anhydrous Na2SO4. Benzene was removed by rotary evaporation. Dry column chromatography silica gel with benzene as solvent was used to purify the products further and to remove anthraquinone-2-sulfonyl acid which was the primary byproduct. Chromatographic fractions were collected and checked for product purity >97% by silica gel thin-layer chromatography.

Four derivatives phenol-ASC abbr. APH pale yellow solid p-tert-butylphenol-ASC abbr. ABP yellow solid 2,4-dichlorophenol-ASC abbr. ACP orange solid and m-methoxyphenol-ASC abbr. AMP dark red were obtained via the above process. They were characterized by nuclear magnetic resonance NMR mass-spectrometry etc and have the structure as follows.

R is resulted from phenols.

1.5 Trace enrichment step

Before use the C18 cartridge was activated by passing methanol 2 mL through it followed by distilled water 5 mL. The water sample 100 mL or less was transferred into a glass beaker. After acidification to pH 2 – 3 by dropwise addition of sulphuric 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 eluate were collected. The acetone extract was dried at room temperature using a gentle stream of nitrogen and the residue was redissolved in 0.5 mL toluene.

1.6 Pre-column 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 tetrabutylammonium chloride 0.05 mol L B 0.5 mL ASC solution 0.05 mol L in toluene C 0.5 mL phenols solution the concentration range of 0.2 µmol L to 200 µmol 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 into the NP or RP chromatographic column.

1.7 Analysis of water samples

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

2 Results and discussion

2.1 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 dichloromethane benz-
zene or acetone containing ASC 0.05 mo/L phenol as a model compound 1 × 10–3 mo/L and saturated potassium hydroxide or other organic bases such as triethylamine pyridine or 4-dimethylamino pyridine did not result in the formation of the corresponding phenolic derivatives. However on addition of 1.0 mL aqueous solution containing 1 mo/L sodium hydroxide and 0.05 mo/L quaternary salt e.g. tetrabutylammonium chloride to the organic phase thus creating a two-phase system very rapid formation of a derivative was observed. Similar results were obtained after the addition of water saturated with ca. 1 mo/L sodium orthophosphate. On the other hand the addition of pure water or water containing 1 mo/L potassium sulphate 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 TBACI TBABr and TBAOH all gave derivatives in the present two-phase derivatization system. However faster kinetics were observed with the more water-soluble TBACI 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 below and TBABr produced a derivative yield lower than that obtained from using TBACI TBACI was selected for further experiments.

Fig. 1 shows the influence of TBACI on the ASC derivatization of phenol. NP-HPLC was used for separation and analysis with benzene as the mobile phase at a flow rate of 1 mL/min. Fig. 1-a shows that no derivatization occurred if TBACI is not present in the two-phase system. Fig. 1-b is the chromatogram of the derivatization mixture using alkali aqueous solution containing 0.05 mo/L TBACI. It is obvious that the existence of TBACI is essential to the conversion of phenols to their derivatives. Quantitative derivatization of phenols could result from the use of a 7.5-fold molar excess of TBACI vs total phenols content. The increase of the reagent beyond this level had no significant effect on conversions. With as low as a 6-fold molar excess of TBACI derivatization resulted in >85% yield with a turbid interface between the organic and aqueous phase. In order to guarantee the derivatization yield the concentration of TBACI in aqueous layer was set at 0.05 mo/L.

The above results suggest that a “phase transfer catalysis” takes place at the interface of the two phases. A proposed reaction mechanism for the two-phase derivatization of phenolic compounds is shown in Fig. 2. The phenolic compounds present in the organic phase are deprotonated at the interface by means of a strong base present in the aqueous phase. The anolyte anion is transferred into the organic phase as an ion pair with the tetrabutylammonium cation as the counter ion. The “naked” anolyte anion reacts with ASC in the organic phase to form the ASC derivatives. A similar mechanism could account for the possible formation of the ASC dimer which only appears when TBAOH is used as the phase transfer catalyst and the concentration of TBACI is too high. Compared with the derivatization process polymerization of ASC to form dimer is a slower one under the reaction conditions described above.

To support the derivatization mechanism proposed above the influence of base concentrations and mixing time of two phases were studied further. When the concentration of base was varied from 0.05 mo/L to 2.0 mo/L and the mixing time was varied form 0 to 5 min the derivatization yield increased to >95% yield. If the base concentration is relatively low i.e. 0.05 mo/L in aqueous solution then the mixing time become important to the quantitative conversion. Even though the reaction is much faster when the surface of the reaction interface is increased by vortex mixing less than 3 min in a system containing base with concentration more than 1.0 mo/L a very low yield can still be obtained if the reaction interface is 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\(^{-1}\) of sodium hydroxide was present in the aqueous solution.

2.2 Solvent effect on derivatization

Benzene, dichloromethane, toluene and other organic solvents were tested for their compatibility as reaction solvents for the derivatization procedures. As a result, toluene was chosen as the solvent because it gave complete derivatization.

2.3 Influence of ASC concentration on derivatization

Derivatization yields of phenols, 1 \times 10^{-3} \text{ mol L}\(^{-1}\) respectively, were examined using different ASC concentrations from 1 \times 10^{-3} \text{ mol L}\(^{-1}\) to 8 \times 10^{-2} \text{ mol L}\(^{-1}\) in 0.5 mL toluene. All reactions were performed in a two-phase system containing 1.0 mL aqueous solution and 1.0 mL organic solution. The concentrations of tetrabutylammonium chloride sodium hydroxide in aqueous solution were 0.05 mol L\(^{-1}\) and 1.0 mol L\(^{-1}\) respectively. Yields as a percentage of the peak area compared to that of standard derivatives recorded at the same chromatograph demonstrated that there is little effect of ASC concentrations in the organic phase in the range from 0.02 mol L\(^{-1}\) to 0.08 mol L\(^{-1}\) so 0.05 mol L\(^{-1}\) ASC in 0.5 mL toluene was finally selected. It was also observed that if the reagent was insufficient to obtain maximum yields, addition of more reagent could reproducibly increase the yield to a maximum.

2.4 Elution solvents for SPE

Acetone, methanol and acetonitrile were chosen from the different solvents usually recommended for the desorption of phenols from SPE C\(_18\) adsorbent. Experiments showed that all of the three solvents were suitable for the purpose. Since acetone is easy to evaporate at room temperature, it was selected as the SPE eluant. To get a maximum enrichment factor, the elution curve recovery % vs volume of acetone for each analyte 1 \times 10^{-7} \text{ mol L}\(^{-1}\) was established. From these curves, the minimum solvent volume needed to remove the adsorbed analytes quantitatively was found to be 1.5 mL. Two milliliter mL methanol was selected in the end to achieve the complete desorption of analytes.
2.5 Building of conditions for separations

NP-HPLC was used to show the optimum results of phenol’s derivatization as the derivatives originally existed in organic phase. Different ratios of benzene-acetone benzene-acetic acid benzene-acetic ether and benzene-hexane were investigated as mobile phases to optimize the separation of four derivatives on a Waters 5 Si-II column. In the end benzene-hexane system was selected because the system could offer good separation see Fig. 3 and the retention times were suitable for analysis.

RP-HPLC is a commonly used mode of chromatography. So it is necessary to investigate the behavior of the derivatives in such a system. Considering four phenol-ASC derivatives are all anthraquinone-2-sulfonate C octylsilane chemically bonded to totally porous silica particles was selected as stationary phase and the mixtures of methanol-water were investigated as mobile phases. To improve the shape of chromatographic peaks sodium perchlorate with the concentration of 0.01 mol L was added to the elution system. The retention time decreased with increasing the percentage of methanol in mobile phase. When volume ratio of methanol-water containing 0.01 mol L NaClO4 was 80:20 all derivatives separated from each other completely and the time for one analysis was less than 15 min see Fig. 4.

2.6 Figures of merit

Figures of merit of the present method were assessed by the following criteria linearity precision stability LOD and limit of quantification LOQ.

The assays exhibited linearity between the response A A A and the corresponding concentration of derivatives C over the range of 2 × 10⁻⁷ mol L - 2 × 10⁻³ mol L using UV detection. Results are presented in Table 1. In all instances the linearity of the calibration was good n = 5 r > 0.999 7. In order to express the sensitivity into concentration units in the chromatogram an area corresponding to a signal to noise ratio around 3 was identified and an LOD of ca. 1 × 10⁻⁷ mol L could be achieved see Table 1. To obtain detection limits as low as 1 × 10⁻⁹ mol L preconcentration of 100 mL water sample on a Waters Sep-Pak plus C₁₈ pre-column is needed.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Calibration plot equation</th>
<th>( r^2 )</th>
<th>LOD ( \times 10^{−7} ) mol L</th>
<th>LOD ( \times 10^{−7} ) mol L</th>
</tr>
</thead>
<tbody>
<tr>
<td>APH</td>
<td>A = 703.7 + 7 + 4393C</td>
<td>0.9999</td>
<td>1.03</td>
<td>0.010</td>
</tr>
<tr>
<td>ACP</td>
<td>A = 16704 + 619C</td>
<td>0.9998</td>
<td>1.11</td>
<td>0.011</td>
</tr>
<tr>
<td>ABP</td>
<td>A = 1307 + 4879C</td>
<td>0.9997</td>
<td>0.97</td>
<td>0.010</td>
</tr>
<tr>
<td>AMP</td>
<td>A = 853.2 + 4753C</td>
<td>0.9999</td>
<td>0.98</td>
<td>0.010</td>
</tr>
</tbody>
</table>

Table 1 Calibration plot equations and detection limits of four derivatives

LOQ are evaluated by the calibration plots as the concentration that can easily be quantified and are around a signal to noise ratio equal to 10. LOQ of 2 × 10⁻⁷ mol L can be achieved under the nominal experimental settings see Experimental. After preconcentration of 100 mL water sample on a Waters Sep-Pak plus C₁₈ pre-column LOQ as low as 2 × 10⁻⁹ mol L is obtained.

Repeatability and reproducibility were 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 the concentration of 2 × 10⁻⁴ mol L to each phenol.
In Table 2 the relative standard deviations (RSDs) ranged from 0.1% to 1.5%.

<table>
<thead>
<tr>
<th>Derivative</th>
<th>Retention time</th>
<th>Peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>within-day</td>
<td>between-day</td>
</tr>
<tr>
<td>APH</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>ACP</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>ABP</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>AMP</td>
<td>0.1</td>
<td>0.3</td>
</tr>
</tbody>
</table>

The stability of derivatives in organic phase was investigated at concentration of $2 \times 10^{-4}$ mol L$^{-1}$ to each phenol. After derivatization the mixtures of the reaction were kept under ambient temperature and daylight over 24 h and the organic phases were analyzed. Nearly not any change happened to chromatograms and the responses of the peaks. This means that the derivatives are stable enough during the storage.

2.7 Tap and river water samples

Tap and river water were spiked with phenols at three concentration levels ranging from $2 \times 10^{-9}$ mol L$^{-1}$ to $2 \times 10^{-5}$ mol L$^{-1}$. The samples were acidified preconcentrated pre-column derivatized and analyzed by HPLC. Fig. 3 and Fig. 4 show the NP and RP chromatograms of a tap water sample spiked with phenol, $p$-tertbutylphenol, 2,4-dichlorophenol and $m$-methoxyphenol at the $2 \times 10^{-6}$ mol L$^{-1}$ level respectively. The recoveries obtained from river water are given in Table 3. Good recoveries for all compounds were obtained. The lower value of APH may be due to the higher polarity of phenol when phenol is concentrated from large amount of water at low concentrations by SP$^+$ [4].

3 Conclusion

In conclusion a new derivatizing reagent ASC was used to tag phenols for HPLC analysis. In the combining of the derivatization with preconcentration phenols in concentrations down to $1 \times 10^{-9}$ mol L$^{-1}$ could be detected. It should be emphasized that the system described above shows a nearly universal UV response for all the phenols at 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 near future.

<table>
<thead>
<tr>
<th>Derivative</th>
<th>Concentrations of phenols added</th>
<th>RSD %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$2 \times 10^{-9}$</td>
<td>$2 \times 10^{-7}$</td>
</tr>
<tr>
<td>APH</td>
<td>87.1</td>
<td>2.5</td>
</tr>
<tr>
<td>ACP</td>
<td>93.3</td>
<td>1.7</td>
</tr>
<tr>
<td>ABP</td>
<td>92.6</td>
<td>1.5</td>
</tr>
<tr>
<td>AMP</td>
<td>91.5</td>
<td>1.2</td>
</tr>
</tbody>
</table>

The recoveries obtained from tap water are similar to those from river water.

References