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Note

Separation and quantification of trace isomeric hydroxyphenols in aqueous solution by high-performance liquid chromatography

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In order to understand fully the reaction of hydroxyl radical, generated radiolytically, with phenol, it was necessary to develop a rapid and sensitive method for the detection and quantification of the low yields of the products formed. In the presence of radical scavenger, *p*-benzoquinone, under certain radiolytic conditions the products are exclusively *o*-, *p*- and *m*-hydroxyphenols. Separation of the isomeric hydroxyphenols on a column of Merckogel PGM 200 has been reported recently[1]. Wulf and Nagel[2] have used a μBondapak C_{18} column for the analysis of phenolic acids and flavonoids by high-performance liquid chromatography. Due to widespread use of the μBondapak C_{18} columns, we have used this stationary phase for the separation of isomeric hydroxyphenols.

EXPERIMENTAL

The main features of the liquid chromatograph used have been previously described[3,4]. The apparatus contained the following components: Waters Assoc. (Milford, Mass., U.S.A.) Model 6000A pump, a 150-μl six-port injection valve. The chromatographic column used was a μBondapak C_{18} obtained from Waters Assoc. The detector was a Vari-Chrom UV–Vis variable-wavelength detector obtained from Varian Instruments (Palo Alto, Calif., U.S.A.). The photometric range of the detector corresponds to 0.005–2.0 a.u.f.s. In most of the studies, 0.02 a.u.f.s., 100 mV, was used. The flow-rate was 1.4 ml/min at an operating pressure of 1000 p.s.i. The detection was primarily carried out at 280 nm, where phenol has a maximum absorbance and all the dihydroxyphenols also have high absorbance. Detection was also made at 260 nm where *p*-benzoquinone has a higher extinction coefficient than at 280 nm. The absorption spectra were taken using a Cary 219 UV–visible spectrophotometer.

RESULTS AND DISCUSSION

Using water containg 0.01 *M* phosphate buffer at pH 7.0 as the mobile phase, the chromatogram obtained at $\lambda = 280$ nm of a synthetic mixture of isometic hydroxyphenols, phenol and benzoquinone is shown in Fig. 1a. As evident from this figure, the retention times of resorcinol and catechol are the same and the rest of the com-

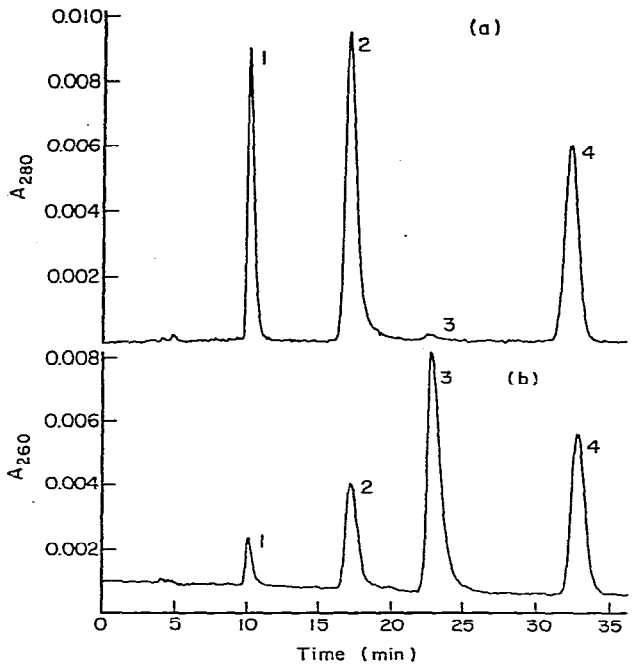

Fig. 1. (a) Separation of isomeric hydroxyphenols from phenol, and benzoquinone on a μBondapak C_{18} column with water containing 0.01 M phosphate buffer at pH 7.0 as the mobile phase. Peaks: 1 = hydroquinone (31 μM), 2 = resorcinol (22 μM) + catechol (37 μM), 3 = benzoquinone (38 μM), 4 = phenol (20 μM). The detection was carried out at 280 nm. (b) Experimental conditions are the same as in (a) except the detection was carried out at 260 nm.

ponents are well separated. Benzoquinone has very low absorbance at this wavelength and, hence, elutes as a small spike. The extinction coefficient of benzoquinone is quite high at 260 nm compared to 280 nm and, hence, in the chromatogram obtained at 260 nm the peak due to benzoquinone is much more pronounced (Fig. 1b). In order to separate resorcinol (*meta* isomer) from catechol (*ortho* isomer), ethanol–water mixtures and acetonitrile–water mixtures as mobile phases were tried and the separation was not achieved, even though all the peaks became sharper compared to the peaks obtained using water as the mobile phase. The separation of resorcinol from catechol was achieved using 4% (v/v) of 1,4-dioxane–water mixture as the mobile phase and the chromatogram obtained at 280 nm is shown in Fig. 2a. The chromatogram obtained at 260 nm is shown in Fig. 2b. The retention times of all the components have become shorter using dioxane–water mixtures as the mobile phase. Moreover, the peaks are much sharper. With increasing concentration of dioxane the retention times become shorter and above 15% dioxane–water mixture, both hydroquinone and benzoquinone eluted very close to the solvent front. It was possible to detect 2 μM of each of the components within $\pm$ 2% accuracy. The peak heights were linear with concentration from 1 μM tp 1 mM. It was possible to detect 10 ppb*

* Throughout this article the American billion (10^9) is meant.

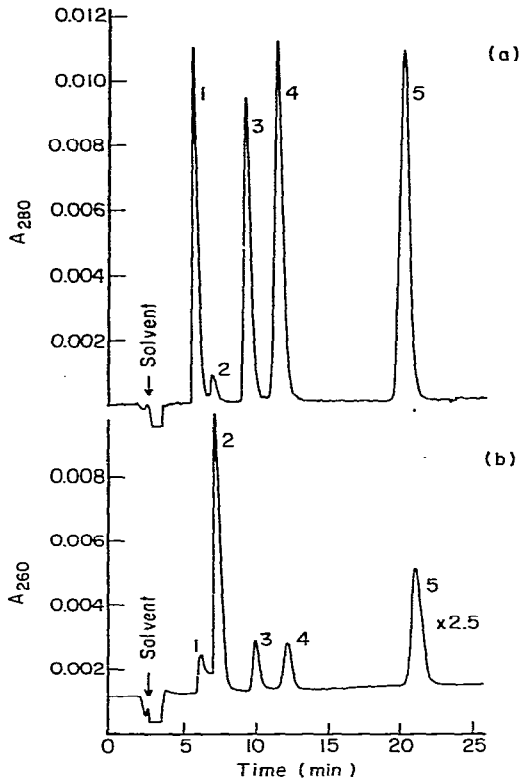

Fig. 2. (a) The mobile phase used was 4% dioxane–water mixture. Peaks: 1 = hydroquinone (31 μM), 2 = benzoquinone (38 μM), 3 = resorcinol (22 μM), 4 = catechol (37 μM), 5 = phenol (20 μM). The detection was carried out at 280 nm. (b) Experimental conditions are the same as in (a) except the detection was carried out at 260 nm.

of phenol in the presence of dihydroxyphenols within $\pm$ 4% accuracy. Similarly, any of the dihydroxyphenols can be detected at a level of 10 ppb within $\pm$ 4% accuracy.

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