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Note

High-pressure liquid chromatographic analysis of isomeric aminophenols with electrochemical detection

LARRY A. STERNSON* and WAYNE J. DeWITTE
Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, Kan. 66045 (U.S.A.)
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In a previous report[1], we described the high-pressure liquid chromatographic (HPLC) separation and quantitation of isomeric hydroxyanilines involving reversed-phase partition chromatography of the mixtures on a C_{18} column with the mobile phase methanol–water (15:85) containing 0.26 *M* ammonium acetate (pH 7) and 0.015 *M* nickel acetate. Spectrophotometric monitoring of the effluent at 254 nm permitted quantitation to *ca.* $5 \cdot 10^{-6}$ *M* in aminophenol. Sensitivity was limited by the molar absorptivity of the aminophenols ($\varepsilon_{para} \approx 10^3$; $\varepsilon_{ortho} \approx 300$) at 254 nm. In this report, the same chromatographic system is used, but advantage is taken of the electrochemical oxidizability of these compounds to provide a more sensitive detection of the aminophenols after HPLC separation, capable of quantitating nanomolar levels.

EXPERIMENTAL

Chromatography was performed on a component system consisting of a Model 6000-A solvent delivery system, Model U-6K septumless injector and Model 440 dual channel absorbance detector operated at 254 nm (Waters Assoc., Milford, Mass., U.S.A.). The HPLC electrochemical cell was purchased from Bioanalytical Systems (West Lafayette, Ind., U.S.A.) and was introduced into the chromatographic system at the eluent collection port of the pump with PTFE tubing. The entire electrochemical detection cell was encased in a Faraday cage. The electrochemical detector ("controller") was laboratory-built and has been described in detail[2–4]. The detector electrode was pre-set at +500 mV *vs.* Ag/AgCl in instances where aniline detection was not required. In systems in which aniline was quantitated, a voltage of +1.0 V was applied. The separation of aniline, *o*-aminophenol and *p*-aminophenol utilized a μBondapak C_{18} column (30 cm × 4 mm O.D.) (Waters Assoc.) operating at ambient temperature at 2.0 ml/min (2000 p.s.i.) with methanol–water (15:85) containing 0.26 *M* ammonium acetate and 0.015 *M* nickel acetate as mobile phase.

RESULTS AND DISCUSSION

o- and *p*-aminophenol undergo facile electrochemical oxidation at stationary

* To whom correspondence should be addressed.

TABLE I

STANDARD CURVES FOR HPLC ANALYSIS OF ANILINE METABOLITES

Each curve represents triplicate analyses of each compound at 6 or 7 concentrations in the range $1 \cdot 10^{-8}$ to $1 \cdot 10^{-4}$ *M*. Analysis performed as described under Experimental.

Compound	V_R* *(ml)*	*UV sensitivity limit (M)*[1]	*Electrochemical sensitivity limit (M)*	*Electrochemical sensitivity / UV sensitivity*	*Linear regression parameters***		
					Slope	*Intercept*	*Correlation coefficient*
p-Aminophenol***	4.2	$1 \cdot 10^{-5}$	$1 \cdot 10^{-8}$	1000	$1.357 \cdot 10^{6}$	0.06	>0.999
o-Aminophenol***	7.8	$5 \cdot 10^{-6}$	$5 \cdot 10^{-8}$	100	$4.31 \cdot 10^{5}$	−0.37	0.999
Aniline§	13.0	$5 \cdot 10^{-6}$	$1 \cdot 10^{-6}$	5	220	0.00	>0.999

* V_R (*p*-methoxyphenol) = 26.0 ml.

** Amount of each compound determined as ratio of peak height of compound relative to internal standard, *p*-methoxyphenol.

*** $E_{applied}$ = +0.50 V (*vs.* Ag/AgCl).

§ $E_{applied}$ = +1.00 V (*vs.* Ag/AgCl).

carbon paste electrodes, in two electron processes yielding the corresponding quinoneimine, which is subject to pH-dependent hydrolysis to give the quinone[5,6]. Amperometric monitoring of column effluent can therefore be used to detect the aminophenols. HPLC column effluent was first passed through a UV detector and then through a thin-layer flow cell containing a carbon paste electrode. The cell was connected to a Ag/AgCl reference electrode and stainless-steel auxiliary electrode. For analysis of aminophenols, the working electrode was polarized to +500 mV. Oxidation of *o*- and *p*-aminophenol occurs at lower potentials ($E_{p/2} \approx +260$ mV) than those at which most other electroactive species respond at an anode, thus reducing interference from potential contaminants. At a potential of +500 mV, aniline did not respond to the detector, even when present in 10^3-fold excess. To monitor aniline concentration, the potential applied between the working and auxiliary electrodes was increased to +1.0 V. By enclosing the detector cell assembly in a Faraday cage, electrical noise is minimized, increasing the signal-to-noise ratio, thus improving sensitivity. Chromatographic behavior of aniline, *o*-aminophenol and *p*-aminophenol with this separation system is presented in Table I. Components were quantitated by measuring peak height relative to 4-methoxyphenol, present as internal standard. A change in applied potential from 500 mV to 1 V causes a dramatic increase in the sensitivity of the detector to 4-methoxyphenol, due to its oxidation potential ($E_{p/2} =$ 660 mV). A linear relationship exists between aminophenol concentrations and peak height ratio in the concentration range of $1 \cdot 10^{-8}$ *M* to $5 \cdot 10^{-5}$ *M* (Table I). This constitutes a 500-fold improvement in sensitivity over previous methods[1,7]. Schwartzfager[8] has similarly demonstrated detection (but not quantitation) of picogram amounts of *p*-aminophenol by electrochemical monitoring of HPLC systems. Analysis gave reproducibility of $\pm 5\%$ or better, where the minimum amount of aminophenol injected was *ca.* 1 pmole. Although N-phenylhydroxylamine was not quantitated by this method, its presence would not interfere with *o*- or *p*-aminophenol, since the chromatographic system resolves the N-hydroxylamine from the phenolic isomers.

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