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Note

Application of amperometric detection to the high-performance liquid chromatographic determination of antipyrine and 4-aminoantipyrine in urine

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Antipyrine and aminopyrine are routinely used as model compounds in the study of hepatic microsomal mixed-function oxidase activity *in vitro* and *in vivo*[1–3] and 4-aminoantipyrine is one of the metabolites of aminopyrine. Antipyrine, aminopyrine and their metabolites can be determined in biological fluids using various chromatographic methods. Thin-layer chromatographic procedures[4,5] have poor sensitivity and yield only semi-quantitative results. Gas chromatography[6,7] and gas chromatography–mass spectrometry[2,8] exhibit sufficient sensitivity and selectivity, but require derivatization. High-performance liquid chromatography (HPLC) with spectrophotometric detection has found wide use in assaying these compounds in plasma, urine and liver microsomes[9–14].

However, biological fluids contain many components that interfere in photometric detection and thus a sample purification step is required prior to the analysis, especially with urine samples. One of the advantages of voltammetric detection is its high selectivity, which often considerably simplifies the sample pre-treatment[15]. This paper describes a simple HPLC method with amperometric detection for determining antipyrine and 4-aminoantipyrine in human urine, which does not require a pre-purification step.

EXPERIMENTAL

Reagents

All the chemicals, including standard antipyrine (AP) and 4-aminoantipyrine (4-AAP), were of analytical-reagent grade from Lachema (Brno, Czechoslovakia) and Merck (Darmstadt, F.R.G.) and were used as received.

Apparatus

The chromatographic apparatus consisted of a Model 2150 HPLC pump, 2151 variable-wavelength UV–VIS detector (both from LKB, Bromma, Sweden), a Rheodyne sampling valve with a 20-μl loop and an EDLC electrochemical detector

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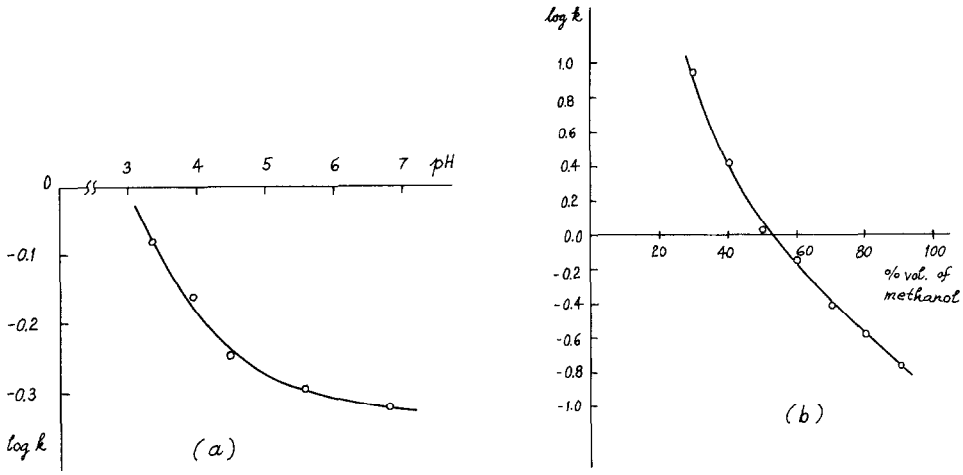

Fig. 1. Depencence of the capacity factor for 4-AAP on (a) mobile phase pH and (b) methanol content. Mobile phase in (a), methanol–0.01 *M* NaH_2PO_4 (75:25); pH adjusted by addition of sodium hydroxide and phosphoric acid solutions; flow-rate, 0.3 ml min^{-1}; UV detection, 254 nm.

(Laboratorní Přístroje, Prague, Czechoslovakia) with a detector cell containing a carbon fibre array working electrode[16], a stainless-steel counter electrode and a silver–silver chloride reference electrode. The photometric and electrochemical detectors were connected in series and their signals were recorded using a TZ 4200 dual-trace chart recorder (Laboratorní Přístroje). The glass analytical column (Separon SGX C_{18}, 7 μm, 150 × 3 mm I.D.) was preceded by a glass pre-column (Separon SGX C_{18}, 7 μm, 50 × 3 mm I.D.), both from Tessek (Prague, Czechoslovakia).

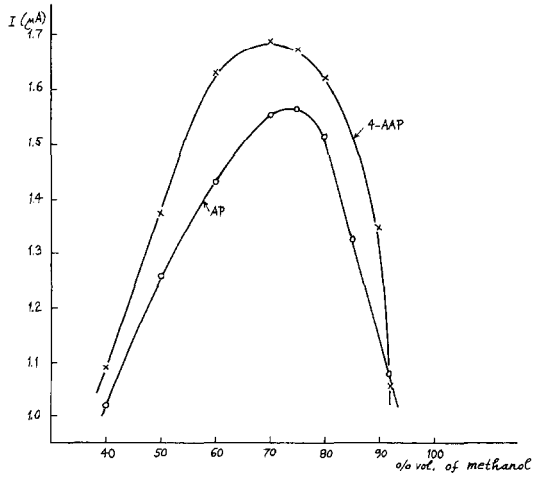

Fig. 2. Dependence of the amperometric signal for 4-AAP and AP on the methanol content in the mobile phase. Mobile phase, methanol–0.01 *M* NaH_2PO_4 (pH 3.0); flow-rate, 0.3 ml min^{-1}. Potentials: 4-AAP, +1.4 V; AP, +1.8 V.

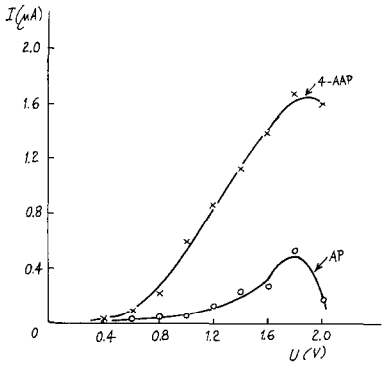

Fig. 3. Hydrodynamic voltammograms of AP and 4-AAP. Mobile phase, methanol–0.01 *M* NaH_2PO_4 (pH 3.0) (75:25); flow-rate, 0.3 ml min^{-1}. The background current was subtracted.

The mobile phase was degassed in an ultrasonic bath and by passage of helium. All the measurements were carried out at laboratory temperature (20 ± 2°C). The electrode potentials are referred to the saturated silver–silver chloride reference electrode.

RESULTS AND DISCUSSION

For separation on the C_{18} chemically bonded reversed phase, aqueous sodium dihydrogenphosphate–methanol was selected as the mobile phase on the basis of our previous experience. The conditions were optimized from the point of view of the capacity factors and the sensitivity of UV photometric and amperometric detection. It was found that the capacity factors decrease with increasing methanol content and increasing pH. The dependences for the two substances are very similar and the curves for 4-AAP are shown in Fig. 1a and b. The sensitivity of detection was maximal at

TABLE I

DETERMINATION OF ANTIPYRINE AND 4-AMINOANTIPYRINE IN URINE

For experimental conditions, see text. R.S.D = Relative standard deviation.

Parameter	*Antipyrine*		*4-Aminoantipyrine*	
	UV (254 nm)	*Amperometric (+1.8 V)*	*UV (254 nm)*	*Amperometric (+1.4 V)*
Correlation coefficient	0.9999	0.9992	0.9998	0.9993
Limit of detection (ng)	0.3	8.6	0.2	5.0
Minimal detectable concentration (mol/l)	$8.0 \cdot 10^{-8}$	$2.3 \cdot 10^{-6}$	$4.9 \cdot 10^{-8}$	$1.2 \cdot 10^{-6}$
Linear dynamic range (ng)	0.3–2500	9–1000	0.2–2500	5.0–1000
R.S.D. (%):				
1.3 ng	10.6	–	5.2	–
6.0 ng	2.7	–	2.6	15.0
260 ng	1.2	1.9	1.0	1.5

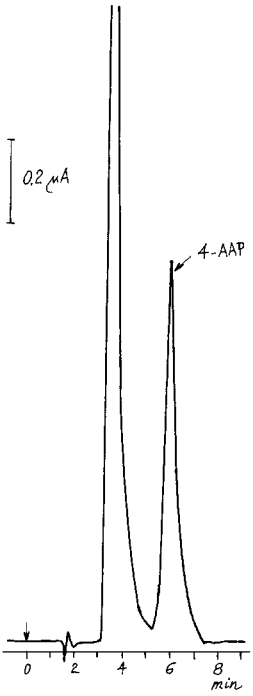

Fig. 4. Chromatogram of a urine sample containing 167 μg of 4-AAP in 1 ml. Experimental conditions as in Fig. 3; amperometric detection at +1.4 V.

a methanol content of *ca.* 70–75% (v/v) (Fig. 2). The optimal mobile phase thus consisted of methanol–aqueous 0.01 *M* NaH_2PO_4 (pH 3.0) (75:25, v/v).

The hydrodynamic voltammograms of AP and 4-AAP obtained in the detection cell under flow conditions and corrected for the background current are given in Fig. 3. The highest signal-to-noise ratio was obtained at potentials of +1.4 and +1.8 V for 4-AAP and AP, respctively. The measurement of 4-aminoantipyrine is more sensitive owing to the presence of the amino group, which is readily oxidized electrochemically. The parameters of the calibration graphs and the limits of detection for photometric detection at 254 nm and amperometric detection at +1.4 V for 4-AAP and +1.8 V for AP are given in Table I.

It can be seen that photometric detection is more sensitive than amperometric detection. However, photometric detection requires pre-purification of urine samples, usually by extraction. On the other hand, urine can be analysed directly with amperometric detection, employing only a short pre-column. The chromatogram of a urine sample containing 4-AAP is shown in Fig. 4.

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