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Note

Determination of amoxicillin by high-performance liquid chromatography with amperometric detection

M. A. BROOKS*, M. R. HACKMAN and D. J. MAZZO
Department of Pharmacokinetics and Biopharmaceutics, Hoffmann-La Roche Inc., Nutley, NJ (U.S.A.)
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Assays for the determination of amoxicillin (α-amino-*p*-hydroxybenzyl penicillin) (Fig. 1) have been described using iodometric titration[1], microbiological method[2], fluorescence[3–6] and polarography[7,8]. Several high-performance liquid chromatographic (HPLC) assays have also been reported using UV detection at 225 nm[9,10], following post-column derivatization to the mercuric mercaptide derivative of the penicillenic acid with UV detection at 310 nm[11] and to the fluorescamine derivative with fluorometric detection at 490 nm following excitation at 385 nm[12].

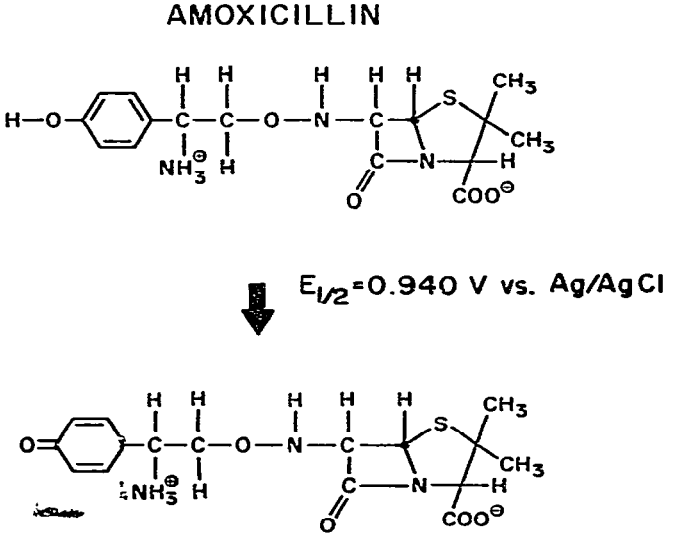

Fig. 1. Postulated electro-oxidation mechanism of amoxicillin.

In the present study, HPLC analysis was used with a UV detector combined with oxidative amperometric (OA) detection using a glassy carbon electrode for the specific determination of amoxicillin and *p*-aminosalicylic acid (the internal standard) in aqueous solution and in the analysis of clinical dosage forms.

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EXPERIMENTAL

Column

The column used was a 30 cm × 3.9 mm I.D. stainless-steel pre-packed reversed-phase column containing 10-μm μBondapak C_{18} (Waters Assoc., Milford, MA, U.S.A.).

Instrumentation

The HPLC system consisted of a Model 6000A pumping system, a Model U6K loop injector and a Model 440 UV absorbance detector operated at 280 nm at an attenuation of 0.01 absorbance units full scale (a.u.f.s.) (Waters Assoc.). A glassy carbon liquid chromatographic detector (Model TL-3) controlled by a potentiostat (Model LC-2A) (Bioanalytical Systems, West Lafayette, IN, U.S.A.) was operated in series with the UV detector. The electrochemical detector was operated in the direct current mode at +1.175 V *vs.* Ag/AgCl at attenuation ranges of 10–500 nA/V. Chromatographic recordings were made on a Model 626 dual-pen strip chart recorder with variable imputs (Leeds & Northrup, North Wales, PA, U.S.A.). The chart speed was 30 in./h using voltage ranges of 10 mV for the UV detector and 1 V for the amperometric detector (effective current ranges of 10–500 nA full scale). Voltammetric scans were performed using a polarograph (PARC Model 174, EG&G Princeton Applied Research Corp., Princeton, NJ, U.S.A.) with a carbon paste (CPE, Bioanalytical Systems) working electrode *versus* a saturated calomel reference electrode (SCE) with a platinum wire auxiliary electrode. A Model 2200-3-3 Omnigraphic x–y recorder (Houston Instruments, Bellaire, TX, U.S.A.) was used with the x-axis at 100 mV/in. and the y-axis at 1 V/in.

Chromatographic conditions

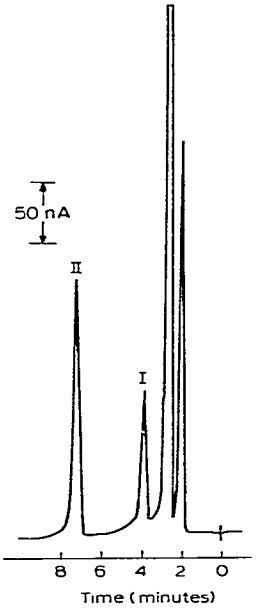
The isocratic mobile phase of water–methanol–acetic acid (74:25:1) at a flow-rate of 1.0 ml/min generates a column head pressure of 750 p.s.i. (5.2 MPa). Under these conditions the retention times of amoxicillin and *p*-aminosalicylic acid (the internal standard) were 3.8 (capacity factor, $k' = 1.0$) and 7.6 ($k' = 2.1$) min, respectively. Under the above assay conditions, 150 ng of amoxicillin and 50 ng of *p*-aminosalicylic acid gave peaks which were 20% and 40% of full scale (500 nA) by OA detection (Fig. 2).

Standard solutions

Stock solution of 1 mg/ml of amoxicillin, 6-[(*R*)-2-amino-2-*p*-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate ($C_{16}H_{19}O_5N_3S \cdot 3H_2O$, mol.wt. 419.45, corrected for 84.9% potency) and *p*-aminosalicyclic acid ($C_7H_7NO_3$, mol.wt. 153.13, m.p. 150–151°C) were prepared in distilled deionized water. Dilutions of these solutions for HPLC analysis were also prepared in distilled deionized water.

Reagents

All reagents were of analytical reagent grade purity. The methanol and acetic acid were purchased from Burdick & Jackson (Muskegon, MI, U.S.A.) and Baker (Phillipsburg, NJ, U.S.A.), respectively.



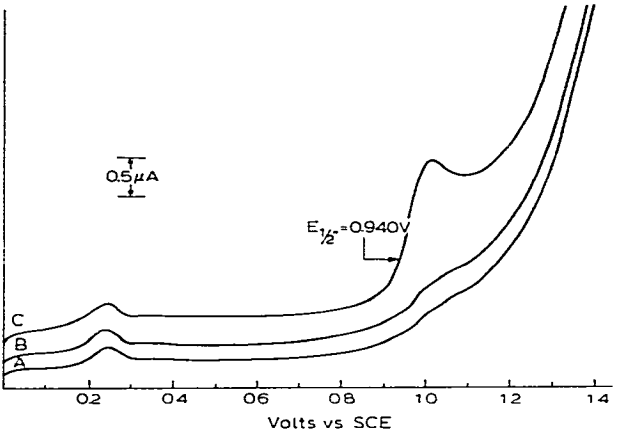


Fig. 2. HPLC chromatogram–OA detection (E applied = +1.175 V *vs.* Ag/AgCl) of 150 ng of amoxicillin (I) and 50 ng of *p*-aminosalicylic acid (II).

Fig. 3. d.c. Voltammograms using the carbon paste electrode in water–methanol–acetic acid (74:25:1) supporting electrolyte: (A) supporting electrolyte, (B) $5 \cdot 10^{-4}$ *M* ampicillin, and (C) $5 \cdot 10^{-4}$ *M* amoxicillin (scan rate = 10 mV/sec; display direction = negative).

RESULTS AND DISCUSSION

Penicillins demonstrate no inherent polarographic activity, and as such must be converted to electro-reducible derivatives prior to analysis[7,8,13]. The penicillins have been assayed polarographically as their penicilloic acid derivatives following basic or enzymatic hydrolysis[7], as 2-hydroxy-3-phenyl-methylpyrazine following acid hydrolysis in the presence of 0.1 % formaldehyde[8,13,14] or as nitroso-derivatives following reaction with $NaNO_2$ in an alkaline media[7]. The electro-oxidative behavior of penicillins has not been previously reported.

Amoxicillin shows one direct current (d.c.) oxidative wave at the carbon paste electrode at $E_{1/2}$ = +0.940 *vs.* SCE (Fig. 3) in a supporting electrolyte of water–methanol–acetic acid (74:25:1). The voltammetric activity is believed to be due to the electro-oxidation of the *p*-hydroxy substituent of amoxicillin to the quinone (Fig. 1) which has been reported for a number of substituted phenols[15,16]. Confirmation of this assignment is based upon the absence of voltammetric activity in ampicillin which is structurally identical to amoxicillin except for the absence of the *p*-hydroxy substituent (Fig. 3).

The combination of HPLC with the oxidative amperometric measurement yields a highly sensitive and specific assay for amoxicillin in the presence of its penicilloic acid ($k' \approx 0.5$) degradation product. High linearity (r = 0.9993) over a concentration range of 42 to 208 ng of amoxicillin injected with an average deviation of 2.5 % (Table I) was demonstrated using linear regression analysis.

TABLE I

CALIBRATION DATA FOR THE ANALYSIS OF AMOXICILLIN

Amount assayed (ng)	*Peak height (cm)*	*Amount found* (ng)*	*Deviation (%)*
208	20.3	206	0.8
180	17.9	180	0.1
152	15.1	151	0.8
124	12.9	127	2.1
97.0	10.2	98.7	1.8
69.3	7.6	71.2	2.8
41.6	4.4	37.8	9.0
		Average	2.5

* Based on the linear regression equation $y = 0.0944x + 0.828$; $r = 0.9993$.

The high degree of correlation between UV detection (280 nm) and OA detection (d.c. at +1.175 V *vs.* Ag/AgCl) in the HPLC analysis of amoxicillin was demonstrated by the analysis of content uniformity of 250-mg capsules of drug (Table II). Equal amounts of drug were determined with the two HPLC detectors placed in series. Coefficients of variation of approximately 3.5% were measured for each detector. In addition, the data are in excellent agreement with the certificate of analysis value of 253.9 mg/capsule reported by iodometric titration. For routine content uniformity analysis, UV detection is preferred because of its long-term stability compared to the OA detector which is subject to contamination.

The utility of HPLC with OA detection for the determination of therapeutic plasma concentrations of drugs is well documented[17–24]. Isolation of the drug from

TABLE II

ANALYSIS OF AMOXICILLIN IN CAPSULES

Larotid (amoxicillin trihydrate) capsules were used. Analyzed by the certified iodometric titration, this batch assayed at 253.9 mg of amoxicillin (anhydrous) per capsule.

Capsule No.	*Amount found (mg)*	
	OA	*UV*
1	250.7	245.0
2	262.4	251.2
3	257.4	252.3
4	262.4	274.0
5	245.6	251.3
6	243.9	251.3
7	245.6	250.2
8	259.1	256.4
9	235.5	240.9
10	249.0	255.4
Mean ± S.D. (C.V.)	251.2 ± 8.9 (3.6%)	252.8 ± 8.7 (3.4%)

the biological matrix prior to HPLC analysis with OA detection has been accomplished using alumina adsorption for L-dopa and dopamine[17] and methyldopa[18], membrane filtration for methyldopa[19], protein precipitation for theophylline[20] and 5-methyltetrahydrofolic acid[21] and selective extraction into an organic solvent for β-cetotetrine[22], acetaminophen[23] and mepindolol[24]. Preliminary studies for the determination of therapeutic concentrations of amoxicillin (0.5 to 10 μg/ml) in plasma have met with little success due to its high water solubility which precludes its quantitative extraction into organic solvents and to difficulties in preparing a sufficiently "clean" protein free filtrate for direct HPLC–OA analysis. In addition, urine samples cannot be analyzed due to the high concentrations of endogenous phenolic compounds in the samples.

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