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Note

Quantitative high-performance liquid chromatographic determination of ampicillin embonate and amoxycillin embonate

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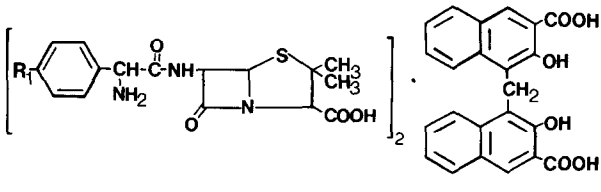
In order to improve the taste and the stability of ampicillin and amoxycillin, nearly water-insoluble embonic acid salts of these semi-synthetic penicillins (1:2) were prepared (Fig. 1)[1]. Owing to the amphoteric character of amoxycillin and ampicillin, it is not possible to extract the components of the salts quantitatively for separate chemical analysis. Moreover, the solubilities of embonic acid and its antibiotic salts are very low in all common solvents.

The quantitation of some sparingly soluble salts formed between organic bases and penicillin G and V has been achieved by assaying the anionic and cationic portions of the salts separately following isolation, and also simultaneously using high-performance liquid chromatography (HPLC)[2–5]. Embonic acid salts of hydroxyzine and noscapine have been quantitated using silica and cyano phase HPLC columns, respectively[6,7]. The retention times of embonic acid on these columns are approximately 13 and 4 min, respectively.

This paper describes a rapid and simple reversed-phase HPLC method for the determination of ampicillin embonate and amoxycillin embonate. The method permits the simultaneous quantitation of the components of amoxycillin embonate. In contrast, the analysis of components of ampicillin embonate require separate assays. By using internal standard methods a good linear range of detection at 240 nm was obtained for all the substances investigated.



	R_1
AMPICILLIN EMBONATE	H
AMOXYCILLIN EMBONATE	OH

Fig 1. Structure of ampicillin embonate (2:1) and amoxycillin embonate (2:1).

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EXPERIMENTAL

Chemicals

Amoxycillin trihydrate (Amphar, Amsterdam, The Netherlands), ampicillin trihydrate (Fermion, Helsinki, Finland) and phenoxymethylpenicillin potassium (Biochemie, Kundel-Tirol, Austria) were kindly supplied by Orion Pharmaceutica. Embonic acid (99%) was obtained from Ega-Chemie (Steinheim, F.R.G.). All other chemicals were of commercial analytical-reagent grade. The identification of the compounds and the preparation methods for ampicillin embonate (2:1) and amoxycillin embonate (2:1) have been described elsewhere[1]. The water contents of ampicillin embonate and amoxycillin embonate were 8.15 and 8.80%, respectively.

Chromatographic conditions

A Hewlett-Packard (HP) 1090 liquid chromatograph, equipped with an HP 85 B computer, a Pye Unicam 4020 UV detector, an HP 3380 A computing integrator and a 5-μl injection loop was used during the study. A reversed-phase column (RP-8, 200 mm $\times$ 4.6 mm I.D., particle size 10 μm, HP 79915 MO) was used at 40°C with a flow-rate of 1.5 ml/min. Detection was carried out at 240 nm. The mobile phase consisted of 1/15 *M* Sörensen phosphate buffer (pH 7.0)[8]–methanol (62:38).

Preparation of calibration graphs

Stock solutions of ampicillin trihydrate (1 mg/ml), embonic acid (30 μg/ml) and amoxycillin trihydrate (600 μg/ml) used as an internal standard were prepared in 1/15 *M* Sörensen phosphate buffer (pH 7.0)–methanol (3:7, v/v) (solvent I) in an ultrasonic bath at room temperature. Dilutions were made using solvent I: the concentration ranges were 50–500 μg/ml for ampicillin trihydrate and 1.5–21 μg/ml for embonic acid. The concentration of the internal standard was 60 μg/ml.

Stock solutions of amoxycillin trihydrate (200 μg/ml), embonic acid (30 μg/ml) and penicillin V potassium (10 mg/ml) used as an internal standard were prepared in solvent I in an ultrasoni bath at room temperature. Dilutions were made using solvent I; the concentration ranges were 10–110 μg/ml for amoxycillin trihydrate and 1.5–21 μg/ml for embonic acid. The concentration of the internal standard was 1 mg/ml.

Sample preparation

The samples of ampicillin embonate and amoxycillin embonate were prepared using solvent I. Dissolution was performed rapidly (about 5 min) in an ultrasonic bath at room temperature. The ampicillin content of the embonate salt was assayed in solutions containing accurately measured amounts of ampicillin embonate (about 350 μg/ml) and 60 μg/ml of amoxycillin trihydrate as an internal standard. The embonic acid content of ampicillin embonate was determined using solutions containing accurately measured amount of ampicillin embonate (about 23 μg/ml) and 60 μg/ml of amoxycillin trihydrate as an internal standard.

The amoxycillin and embonic acid contents of amoxycillin embonate were determined using solutions containing accurately measured amount of amoxycillin embonate (about 42 μg/ml) and 1 mg/ml of penicillin V potassium as an internal standard.

RESULTS AND DISCUSSION

The most recent HPLC methods for β-lactam antibotic anlysis have employed reversed-phase or ion-pair reversed-phase techniques[9,10]. Penicillins containing a side-chain amino group have mostly been analysed with or without a pairing ion using methanol–phosphate buffer mixtures or gradients, or acetonitrile–phospate buffer mixtures over the pH range 4.5–7.5.

Embonic acid and the prepared ampicillin embonate and amoxycillin embonate were poorly soluble in mixtures of acetonitrile and phosphate buffers. The embonates dissolved easily in the mixtures of methanol and 1/15 *M* Sörensen phosphate buffer (pH 7.0) (at pH <7.0 the solubilities decreased rapidly). The latter mixtures gave baseline resolution for the penicillins and embonic acid on an RP-8 column within a few minutes (Fig. 2). Increasing the methanol content in the mobile phase accelerated the passage of embonic acid through the column. Ampicillin and amoxycillin had very short retention times; the methanol content of the eluent had very little influence on the retenton times of the penicillins.

The lack of intrinsic absorption is the major problem in the HPLC of penicillins[10]. Further, the absorption of embonic acid at all UV wavelengths is much higher than that of ampicillin and amoxycillin. At 240 nm the molar absorptivity ratios of embonic acid and amoxycillin trihydrate and embonic acid and ampicillin

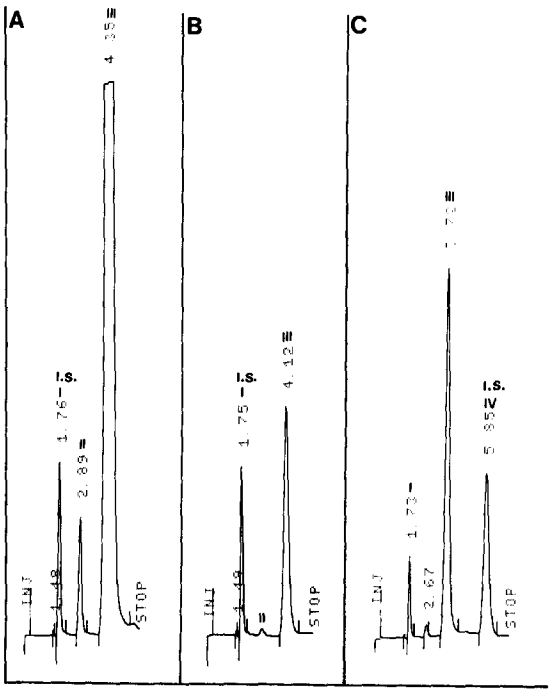

Fig. 2. HPLC traces of ampicillin embonate (A, 350 μg/ml; B, 23 μg/ml) and amoxycillin embonate (C, 42 μg/ml). Peaks: I = amoxycillin; II = ampicillin; III = embonic acid; IV = penicillin V (I.S. = internal standard). For chromatographic conditions see Experimental. Time scale in minutes.

trihydrate were about 10 and 45, respectively. However, UV detection gave a sensitivity that was adequate for the determination of the contents of the salt components and for evaluating the salt syntheses. The analysis of the components of ampicillin embonate required separate assays owing to the tailing peaks of embonic acid and the low detector response to ampicillin (Fig. 2A and B).

The reference standards and salts were dissolved rapidly in solvent I in order to avoid degradation of the penicillins. It has been concluded that solvent I reduces the degradation of penicillin G and V salts[3]. No degradation was detected when ampicillin trihydrate, amoxycillin trihydrate or embonate salts were dissolved in solvent I in thin-layer chromatographic experiments[11]. The internal standard method was used owing to its better linearity compared with the external standard method.

Linearity of the HPLC metod for the salt components

A linear response was obtained for both components of embonates; the equations of the calibration graph for six solutions were $y = 0.0065x + 0.0225$ ($r = 0.998$) for amoxycillin trihydrate, $y = 0.0048x - 0.0514$ ($r = 0.999$) for ampicillin trihydrate and $y = 0.1336x - 0.0441$ ($r = 0.999$) (penicillin V potassium as internal standard) and $y = 0.3543x - 0.1436$ ($r = 0.998$) (amoxycillin trihydrate as internal standard) for embonic acid, where x is the concentration of salt component injected (μg/ml) and y the corresponding peak-area ratio. The precision at the lowest and highest levels were, on average, 1.8 and 1.4%, respectively.

Determination of ampicillin embonate and amoxycillin embonate

The penicillin and embonic acid contents were determined by assaying six samples of the salts *versus* the standards. The mean recoveries (anhydrous) of ampicillin embonate and amoxycillin embonate were 101.6 and 103.1%, respectively (Table I). The ampicillin and amoxycillin contents of the salts are close to the theoretical values (64.3 and 65.3%, respectively). The results suggest that both of the

TABLE I

DETERMINATION OF AMPICILLIN EMBONATE (I) AND AMOXYCILLIN EMBONATE (II) IN BULK

S.D. = Standard deviation.

Sample No.	*Recovery of salt components (%)**			
	I		*II*	
	Ampicillin	*Embonic acid*	*Amoxycillin*	*Embonic acid*
1	65.1	37.8	66.1	34.5
2	63.6	38.9	64.9	39.8
3	62.7	37.4	65.5	36.1
4	64.5	38.3	64.6	38.3
5	64.3	38.1	65.0	38.9
6	63.5	36.0	67.1	38.2
Mean ± S.D.	63.9 ± 0.85	37.7 ± 0.99	65.5 ± 0.93	37.6 ± 1.9

* Calculated from anhydrous salts.

prepared salts may contain some unreacted embonic acid. A suitable washing system must be found in order to obtain exact stoichiometry of 2:1 for these embonates.

The applicaton of these HPLC methods is not limited to the quality control of the pure penicillin salts; the methods can also be used for pre-formulation studies, *e.g.*, studies on *in vitro* solubility and dissolution behaviour of ampicillin embonate and amoxycillin embonate as a function of pH[12].

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