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Note

Identification and purity determination of benzathine and embonate salts of some β-lactam antibiotics by thin-layer chromatography

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The organic salts of both ampholytic β-lactam antibiotics and their prodrug esters are nearly always precipitated from aqueous solutions[1]. In such solutions, penicillins and cephalosporins can decompose in a number of ways[2,3]. Moreover the salt-forming agents and alcoholic solvents, being nucleophiles, may even interact with penicillins and cephalosporins to form amides or esters. However, no thin-layer chromatographic (TLC) or other procedure has yet been proposed for purity tests on the salts in question.

Some of the degradation products of benzathine salts of penicillin G and V have been separated and identified by a TLC system developed for identifying these salts[4]. According to the TLC studies of Fooks and Mattok[5], procaine penicillin G may contain, in addition to procaine and penicillin G, benzylpenicilloic acid, benzylpenillic acid and corresponding alkyl α-D-penicilloate.

The present paper describes a number of TLC systems and spray reagents which are capable of separating and identifying the cation and anion parts of benzathine and embonic acid salts of ampicillin, amoxycillin and cephalexin and talampicillin embonate. Since all of these TLC methods have been described earlier in connection with studies on degradation products of penicillins or cephalexin, they have now been used for assessment of the purity of the synthesized embonate and benzathine salts of β-lactam antibiotics[4,6–11].

EXPERIMENTAL

Materials

Amoxycillin trihydrate (Amphar, Amsterdam, The Netherlands), ampicillin trihydrate (Fermion, Helsinki, Finland) cephalexin monohydrate (Fermion) and talampicillin (Gechim, Milan, Italy) were kindly supplied by Orion Pharmaceutica. Benzathine diacetate (pure) was obtained from Koch-Light Laboratories (Colnbrook, U.K.), and embonic acid (99%) from Ega-Chemie (Steinheim, F.R.G.). All the other chemicals were of commercial analytical grade. The identification of the antibiotics and salt-forming agents and the preparation methods for benzathine and embonate salts of ampicillin, amoxycillin and cephalexin (1:2) and talampicillin embonate (2:1) have been presented elsewhere[12].

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Thin-layer chromatography

Commercially available precoated silica plates (Kieselgel 60 or Kieselgel 60 F_{254}: Merck, Darmstadt, F.R.G.), 20 cm × 20 cm (chromatographic chamber, 22 cm × 22 cm × 10 cm, lined with filter paper) or cut to 10 cm × 10 cm (Camag chamber, 13 cm × 12 cm × 5 cm) were used. The salts and reference substances were rapidly dissolved in methanolic Sörensen phosphate buffers (1/15 *M*, pH 7.0) (7:3, v/v) in an ultrasonic bath at room temperature[13]. A 5-μl aliquot of each of the solutions (0.5, 1 or 2 mg/ml) was applied to the plates (n = 6–10) and the plates were developed in saturated chambers at room temperature. The spots were inspected under UV light (366 nm) and developed with iodine vapour or by uniformly spraying the plate with some of the spray reagents. Mobile phases: (A) 1-butanol–acetic acid–water (4:1:1, v/v/v); (B) ethyl acetate–acetic acid–water (70:15:15, v/v/v); (C) ethyl acetate–acetic acid–water (7:2:1, v/v/v/); (D) ethyl acetate–acetic acid–water (3:1:1, v/v/v); (E) acetic acid–butyl acetate–1/15 *M* Sörensen buffer pH 5.6–1-butanol–absolute ethanol (20:40:12:5:7.5, v/v); (F) 1-butanol–formic acid–water (80:3:1, v/v/v); (G) chloroform–ethanol–acetic acid (9:1:0.2, v/v/v); (H) water–acetic acid–acetone–ethyl acetate (1:2:2:5, v/v). Spray reagents: (1) 0.2% ninhydrin in ethanol and heating at 105°C; (2) modified chloroplatinic reagent: 0.1 ml 1 *M* hydrochloric acid, 300 μl 10% hexachloroplatinate (IV)–acid solution, 20 ml acetone and 0.2 ml 20% potassium iodide–water solution[4,6]; (3) 1% starch solution–acetic acid–0.1 *M* iodine solution (100:8:1, v/v/v)[7].

RESULTS AND DISCUSSION

In order to minimize decomposition of the parent antibiotics, the preparation of the organic salts of β-lactam antibiotics was mainly carried out at low temperatures and at neutral pH region[1]. However, aminopenicillins, and cephalexin can react with water molecules or undergo intra- or intermolecular aminolysis in neutral media[2]. Since no general purity test is available for penicillins, a large number of procedures were used in the present study to determine possible degradation products of the benzathines and embonates synthesized.

A mixture of Sörensen phosphate buffer pH 7.0 and methanol (3:7) was selected for the sampling solvent because it did not give any shadow spots with the samples of the antibiotics and their salts. Moreover, this kind of mixture has been shown to reduce the degradation of penicillin G and V[14].

Methanol proved to be unsuitable as the sampling solvent because aminopenicillins and their salts gave shadow spots when dissolved in methanol. According to an earlier report, decomposition of penicillin G occurs when alcoholic solutions are used for spotting the chromatograms, owing to the formation of the corresponding alkyl α-D-penicilloate[5]. Only penicillin G was detected when freshly prepared aqueous solutions were used. However, no data have been published to indicate that aminopenicillins would behave in the same way as penicillin G.

The best separation and identification of the components of the benzathines were achieved with mobile phases B, E and F (Table I). Mobile phase A separated benzathine and cephalexin completely, but the components of benzathine ampicillin and benzathine amoxycillin were also identified on the basis of the different colours of the benzathine and penicillin spots. Iodine dyes benzathine brown and penicillins and

TABLE I

TLC hR_F VALUES OF BENZATHINE AND EMBONIC ACID SALTS OF AMPICILLIN, AMOXYCILLIN AND CEPHALEXIN AND TALAMPICILLIN EMBONATE AND THEIR REFERENCE SUBSTANCES IN DIFFERENT SOLVENT SYSTEMS

Identification of the impurity spots is explained in the text. – = not sampled.

Sample	*Mobile phase*						
	A	*B*	*C*	*D*	*E*	*F*	*G*
Ampicillin trihydrate	40	14	31	–	34	32	0
Amoxycillin trihydrate	40	11	–	39	32	37	–
Cephalexin monohydrate	29	9	–	34	27	15	–
Talampicillin	69	86	92	–	–	39	55
	53*	38*	58*			32*	24
	39	15	32				19
	32	12					12
		9					0*
Benzathine diacetate	38	19	39	49	39	4	–
Embonic acid	65	83	93	95	–	73	29
Benzathine ampicillin	40	19	42	–	39	32*	–
	38	14	31		34	4.5	
						4	
Benzathine amoxycillin	46	19	–	86	41	37*	–
	40*	16		57	38	9	
	38	11*		52	32*	4	
				48			
				39*			
Benzathine cephalexin	38	19	–	47	39	15	–
	29	9		34	27	4	
Ampicillin embonate	65	83	92	–	–	73	–
	40	15	31			32	
Amoxycillin embonate	65	83	–	95	–	73	–
	40	12		39		37	
Cephalexin embonate	65	83	–	96	–	73	–
	28	10		34		15	
Talampicillin embonate	65	83	90	–	–	73	29
	52	37				32	0

* The main spot of the chromatogram containing additional spots.

cephalexin yellow; ninhydrin dyes benzathine reddish brown and penicillins and cephalexin red. Mobile phase B permits rapid and convenient identification of the benzathines and embonates investigated; it distinguishes them all. The R_F values of embonic acid (UV 366 nm) differ considerably from those of penicillins and cephalexin (Table I, mobile phases B–D); the presence of embonic acid probably does not disturb the determination of the degradation products of embonates.

Ampicillin and its penicilloic acid were separated by five different solvent systems: 1-butanol–formic acid–water (80:3:1), 1-butanol–acetic acid–water (4:1:1), 1-butanol–water–ethanol-acetic acid (5:2:1.5:1.5), acetone–acetic acid (95:5) and 85% aqueous acetone[7,8]. Of these, only the first and second phases (A and F) gave good spots for benzathine and ampicillin; all the others yielded tailing spots.

Mobile phase F, used together with spray reagent 3, gave only one very small

impurity spot for benzathine ampicillin; according to its R_F value, 0.045, this spot may be the penicilloic acid of ampicillin[7]. Spray reagent 3 does not make benzathine visible, but iodine vapour (brown) or ninhydrin (reddish brown) can colour it. In the TLC experiment carried out by Vandamme and Voets[8] the penicilloic acid of ampicillin was dyed red-purple by ninhydrin; this colour was not detected in this experiment although other ninhydrin-positive substances, *e.g.*, aminopenicillins and some impurities of benzathine amoxycillin, were distinguished from benzathine quite clearly according to their different colours.

Some oligomers and the piperazinedione of ampicillin were separated from ampicillin by mobile phases C and F[7,9]. Benzathine ampicillin and ampicillin embonate did not give any spots at the published R_F values of the above-mentioned degradation products. No impurity spot was detected when the samples of benzathine cephalexin (R_F 0.51 and 0.40) or cephalexin embonate (R_F 0.41 and 0.97) were chromatographed by the method published for the determination of the piperazinedione of cephalexin (mobile phase H)[10].

Benzathine amoxycillin gave one impurity spot with mobile phases A (R_F 0.46), B (R_F 0,16), E (R_F 0.41) and F (R_F 0.09) with all the visualization methods. The spots were not identified. Using mobile phase D, benzathine amoxycillin (2 mg/ml) gave four impurity spots with iodine (Table I). In comparison with the published R_F values, these impurities may be piperazine-2,5-dione (R_F 0.86) and oligomers (R_F 0.48, 0.52, and 0.57) of amoxycillin[9]. Ninhydrin solution dyed the spots at R_F 0.48, 0.52 and 0.57 red like the amoxycillin spot; the benzathine spots were brown. The spot at R_F 0.86 was not detected by ninhydrin, only by iodine.

Talampicillin showed traces of impurities with all the methods. The impurity spot with R_F 0.55 (Table I, mobile phase G) may be due to phthalaldehydic acid[11]. One of the impurities of talampicillin may be ampicillin (Table I, mobile phases A–C). Talampicillin embonate samples produced only two intense spots with mobile phases A, B, F and G; the impurities of talampicillin were not detected in talampicillin embonate.

The results of these TLC studies indicate that the synthesized benzathine and embonic acid salts of antibiotics are very pure and homogeneous. The one exception is benzathine amoxycillin, which contains many degradation products. It has been concluded that amoxycillin forms polymers very rapidly, and that these are much more soluble in water than the corresponding ampicillin derivatives[9].

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