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IDENTIFICATION OF PENICILLINS BY THIN-LAYER CHROMATOGRAPHY

S. HENDRICKX*, E. ROETS, J. HOOGMARTENS and H. VANDERHAEGHE
Katholieke Universiteit Leuven, Laboratorium voor Farmaceutische Chemie, Instituut voor Farmaceutische Wetenschappen, Van Evenstraat 4, B-3000 Leuven (Belgium)
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SUMMARY

Results are reported of a study on thin-layer chromatography of eighteen penicillins on silica gel and silanized silica gel, using thirty-five mobile phases. Silanized silica gel allows better separations than silica gel. Each penicillin can be separated from all others with an appropriate mobile phase. Any of the penicillins examined can be identified by combining the results obtained with a few mobile phases.

INTRODUCTION

Paper chromatography and earlier thin-layer chromatography (TLC) of penicillins was reviewed by Hughes *et al.*[1]. To the references cited in that review one should add the publications of Auterhoff and Kienzler[2], who performed TLC before and after alkaline hydrolysis, and of Overvliet *et al.*[3], who used silanized silica gel.

Thijssen also used silanized silica gel for TLC of oxacillin, cloxacillin, dicloxacillin, flucloxacillin and their derivatives[4]. Wilson *et al.* described the identification on silica of benzathine salts of benzylpenicillin and phenoxymethylpenicillin[5]. Cruceanu *et al.*[6] reported the TLC of fifteen penicillins on silica using the mobile phase described by Nussbaumer[7]. Gerold and Heinisch reported the identification of penicillins by TLC on silica of the carboxamide derivatives, obtained after heating the penicillins in sodium hydroxide solution containing copper sulphate[8]. The British Pharmacopoeia (BP) addendum 1982 prescribes TLC on silica for the identification of amoxicillin[9].

In this paper we report the results of a TLC study of eighteen penicillins on silica gel and on silanized silica gel, using 35 mobile phases.

EXPERIMENTAL

Samples

The structures of the penicillins examined are given in Table I. Compounds II, III, VI, X, XI, XII, XIV, XVII and XVIII were obtained from Beecham (Heppignies, Belgium and Worthing, U.K.); IX and XV from Bristol-Myers (Syracuse, NY, U.S.A.); I, IV, VIII and XIII from Gist-Brocades (Delft, The Netherlands); VII and

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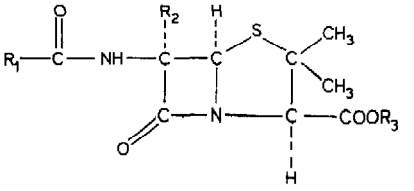
TABLE I

PENICILLIN STRUCTURES



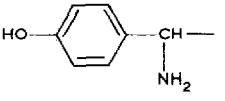
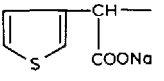
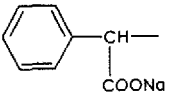
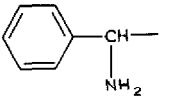
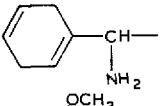
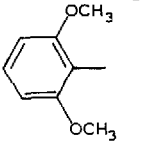
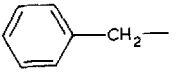
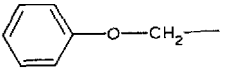
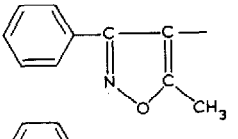
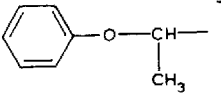
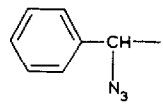
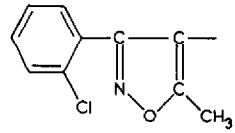
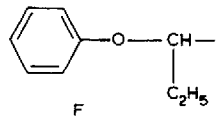
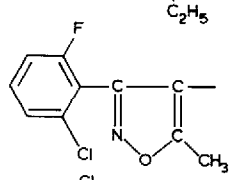
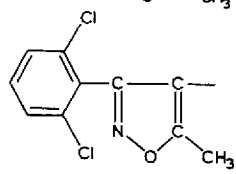
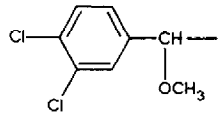
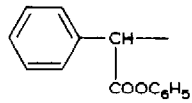
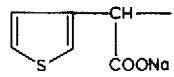
	*Generic name, INN**	R_1	R_2	R_3
I	Amoxicillin		H	H
II	Ticarcillin		H	Na
III	Carbenicillin		H	Na
IV	Ampicillin		H	Na
V	Epicillin		H	H
VI	Methicillin		H	Na
VII	Benzylpenicillin		H	Na
VIII	Phenoxymethylpenicillin		H	K
IX	Oxaxillin		H	Na
X	Phenethicillin		H	K

TABLE I (*continued*)

	*Generic name, INN**	R_1	R_2	R_3
XI	Azidocillin		H	K
XII	Cloxacillin		H	Na
XIII	Propicillin		H	K
XIV	Flucloxacillin		H	Na
XV	Dicloxacillin		H	Na
XVI	Clometocillin		H	Na
XVII	Carfecillin			
XVIII	Temocillin		OCH_3	Na

* International Non-proprietary Names (INN), the nomenclature of the World Health Organization, has been used.

XVI from Smith Kline-RIT (Genval, Belgium) and V from Squibb (Princeton, NJ, U.S.A.).

Stationary phases

Precoated silica gel plates were used, *i.e.* Stratochrom SIF_{254} (Carlo Erba, Milan, Italy). Precoated silanized silica gel plates, Fertigplatten Kieselgel 60 F_{254} silanisiert, were obtained from E. Merck (Darmstadt, F.R.G.). Laboratory-made

silanized silica gel plates were prepared using a suspension of 35 g of Kieselgel 60 HF_{254} silanisiert (E. Merck) in 60 ml of water–methanol (2:1). After initial drying at room temperature for several hours, the layers were further dried at 50°C overnight. All layers used were 0.25 mm thick.

Mobile phases

For use with silica gel: Si-A, 0.5 *M* sodium chloride solution; Si-B, ethyl acetate–acetic acid–water (3:1:1); Si-C, acetonitrile–water (4:1); Si-D, ethyl acetate–methanol–acetic acid (100:50:5); Si-E, chloroform–ethanol–acetic acid (100:50:7.5); Si-F, ethyl acetate–acetone–water (2:4:2); Si-G, upper layer of: isoamyl acetate–methanol–formic acid–water (65:20:5:10); Si-H, acetone–acetic acid (95:5); Si-I, ethyl acetate–acetone–acetic acid–water (5:2:2:1); Si-J, *n*-butanol–acetic acid–water (4:1:1); Si-K, acetone–benzene–water–acetic acid (65:14:14:7); Si-L, upper layer of: *n*-butyl acetate–*n*-butanol–acetic acid–0.066 *M* phosphate buffer pH 6.0 (90:9:25:15); Si-M, *n*-butyl acetate–*n*-butanol–acetic acid–0.1% (w/v) disodium edetate in 5% (w/v) solution of sodium dihydrogen phosphate (10:1:6:2).

For use with silanized silica gel: RP-A, buffer pH 6.2–methanol (85:15); RP-B, buffer pH 6.2–acetonitrile (85:15); RP-C, buffer pH 6.2–methanol–acetonitrile (70:10:20); RP-D, buffer pH 6.2–acetone–ethanol (70:20:10); RP-E, buffer pH 6.2–acetone–ethanol (60:30:10); RP-F, buffer pH 6.2–methanol–ethanol (50:40:10); RP-G, buffer pH 5.0–acetonitrile (85:15); RP-H, buffer pH 5.0–ethylene glycol monoethyl ether (80:20); RP-I, buffer pH 5.0–acetonitrile–ethylene glycol monoethyl ether (80:10:10); RP-J, buffer pH 5.0–tetrahydrofuran (75:25); RP-K, buffer pH 5.0–acetone–ethyleneglycol monoethyl ether (75:15:10); RP-L, buffer pH 5.0–ethanol (75:25); RP-M, buffer pH 5.0–ethanol–ethylene glycol monoethyl ether (75:15:10); RP-N, buffer pH 5.0–ethylene glycol monoethyl ether (75:25); RP-O, buffer pH 5.0–acetone (70:30); RP-P, buffer pH 5.0–ethanol–ethylene glycol monoethyl ether (70:10:20); RP-Q, buffer pH 5.0–ethanol (65:35); RP-R, buffer pH 5.0–methanol–acetonitrile (60:15:25); RP-S, buffer pH 5.0–methanol–ethanol (50:40:10); RP-T, 0.3 *M* sodium chloride in 0.05 *M* potassium phosphate buffer pH 5.6–acetone (2:1); RP-U, 0.1 *M* sodium chloride solution–acetone (2:1); RP-V, 0.05 *M* potassium phosphate buffer pH 6.0–acetone (4:1).

Buffer solutions pH 5.0 and pH 6.2 used in mobile phases RP-A to RP-S were prepared by adjusting a 2 *M* solution of ammonium acetate to the desired pH with glacial acetic acid.

Chromatographic procedure

Aqueous solutions (0.5 μl) containing 10 mg/ml of the compound were applied to the plates by means of a microsyringe, except for III and V, where a 0.1 *M* potassium hydrogen carbonate solution was used as the solvent, and for I, where a saturated aqueous solution was used. The plates were developed over a distance of *ca.* 15 cm in filter-paper-lined chromatographic tanks, which had been saturated for at least 1 h. The plates were dried in a stream of hot air and placed in a tank saturated with iodine vapour for detection.

RESULTS AND DISCUSSION

Table II shows the R_F values obtained on silica gel. All the mobile phases have been described previously for TLC of penicillins or cephalosporins: Si-A[10]; Si-B[11]; Si-C, D, E and F[12]; Si-G and H[13,14]; Si-I[15]; Si-J[16,17]. Mobile phases similar to Si-L were described by Wilson *et al.*[5], Cruceanu *et al.*[6], Nussbaumer[7] and Saccani[18]. A mobile phase similar to Si-I was recently used by Fabre and Hussam-Eddine[19]. Mobile phase Si-M is prescribed by the BP 1980 (addendum 1982) for the identification of amoxicillin[9]. Mobile phases Si-A to Si-L have also been examined for the identification of cephalosporins[20]. System Si-A, which had shown a good separation of many cephalosporins[20], gives a much inferior result with the penicillins. The other silica gel systems also show a poor distribution of the penicillins. In some systems the more polar compounds form badly streaking spots. It is therefore concluded that, although some systems can be useful for the identification of certain penicillins, TLC on silica gel is not a valuable general method. Some systems may be useful for the identification of certain penicillins, *e.g.* system Si-M for amoxicillin.

The results obtained with reversed-phase (RP) systems are shown in Fig. 1. Some of these systems have been used before: RP-A and B for the identification of cephalosporins[20], RP-T and U for the separation of IX, XII, XIV and XV[4,21,22]. RP-V was prescribed by the BP 1980 for the identification of I. The results reported were obtained with laboratory-made thin layers. Precoated plates were also used with most mobile phases, and although small differences in R_F values were noticed, the separation pattern was observed to be the same. The influence of small differences of the pH of the ammonium acetate buffer (0.1 pH unit) was also examined, but here

TABLE II

R_F × 100 VALUES FOR PENICILLINS CHROMATOGRAPHED ON SILICA GEL

Samples	*Mobile phases*												
	Si-A	*Si-B*	*Si-C*	*Si-D*	*Si-E*	*Si-F*	*Si-G*	*Si-H*	*Si-I*	*Si-J*	*Si-K*	*Si-L*	*Si-M*
I	83	26	19	*	*	39	09	08	42	50	41	04	16
II	60	43	22	*	*	39	24	*	28	24	23	04	51
III	58	43	24	*	*	37	24	*	28	24	23	04	51
IV	55	30	22	*	*	41	12	13	44	50	44	06	19
V	52	28	19	*	*	38	13	09	42	48	43	06	20
VI	31	85	42	60	82	53	50	65	84	65	91	47	51
VII	46	92	41	65	82	59	58	70	86	67	93	57	60
VIII	45	92	43	67	83	60	59	70	86	67	93	58	61
IX	43	92	43	67	83	60	60	71	88	67	95	60	62
X	47	93	43	68	83	60	60	72	88	68	95	59	62
XI	48	93	46	68	83	65	61	72	88	68	95	61	64
XII	39	93	45	67	83	61	60	71	88	67	95	60	61
XIII	44	95	45	69	85	62	61	71	88	69	95	59	62
XIV	42	95	45	67	83	62	60	71	88	67	95	59	61
XV	35	95	46	67	83	62	60	71	88	67	95	59	61
XVI	29	95	46	67	83	62	60	71	88	67	95	55	61
XVII	33	96	50	67	83	63	62	70	88	69	96	55	64

* Streaking from the start point.

too the general separation pattern remained unchanged. Good results were also obtained when thin layers were dried at 80°C for 3 h, instead of 50°C overnight. Most separations were carried out with mobile phases at pH 5.0, where the shape of the spots was found to be better. Temocillin (XVIII) was chromatographed with a restricted number of mobile phases. The penicillins do not migrate very well in systems RP-A and B, which were found to be useful for the identification of the cephalosporins. More than ten penicillins can be separated by eight of the mobile phases examined. Twelve compounds are separated by RP-H and N, and thirteen by RP-K. The last three mobile phases all contain ethylene glycol monoethyl ether. It should be mentioned that some difficulties were experienced with some batches of ethylene glycol monoethyl ether. Distillation of the solvent restored its quality. The problem observed could be related to the presence of peroxides.

It is obvious from Fig. 1 that no system will separate all penicillins. For this reason, groups of related products will be considered.

Amoxicillin (I)–ampicillin (IV) and benzylpenicillin (VII)–phenoxymethylpenicillin (VIII) may be differentiated with most mobile phases. Oxacillin and its derivatives (IX, XII, XIV, XV) can be separated by seven RP systems, but if one adds this series to the aforementioned penicillins (I, IV, VII, VIII) only systems RP-K, O, U and V seem to have the necessary qualities. It should be noted that system RP-V, which was used in the BP 1980 for the identification of amoxicillin (I), was replaced by system Si-M in the BP addendum 1982. The reason probably was that amoxicillin was not separated from all other penicillins. The shape of the spots obtained with

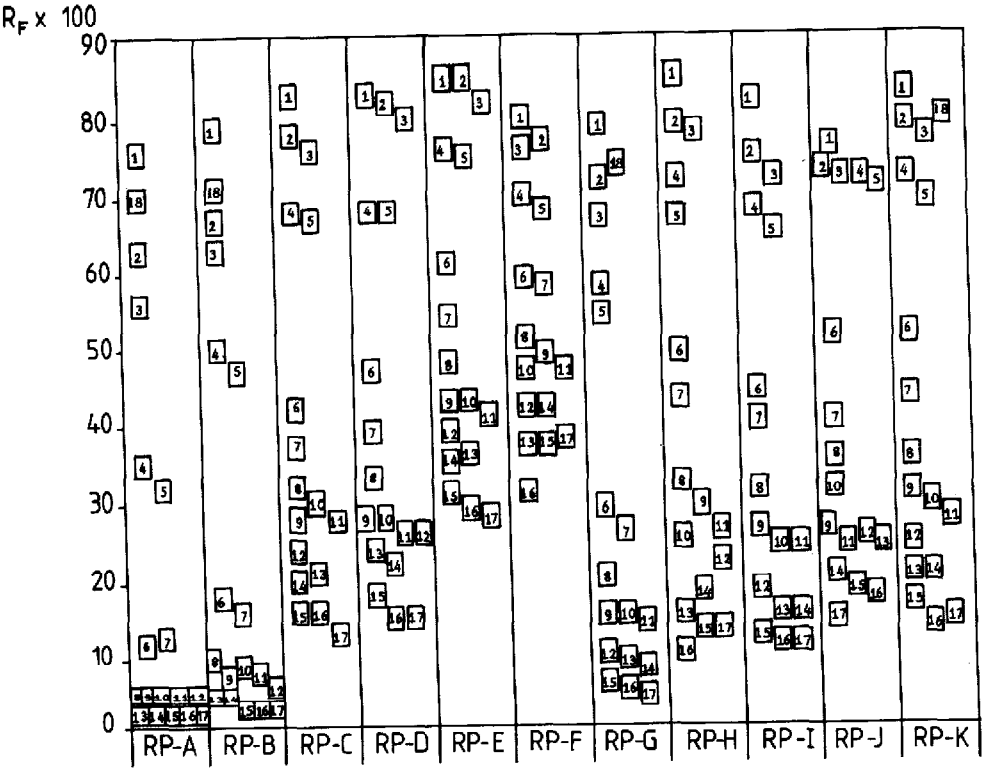


Fig. 1.

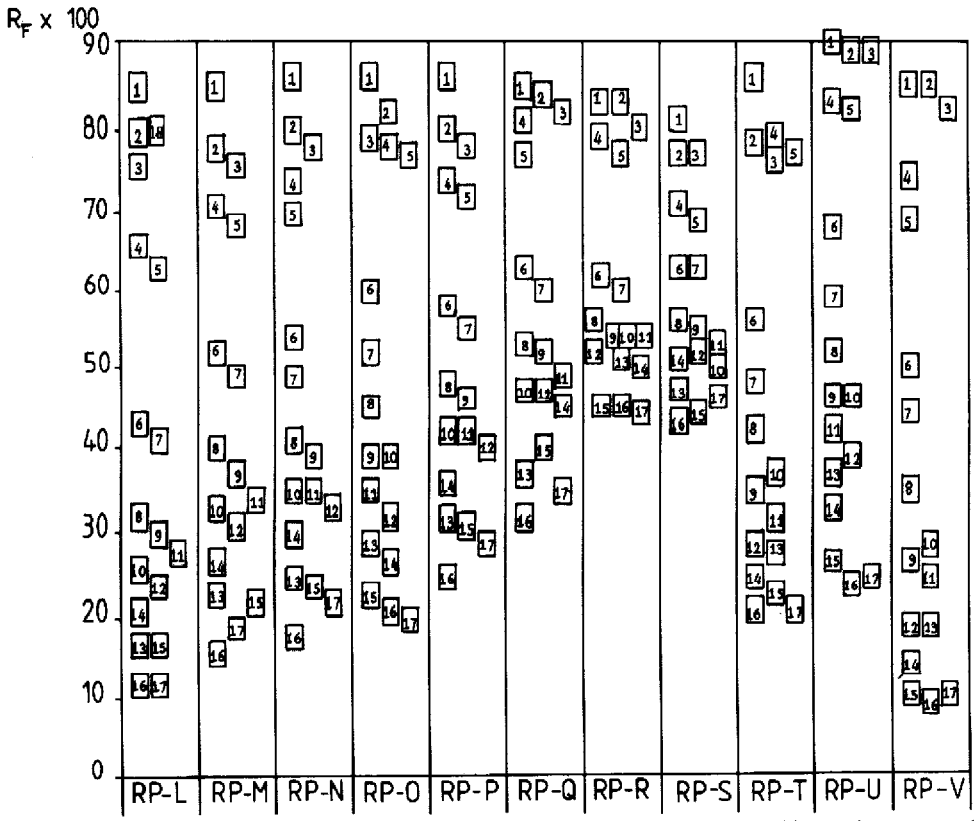


Fig. 1. R_F × 100 values of various penicillins chromatographed in RP systems. Arabic numbers are used for clarity and correspond to the roman numbers in Table I. See Experimental for chromatographic conditions.

RP-V seemed to be inferior to that obtained with RP-K, O, U. With system RP-U, amoxicillin (I) migrates very close to the front. Of the remaining two systems RP-O is preferred because it uses the more commonly available acetone instead of ethylene glycol monoethyl ether.

Phenoxymethylpenicillin and its related compounds (VIII, X, XIII) are separated in most systems. For the separation of epicillin (V) and ampicillin (IV), systems RP-G, H, N, V are appropriate. For penicillins with a free carboxyl group in the side-chain (II, III, XVIII), systems A and B give satisfactory results. Carbenicillin (III) is separated by all the RP systems from benzylpenicillin (VII), which can be formed from III by decarboxylation. Clometocillin (XVI) is separated from all other penicillins by systems RP-N and P.

All penicillins examined may be separated on silanized silica gel with an appropriate mobile phase, which is not the case with straight silica gel. TLC on C_8 or C_{18} RP materials also would probably give satisfactory results, but these materials are more expensive.

It should be mentioned that the separation pattern obtained on silanized silica gel for oxacillin and its related compounds (IX, XII, XIV, XV) corresponds with the partition coefficients reported by Thijssen[21]. The separation pattern obtained with our RP systems also corresponds to the partition coefficients reported for eight pen-

icillins (VI–X and XII–XV) by Bird and Marshall[23], who performed also reversed phase TLC on cellulose impregnated with octanol. It is also worthwhile to compare our results with those obtained by Biagi *et al.*[24,25] for a rather important group of penicillins on silica gel impregnated with silicone oil. Again the sequence is similar.

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