ACTA CHROMATOGRAPHICA, NO. 16, 2006

THIN-LAYER CHROMATOGRAPHIC SEPARATION OF PENICILLINS ON STANNIC ARSENATE–CELLULOSE LAYERS

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SUMMARY

Thin-layer chromatographic separation of penicillins has been performed on stannic arsenate–cellulose layers. R_F values of penicillins were determined after use of different mobile phases. Amoxycillin and ampicillin were selectively separated from binary and ternary synthetic mixtures. Amoxycillin and ampicillin in commercially available drugs were also separated and were quantitatively determined.

INTRODUCTION

Antibiotics are chemical substances, produced by microorganisms, that can inhibit the growth of, or even destroy, other microorganisms. Penicillin, the first antibiotic, was discovered by Alexander Fleming (1929) in the mould *Penicillium notatum*. Ampicillin and amoxycillin are semi-synthetic modifications of penicillin. The structures of some penicillins are shown in Fig. 1.

The TLC of antibiotics has been widely studied [1–5] and methods for separation of natural and semi-synthetic antibiotics and their degradation products by TLC have been reported [6–8]. A simple bacteriological and TLC method for determination of individual drug concentrations treated with penicillin G in combination with one of the glycosides has been reported by Shin Yuh Lin [9]. Silanized silica gel has been used by Overliete [10] and Thijssen [11] for chromatographic study of oxacillin, cloxacillin, dicloxacillin, flucloxacillin, and their derivatives. Wilson et al. [12] and Cruceanu [13] used silica gel for TLC of antibiotics. Bystrova [14] has reported the TLC behaviour of seven penicillins. Agarwal and Nwaiwu [15] used several spray reagents for detection of penicillins. Hendrickx [16] et al. used silica gel and silanized silica gel, and thirty-five mobile phases, for study of eighteen penicillins.

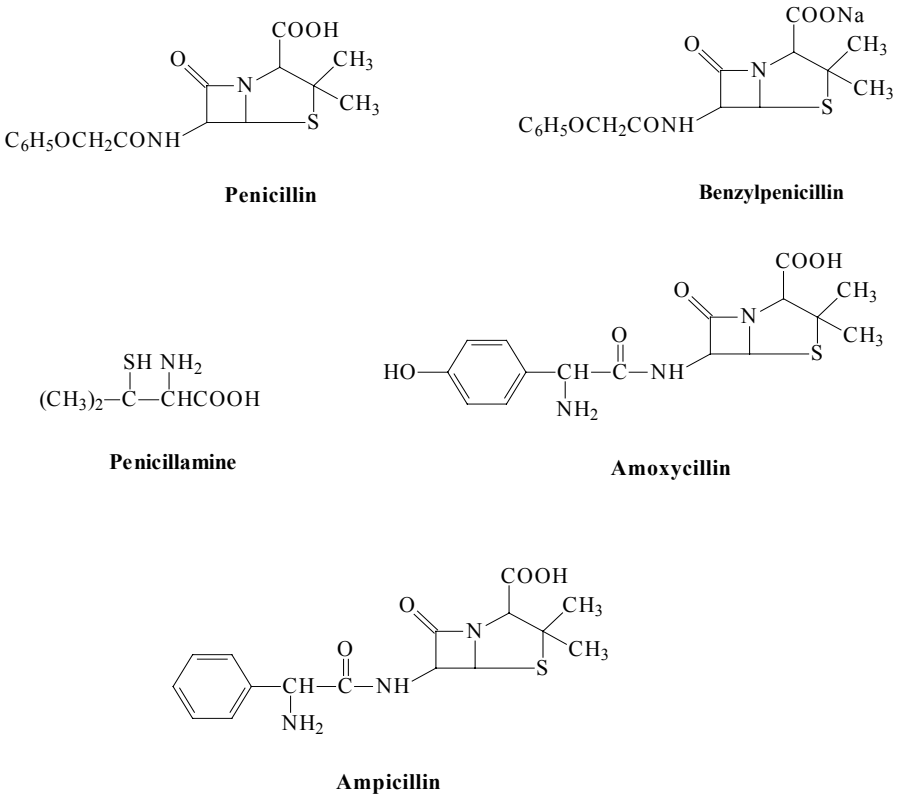

Fig. 1

The structures of the penicillins

This paper describes the TLC separation of penicillins on stannic arsenate–cellulose layers with new mobile phases. Quantitative determination of the compounds, both singly and in combination, in commercial products, was performed by the method of Askal et al. [17] using 2,3-dichloro-5,6-dicyano-*p*-benzoquinone as detection reagent.

EXPERIMENTAL

Reagents and Chemicals

Stannic chloride pentahydrate was from Loba Chemie (India), sodium arsenate and cellulose powder were from E. Merck (India), ampicillin capsules were from Cadila Healthcare, amoxycillin capsules were from Moxiwell Carewel Laboratories, penicillamine capsules were from Artamin

Biochemie (Austria), and benzylpenicillin and penicillin vials were from Alembic. All reagents used were AR grade. Iodine was used for the detection of the penicillins.

Preparation of Ion-Exchange Material

Stannic arsenate was synthesized by slowly adding 0.1 M aqueous sodium arsenate to a 2:1 mixture of 0.1 M stannic chloride solution and 0.1 M HCl. The mixture was intermittently shaken during mixing and the resulting precipitate was finally left for 24 h at room temperature. The mixture was washed several times with distilled water, by decantation, and then filtered under vacuum. The product was then dried in an oven at 50 ± 2°C. The dried product was broken into small granules simply by immersing in distilled water. The granules were again washed several times with distilled water and finally dried at 50°C. The cation-exchange capacity of the ion-exchange material for Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} was found to be 0.77, 1.04, 1.03, and 1.31 mmol g^{-1}, respectively.

Preparation of TLC Plates

Granules of stannic arsenate were well powdered in a mortar, mixed with cellulose powder in 1:4 ratio, and 10% $CaSO_4$ was added as binder. A slurry of this mixture (10 g) in water (50 mL) was prepared and spread over glass plates, by means of applicator, to form uniform thin layers 0.2 mm thick. The plates were then dried in an oven at 60°C.

TIC Procedure

For qualitative analysis approximately 0.05 mL of solutions of the penicillins in methanol were applied to the plates by means of fine glass capillaries. After drying of the spots the plates were developed with the desired mobile phase, to 15 cm from the point of application, by the ascending technique. The plates were dried in air and then placed in iodine chambers for detection of the spots.

Quantitative Separation of Penicillins in Synthetic Mixtures

Mixtures of penicillins were spotted on the plates by means of a syringe and the plates were developed in usual manner. Pilot chromatograms were run under similar experimental conditions to ascertain the actual positions of the spots on the plates. The adsorbent was scraped from the same regions of the sample plates and the penicillins present in the adsorbent was extracted with 10 mL methanol. The extracts were then determined

spectrophotometrically, with a Spectronic 20-D Genesys spectrophotometer (USA), by measuring the absorbance at 460 nm using 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) as reagent.

RESULTS AND DISCUSSION

Chromatography on thin layers of stannic arsenate mixed with cellulose resulted in differential migration of the penicillins studied. Several mobile phases were investigated to observe the behaviour of the penicillins (Table I). The R_F values obtained for the penicillins are listed in Table II. As a result of these investigations several binary and ternary separations were achieved on this adsorbent (Table III).

Table I

The mobile phases used

Components	Volume ratio	Abbreviation
Butanol–acetic acid–water	4:1:1	S1
Acetonitrile–water	3:5	S2
Acetic acid–chloroform–methanol	2:3:2	S3
Butanol–acetic acid–chloroform	1:2:5	S4
Acetone–acetic acid–chloroform	1:1:6	S5
Butanol–acetic acid–carbon tetrachloride	1:1:6	S6
Acetonitrile–acetic acid–chloroform	1:1.5:4.5	S7
Acetonitrile–acetic acid–carbon tetrachloride	1:1:5	S8
Ethanol–acetic acid–carbon tetrachloride	1:1:6	S9
Acetone–acetic acid–carbon tetrachloride	1:1:5	S10
Acetone–acetic acid–chloroform	5:5:6	S11

With most of the mobile phases either tailing was observed or the drugs moved with the mobile phase front. The drugs remained at the point of application when butanol–dioxane, methanol–chloroform, acetic acid–butanol–dioxane, chloroform–acetonitrile, carbon tetrachloride–methanol, and chloroform–acetone–acetic acid mixtures were used as mobile phases. It was observed that the drugs migrated when chromatographed with solvents of relatively high polarity. With some mobile phases, for example ethyl methyl ketone–acetic acid–chloroform and acetone–acetic acid–chloroform differential movement of the drugs was observed. With ethyl methyl

Table II

R_F values of the penicillins on stannic arsenate–cellulose (1:4) layers

Mobile phase	Penicillin	Benzyl-penicillin	Amoxycillin	Penicillamine	Ampicillin
S1	0.98	1.0	1.0	0.86	1.0
S2	0.98	0.88	1.0	0.87	1.0
S3	0.91	0.85	0.80	0.92	0.91
S4	0.97	0.95	0.10	T[a]	T
S5	0.94	0.96	0.08	T	0.06
S6	0.80	0.90	0.28	0.63	0.57
S7	0.92	0.91	0.18	0.48	0.17
S8	0.90	0.71	0.13	0.62	T
S9	1.0	1.0	0.94	0.96	0.96
S10	0.15	0.20	0.13	0.17	0.06
S11	1.0	0.97	0.11	0.27	0.07

[a]Tailing

Table III

Binary and ternary separations of the penicillins on stannic arsenate–cellulose (1:4) layers

Penicillins	R_F (= R_L − R_T)[a]	Mobile phase
Amoxycillin–penicillin	(0.33–0.0) (1.0–0.86)	S7
Amoxycillin–benzylpenicillin	(0.32–0.0) (1.0–0.87)	S7
Amoxycillin–penicillamine	(0.30–0.0) (0.60–0.38)	S7
Ampicillin–penicillin	(0.25–0.06) (1.0–0.81)	S7
Ampicillin–benzylpenicillin	(0.26–0.06) (1.0–0.86)	S7
Ampicillin– penicillamine	(0.28–0.05) (0.60–0.40)	S7
Amoxycillin–cloxacillin	(0.30–0.0) (1.0–0.88)	S7
Amoxycillin–penicillamine–benzylpenicillin	(0.24–0.0) (0.36–0.20) (1.0–0.83)	S11
Amoxycillin–penicillamine–penicillin	(0.22–0.0) (0.38–0.19) (1.0–0.87)	S11
Ampicillin–penicillamine–benzylpenicillin	(0.17–0.0) (0.36–0.20) (1.0–0.96)	S11
Ampicillin–cloxacillin	(0.16–0.0) (1.0–0.92)	S11
Amoxycillin–ampicillin–penicillin	(0.24–0.0) (0.16–0.0) (1.0–0.88)	S11

[a]R_L and R_T are, respectively, the R_F values of the leading and trailing edges of the spots

ketone–acetic acid–chloroform R_F values of penicillin and benzylpenicillin were very high whereas ampicillin and amoxycillin remained almost at the point of application. Penicillamine moved to some extent, but with tailing. Clean separations of binary and ternary mixtures were achieved with acetone–acetic acid–chloroform and acetonitrile–acetic acid–chloroform. With these mobile phases R_F values of benzylpenicillin and penicillin were high, penicillamine migrated to the middle of the plate, and amoxycillin and ampicillin remained almost at the point of application. With acetonitrile–acetic acid–chloroform and acetone–acetic acid–chloroform mobile phases R_F values of amoxycillin and ampicillin were low compared with those of penicillamine, penicillin, and benzylpenicillin. It is possible that the presence of dipolar groups, which facilitates interaction with the ion-exchange material, resulted in retardation of the migration of these drugs. For this reason these mobile phases were chosen for selective separations of amoxycillin and ampicillin from synthetic mixtures of penicillins. The results obtained are listed in Tables IV and V.

Table IV

Quantitative separation of penicillins using mobile phase S11

Separations achieved	Amount taken (μg)	Amount found (μg)[a]	Error (%)[a]
Amoxycillin	100	99.80	−0.20
Ampicillin	100	100.00	0.00
Amoxycillin	100	99.90	−0.10
Penicillin	100	99.75	−0.25
Amoxycillin	100	100.00	0.00
Benzylpenicillin	100	99.85	−0.15
Amoxycillin	100	99.90	−0.10
Penicillamine	100	99.85	−0.15
Ampicillin	100	100.00	0.00
Penicillin	100	99.75	−0.25
Ampicillin	100	99.70	−0.30
Benzylpenicillin	100	100.00	0.00
Ampicillin	100	99.70	−0.30
Penicillamine	100	99.85	−0.15

[a]Average from five replicate determinations

Table V

Quantitative separation of penicillins using mobile phase S7

Separations achieved	Amount taken (μg)	Amount found (μg)[a]	Error (%)[a]
Ampicillin	10.0	9.85	−1.5
Benzylpenicillin	10.0	9.90	−1.0
Ampicillin	18.0	17.90	−1.0
Benzylpenicillin	12.0	11.9	−1.0
Ampicillin	26.8	26.8	0.0
Benzylpenicillin	13.2	13.2	0.0
Ampicillin	36.0	35.7	−3.0
Benzylpenicillin	14.0	13.8	−2.0
Ampicillin	45.0	44.90	−1.0
Benzylpenicillin	15.0	15.0	0.0
Amoxycillin	10.0	10.0	0.0
Penicillin	10.0	9.8	−2.0
Amoxycillin	18.0	17.80	−2.0
Penicillin	12.0	11.85	−1.5
Amoxycillin	26.8	26.8	0.0
Penicillin	13.2	13.10	−1.0
Amoxycillin	36.0	35.95	−0.5
Penicillin	14.0	13.85	−1.5
Amoxycillin	45.0	44.90	−1.0
Penicillin	15.0	14.75	−2.5

[a]Average from five replicate determinations

Table VI

Quantitative separation and determination of amoxycillin in the commercially available drug hipenox capsule (amoxycillin and cloxacillin) using mobile phase S11

Labelled amount of drug (mg)	Amount taken (μg)	Amount found (μg)[a]	Mean recovery (% ± S.D.)[a]
250	10	9.97	99.67 ± 0.08
250	15	14.83	98.87 ± 0.07
250	20	19.95	99.73 ± 0.09
250	25	24.96	99.85 ± 0.13
250	30	29.66	98.87 ± 0.12

[a]Average from five replicate determinations

Table VII

Quantitative separation and determination of ampicillin from the commercially available drug megapen vial (ampicillin and cloxacillin) using mobile phase S7

Labelled amount of drug (mg)	Amount taken (μg)	Amount found (μg)[a]	Mean recovery (% ± S.D.)[a]
125	10	9.96	99.64 ± 0.09
125	15	14.83	98.88 ± 0.09
125	20	19.79	98.93 ± 0.13
125	25	24.96	99.83 ± 0.08
125	30	29.94	99.81 ± 0.12

[a]Average from five replicate determinations

The practical utility of this method was also demonstrated by quantitative separation of penicillins from the commercial drugs megapen vial and hipenox capsule (Cadila Healthcare). The recoveries obtained are shown in Tables VI and VII. The method has been found to be useful in practice because of its accuracy and reproducibility.

ACKNOWLEDGEMENT

The authors are grateful to the chairman, Department of Chemistry, Aligarh Muslim University, Aligarh, India, for providing the necessary research facilities.

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