Thin-layer chromatography of erythromycins

Ascending paper chromatography has been used in the analysis of erythromycins[1–3]. In our laboratory, however, it became desirable to develop a method of chromatographic analysis which would be more rapid, sensitive, and less subject to temperature variation. As a result, a thin-layer chromatographic procedure for separating erythromycins on silica gel plates was developed.

A method for the thin-layer chromatographic separation of cardiac glycosides using methylene chloride–methanol–formamide (80:19:1) was described by STAHL[4]. Since the erythromycin molecule contains sugar residues similar to the cardiac glycosides, attempts were made to use the above solvent system for the thin-layer chromatographic separation of erythromycins. Satisfactory results were obtained after the solvent system was modified to include benzene and variable amounts of formamide.

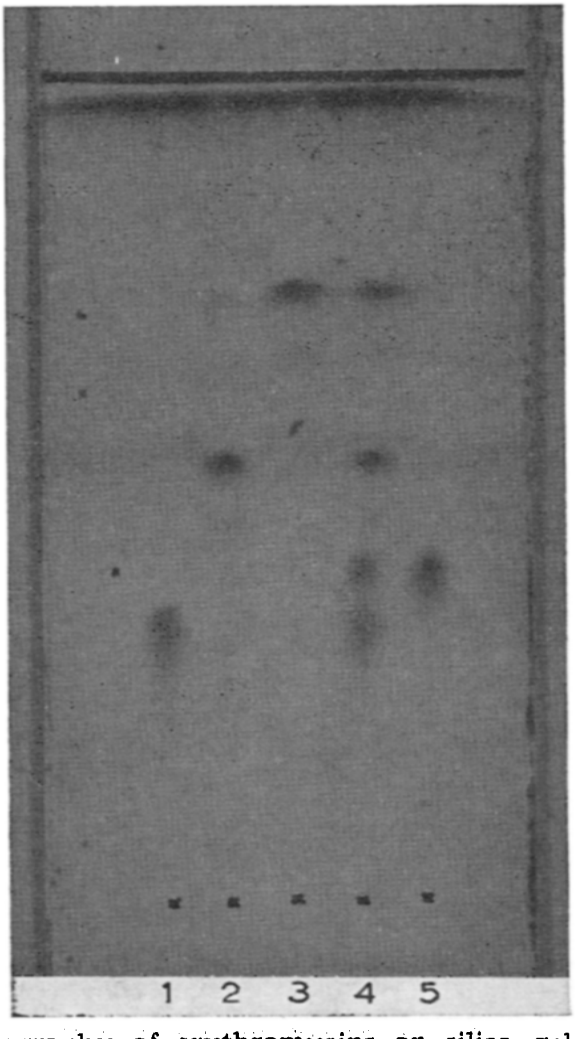

Fig. 1. Thin-layer chromatography of erythromycins on silica gel G plates. Solvent system: methylene chloride–methanol–benzene–formamide (80:20:20:3); (relative humidity 26%). (1) erythromycin A (5 γ); (2) 4″-acetylerythromycin A* (5 γ); (3) 2′,4″-diacetylerythromycin A* (5 γ); (4) mixture of 1, 2, 3 and 5 (10 γ); (5) erythromycin A "Hemiketal"[2] (5 γ). Time of development 30 min; spot detection with 50% aqueous H_2SO_4 spray and charring.

* Prepared by Dr. P. H. JONES, Abbott Laboratories; procedure to be published.

Glass plates (5 × 20 cm) were coated and activated according to the method of STAHL[5], using silica gel G (Merck) as the adsorbent layer. The solvent system used for the development of the plates consisted of methylene chloride–methanol–benzene–formamide (80:20:20:2–5 v/v/v/v). The amount of formamide in the system was varied according to the humidity conditions present in the laboratory. At a relative humidity of 20%, 5 volumes of formamide in the solvent system gave clear separation of erythromycin A and B. Higher relative humidity conditions (30–40 %) required 3 or 2 volumes of formamide for separation.

The time of development varied from 30–40 min. Chromatographic separation was completed when the solvent front advanced to 10 cm from the origin.

Detection of the spots was carried out by spraying the plate with 10 % phosphomolybdic acid in ethanol. Upon heating on a hot plate, blue spots appeared on a yellow background. Spraying the plate with 50 % aqueous H_2SO_4 and heating on a hot plate charred the erythromycins. Although the phosphomolybdic acid spray is

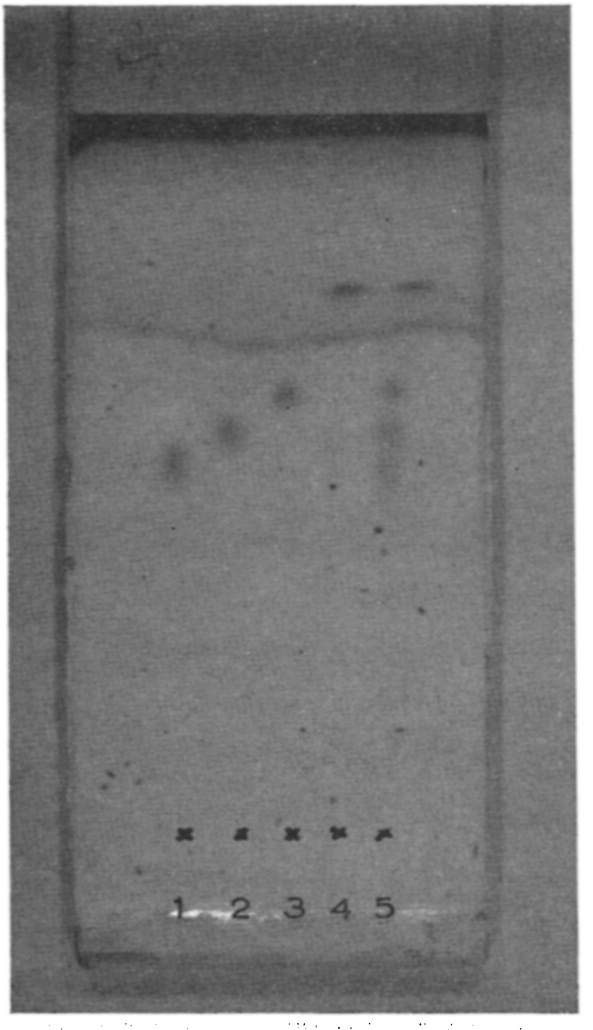

Fig. 2. Thin-layer chromatography of erythromycins on silica gel G plates. Solvent system: methylene chloride–methanol–benzene–formamide (80:20:20:4); (relative humidity 25 %). (1) erythromycin A (2 γ); (2) erythromycin B (2 γ); (3) anhydroerythromycin[6] (2 γ); (4) 2′-acetylerythromycin[7] (2 γ); (5) mixture of 1, 2, 3 and 4 (4 γ). Time of development 40 min; spot detection with 50 % aqueous H_2SO_4 spray and charring.

more sensitive, the spots tend to fade rapidly one to two hours after spraying. Charring with H_2SO_4 is preferable if the spots should remain visible for documentation.

Erythromycin A and B, erythromycin A "Hemiketal"[2], anhydroerythromycin[6], and several erythromycin acetates were separated using the methylene chloride–methanol–benzene–formamide solvent system (Figs. 1 and 2).

Thin-layer chromatography on silica gel G plates using the methylene chloride–methanol–benzene–formamide system has proven to be an efficient and sensitive method for the separation of erythromycins. This procedure is preferable to paper chromatography since the time of analysis is greatly decreased.

Acknowledgement

The author wishes to thank Dr. P. H. JONES for supplying the erythromycin samples and giving guidance and encouragement.

Organic Chemistry Department, Research Division,
Abbott Laboratories, North Chicago, Ill. (U.S.A.) TAIMI T. ANDERSON

[1] M. PIECHOWSKA AND B. OSTROWSKA-KRYSIAK, *Med. Doswiadczalna Mikrobiol.*, 13 (1961) 167.
[2] V. C. STEPHENS AND J. W. CONINE, *Antibiot. Ann.*, (1958–1959) 346.
[3] V. ŠEVČÍK, M. PODOJIL AND A. VRTIŠKOVÁ, *Folia Biol. (Prague)*, 3 (1957) 218.
[4] E. STAHL AND U. KALTENBACH, *J. Chromatog.*, 5 (1961) 458.
[5] E. STAHL, *Chemiker Ztg.*, 82 (1958) 323.
[6] P. F. WILEY, K. GERZON, E. H. FLYNN, M. V. SIGAL, O. WEAVER, U. C. QUARCK, R. R. CHAUVETTE AND R. MONAHAN, *J. Am. Chem. Soc.*, 79 (1957) 6062.
[7] V. C. STEPHENS, *Union of South Afrika Patent* 2616/58, July 11, 1958.

Received August 5th, 1963

J. Chromatog., 14 (1964) 127–129

Bioautography of antibiotic spread-layer chromatograms

Thin-layer chromatography (TLC) of antibiotics is receiving increasing attention[1–6]. In comparison with paper chromatography, TLC has greater resolving power, is the more rapid of the two techniques and can be more easily scaled up to preparative scale. A necessary adjunct to any chromatographic technique is a means of detection; with TLC, U.V. absorption, U.V. fluorescence, color producing reagents, charring with mineral acids and heat, and bioautography are methods in use. For antibiotics, bioautography is preferable and necessary because it is usually more sensitive than chemical methods and detects only biologically active components.

Several reports in the literature describe bioautography of antibiotics separated by TLC. NICOLAUS, CORONELLI AND BINAGHI[1] poured agar seeded with *Bacillus subtilis* over the developed chromatographic plate, while BICKEL *et al.*[3] and BRODASKY[5] pressed the chromatographic plate onto seeded agar plates. These investigators used adsorbents containing binder. In our studies, we have used the spread-layer technique[7–9], in which no binder is present in the adsorbent. Some advantages of this method are: alumina plates can be prepared and used within a matter of minutes, alumina of the desired activity can be used and the need for costly spreading devices