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Note

A simple thin-layer chromatographic identification procedure for erythromycin base, stearate, estolate and ethylsuccinate

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Erythromycin, a broad-spectrum antibiotic, is the most important member of the macrolide antibiotic group and is produced in fermentation by *Streptomyces erythreus*[1]. Anti-bacterial activity is highest against gram-positive bacteria with a lower order of activity against gram-negative strains and the drug is mainly used for treating some disease states in patients who are allergic to penicillin. Erythromycin is marketed in the form of the base or as salts, esters or an ester–salt combination (stearate, gluceptate, lactobionate, ethylsuccinate, ethylcarbonate and estolate). Erythromycin estolate is the generic name for the lauryl sulfate salt of propionyl erythromycin.

Erythromycin base is susceptible to acid hydrolysis in the stomach, therefore acid-resistant coatings of the tablets or chemical derivatization of the basic drug as esters or salts are required for oral administration. The estolate is acid-stable and is hydrolyzed in the small intestine to the free base which is then readily absorbed.

The present pharmacopoeial procedure for the analysis of this drug is microbiological[2,3]. Esters are hydrolyzed to yield free base prior to the analysis and the results are calculated and expressed in terms of equivalence to erythromycin base.

In our current program of developing more specific and less time-consuming identification methods for antibiotics, we reported thin-layer chromatographic (TLC) methods which differentiate erythromycin base from erythromycin estolate and ethylsuccinate[4] and recently a system that allows the detection of both the stearic acid and erythromycin portions of the erythromycin stearate molecule and thus differentiates this salt from other erythromycin derivatives[5]. However, differentiation between estolate and ethylsuccinate[4] by TLC was inconclusive, there being difference in R_F of only 0.02.

In the present publication we wish to report a new TLC procedure, employing three solvent systems, capable of differentiating between erythromycin base, stearate, estolate and ethylsuccinate. Commercially available TLC plates are employed and sodium acetate buffering of plates is unnecessary[4]. The procedure is simple, rapid and applicable to both bulk drug and formulations.

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EXPERIMENTAL

TLC plates

Commercially available pre-coated silica gel 60 F_{254} (Merck) plates (20 × 20 cm, 0.25 mm thickness) were employed. Plates were activated for 30 min at 130° prior to use.

Solvent systems

Three solvent systems: A, methanol; B, chloroform–methanol–acetic acid (90:10:1), and C, methanol–chloroform–acetic acid (90:5:5) were employed.

Spray reagent[6]

Potassium dichromate (5.0 g) dissolved in 40% (v/v) sulfuric acid (100 ml) was used as the spray reagent.

Solutions for TLC

Standard solutions employed were as follows: For erythromycin estolate, ethylsuccinate and stearate, solutions of 50 mg/ml in chloroform were prepared. In the case of erythromycin base, chloroform–methanol (2:1) was used. Stearic acid (Applied Research Labs.) and sodium lauryl sulfate (Sigma) were dissolved in chloroform and water, respectively (20 mg/ml).

Sample solutions used were as follows. Capsules: the contents of one capsule (equivalent to 250 mg erythromycin) were transferred to a 15-ml glass-stoppered centrifuge tube, and a 5.0-ml portion of chloroform was added; vigorous shaking for several minutes and centrifugation at 400 *g* for 5 min gave a clear supernatant for application to the TLC plate. Tablets: one tablet (equivalent to 250 mg erythromycin) was ground using a mortar and pestle; the powder was then transferred to a 15-ml glass-stoppered centrifuge tube and processed as described above for capsules. Oral Suspensions: a 5.0-ml portion of the well shaken liquid formulation (equivalent to 250 mg erythromycin) was transferred to 125 ml separatory funnel and diluted with 10.0 ml of distilled water; a 5.0-ml portion of chloroform was added, and the organic layer was separated for application to a TLC plate.

Chromatographic procedure

Solutions (1 μl) containing 50 μg of the drug (20 μg for stearic acid and sodium lauryl sulfate) were applied to the plates by means of micropipettes and the plates were placed in a filter paper-lined chromatographic chamber which had been saturated with solvent vapour for 1 h prior to use. The plates were developed to a height of 15 cm, then removed from the chamber and dried at 130°. Visualization was achieved by spraying the plates with the spray reagent and charring. Systems A and C required only 20 min heating at 150°, while B required heating for 1 h at 150°.

RESULTS AND DISCUSSION

Although there is a TLC method already reported[4], we feel that our new procedure, employing three solvent systems, is more specific and makes possible the conclusive differentiation between erythromycin base and stearate and between erythro-

mycin estolate and ethylsuccinate. A schematic representation of the method is shown in Fig. 1. TLC system A differentiates between base (including stearate) and esters (including estolate). Erythromycin base and stearate are then differentiated in system B[5]. Erythromycin esters (ethylsuccinate and estolate) are subsequently differentiated in system C. Erythromycin estolate in this system separates into two spots, one with R_F 0.80 due to lauryl sulfate and the other one with R_F 0.42 due to erythromycin propionate while erythromycin ethylsuccinate gives only one spot with R_F 0.44.

The corresponding R_F values for various erythromycin derivatives and stearic acid and sodium lauryl sulfate in three solvent systems are listed in Table I. The dif-

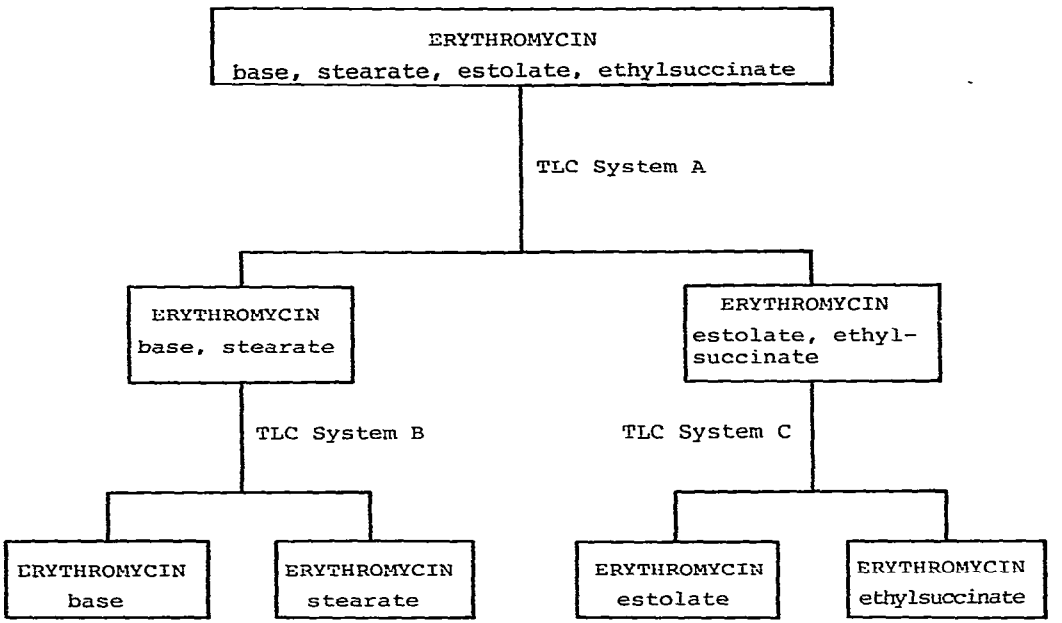

Fig. 1. Schematic representation of identification procedure enabling differentiation of erythromycin derivatives by TLC.

TABLE I

R_F VALUES OF ERYTHROMYCIN DERIVATIVES AND STEARIC ACID AND SODIUM LAURYL SULFATE ON SILICA GEL 60 IN SOLVENT SYSTEMS A, B AND C

R_F values are averages of 5 plates. N.D., not detectable after heating for 20 min at 150°. Solvent systems: see text.

Compound	*R_F value*		
	A	*B*	*C*
Erythromycin	0.29	0.05	0.40
Erythromycin stearate	0.29	0.04, 0.68	0.38
Stearic acid	N.D.	0.69	N.D.
Erythromycin estolate	0.73, 0.93	0.10, 0.06	0.42, 0.80
Sodium lauryl sulfate	0.93	0.06	0.80
Erythromycin ethylsuccinate	0.74	0.10	0.44

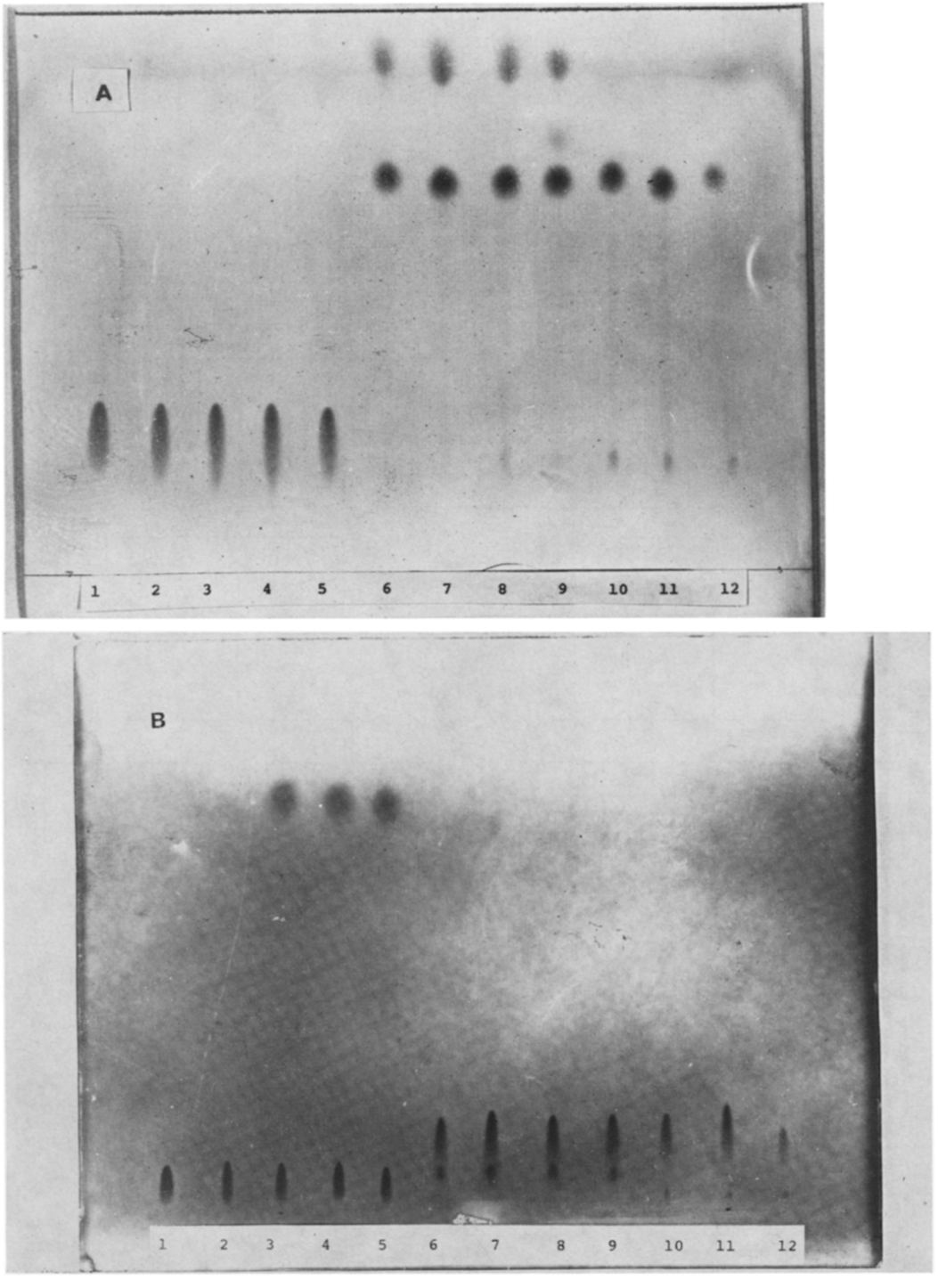

Fig. 2.

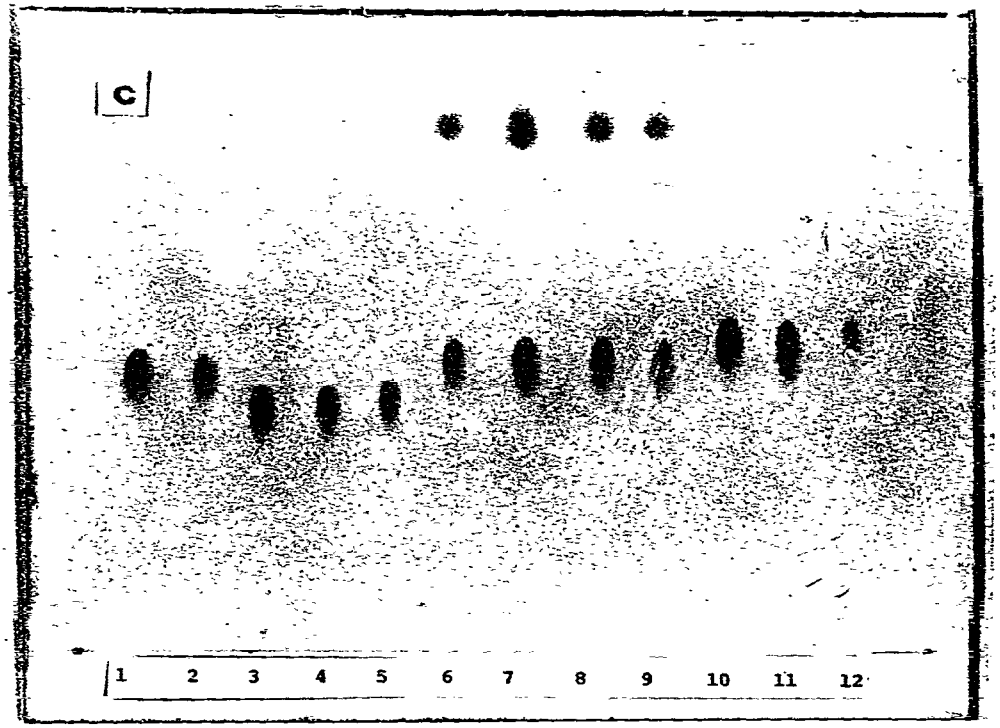

Fig. 2. Chromatograms of erythromycin formulations on Silica gel 60 in solvent systems A, B and C. 1 = Erythromycin reference standard[7]; 2 = erythromycin tablets[7]; 3 = erythromycin stearate tablets[7]; 4 = erythromycin stearate oral suspension, manufacturer's standard; 5 = erythromycin stearate reference standard[7]; 6 = erythromycin estolate reference standard[8]; 7 = erythromycin estolate capsules[9]; 8 = erythromycin estolate oral suspension[9]; 9 = erythromycin estolate sulfa tablets, manufacturer's standard; 10 = erythromycin ethylsuccinate reference standard[7]; 11 = erythromycin ethylsuccinate tablets[8]; 12 = erythromycin ethylsuccinate for oral suspension[7].

ferences in R_F values and the presence of additional spots in the case of stearate (system B) and estolate (system C), corresponding to stearic acid and sodium lauryl sulfate, provided positive identification for the respective compounds.

In Fig. 2, typical chromatograms obtained with eight formulations and erythromycin base, stearate, estolate and ethylsuccinate standards are shown. Excipients did not interfere with the method. The method is also capable of detecting the presence of sulfa drugs in the erythromycin estolate formulation containing sulfadiazine, sulfamerazine and sulfamethazine. All three sulfa drugs run with the same R_F in system C (0.72), are visible under short-wave UV light (254 nm) and can be detected as a grayish-brown spot which appears immediately after spraying with the spray reagent but disappears on charring.

Our proposed TLC identification procedure is well suited for survey programs because of its simplicity and the ease with which conclusive differentiation of erythromycin base, stearate, estolate and ethylsuccinate can be obtained.

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