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Note

Simple thin-layer chromatographic identification method for erythromycin stearate

K. C. GRAHAM, W. L. WILSON and A. VILIM*

Drug Research Laboratories, Health Protection Branch, Health and Welfare Canada, Ottawa K1A 0L2 (Canada)

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The broad-spectrum antibiotic erythromycin is formulated as salts (stearate, gluceptate, lactobionate) and esters (propionate, ethyl succinate, ethyl carbonate) as well as the free base and an ester–salt combination (estolate). Their structures are illustrated in Fig. 1.

The methods for analysis of these products in the British Pharmacopoeia[1] and Code of Federal Regulations[2] are microbiological with prior hydrolysis required in the case of the esters. In all cases, the results are calculated and expressed in terms of equivalence to erythromycin base and give no indication of the form of erythromycin assayed. This is determined by identity methods: colour tests in the British Pharmacopoeia for the base, salts, and esters as well as paper chromatography for the estolate, and infrared spectroscopy in the Code of Federal Regulations (for U.S.P. and N.F. products) for all erythromycin bulk drug materials.

In our search for more specific and less time-consuming identity methods, we reported a thin-layer chromatographic (TLC) method[3] which differentiated erythromycin base from erythromycin estolate and erythromycin ethyl succinate. The system, however, could not separate erythromycin base from the stearate, gluceptate or lactobionate salts, all of which chromatographed as the free base, nor could the stearate portion be detected.

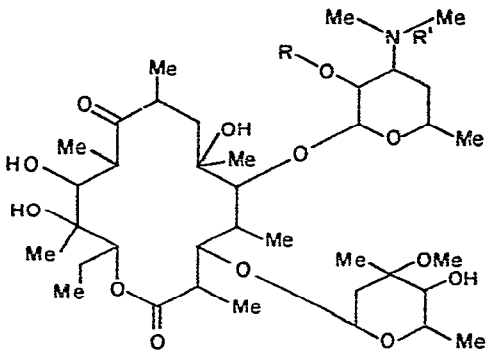
We wish to report here a new TLC method that allows the detection of both the stearic acid and erythromycin portions of the erythromycin stearate molecule and thus differentiates this salt from other erythromycin derivatives. The method is simple, rapid, and applicable to both bulk drug and formulations.

EXPERIMENTAL

TLC plates

Commercially available pre-coated silica gel 60 (Merck) plates (20 × 20 cm, 0.25 mm thickness) were employed. The plates were activated for 30 min in the oven at 130° prior to use.

* To whom correspondence should be addressed.



	R	R'
Erythromycin	H	
Propionyl erythromycin	CH_3CH_2CO	
Erythromycin estolate	CH_3CH_2CO	$C_{12}H_{25}OSO_3H$
Erythromycin stearate	H	$C_{17}H_{35}COOH$
Erythromycin lactobionate	H	$C_{11}H_{21}O_{10}COOH$
Erythromycin gluceptate	H	$C_6H_{13}O_6COOH$
Erythromycin ethyl succinate	$CH_3CH_2OOCCH_2CH_2CO$	
Erythromycin ethyl carbonate	CH_3CH_2OOC	

Fig. 1. Structural formulae of erythromycin derivatives. Me = CH_3.

Spray reagent[4]

Potassium dichromate (5.0 g) was dissolved in 40% sulfuric acid (100 ml).

Solutions for spotting

Standard solutions. A solution (50 mg/ml) of each of the erythromycin compounds (Table I) was prepared in chloroform or, in the case of erythromycin base, gluceptate and lactobionate, in cloroform–methanol (2:1). Stearic acid (Applied Research Labs., Rochester, New York, U.S.A.) was dissolved in chloroform (20 mg/ml).

Sample solutions. In the case of tablets, one tablet (equivalent to 250 mg erythromycin activity) was ground using a mortar and pestle, the powder was then transferred into a 15-ml stoppered glass centrifuge tube, and a 5.0-ml portion of chloroform was added. Vigorous shaking for several minutes and centrifugation at 300 rpm for 5 min gave a clear supernatant solution for spotting on the TLC plate.

In the case of oral suspensions, a 5.0-ml portion of the well shaken liquid formulation (equivalent to 250 mg erythromycin activity) was transferred into a 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 separated for spotting on a TLC plate.

TABLE I

R_F VALUES OF ERYTHROMYCINS AND STEARIC ACID ON SILICA GEL 60

Solvent system: Chloroform–methanol–acetic acid (90:10:1).

Compound	*R_F value**
Erythromycin	0.05
Erythromycin stearate**	0.04, 0.68
Stearic acid	0.69
Erythromycin ethyl succinate	0.10
Erythromycin estolate	0.10
Erythromycin gluceptate	0.04
Erythromycin lactobionate	0.04

* Average of five plates.

** Two spots, *viz.* erythromycin free base and stearic acid.

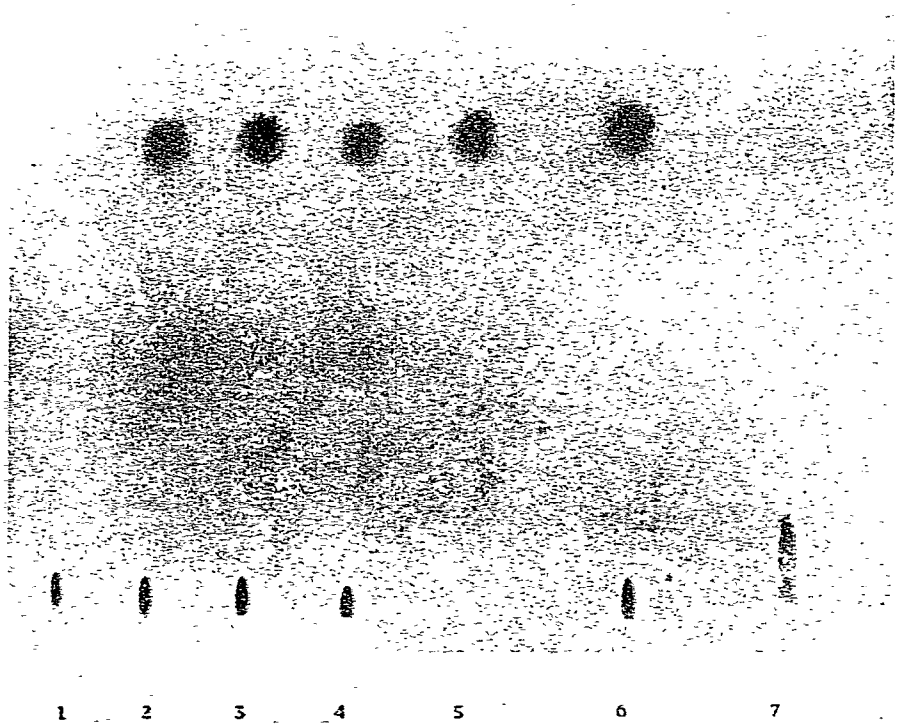


Fig. 2. Chromatogram of erythromycin formulations on silica gel 60. 1 = Erythromycin tablets, U.S.P.; 2 = erythromycin stearate tablets, B.P.; 3 = erythromycin stearate tablets, U.S.P.; 4 = erythromycin stearate reference standard, U.S.P.; 5 = stearic acid standard; 6 = erythromycin stearate oral suspension, manufacturer's standard; 7 = erythromycin estolate oral suspension, N.F.

Chromatographic procedure

Sample solutions (1 μl) representing 50 μg (20 μg for stearic acid) were applied to the plate by means of micropipettes and the plates were inserted into a filter paper-lined chromatographic chamber which had been saturated with solvent vapour for 1 h prior to use. The solvent system employed was chloroform–methanol–acetic acid

(90:10:1). The plates were developed to a height of 15 cm (approx. 75 min), then removed from the chamber, dried at room temperature, then uniformly sprayed with the spray reagent and heated for 1 h at 150°.

RESULTS AND DISCUSSION

Chromatography of erythromycin stearate in our solvent system produces two spots on a TLC plate after spraying with chromic acid–sulphuric acid reagent and charring, one due to stearic acid and the other to erythromycin moiety. Acetic acid, being a stronger acid than stearic acid, displaces the stearate anion, which is then protonated to release free fatty acid. The corresponding R_F values for various erythromycin derivatives and stearic acid are listed in Table I.

In Fig. 2, a typical chromatogram obtained with five formulations and erythromycin stearate and stearic acid standards is shown. The formulations included erythromycin tablets, erythromycin stearate tablets (both B.P. and U.S.P.), erythromycin estolate liquid, and erythromycin stearate liquid. As can be seen from Fig. 2, pharmaceutical excipients do not interfere with the method, both the stearate and erythromycin portions of the molecule being well resolved from each other.

The use of the chromic acid–sulphuric acid spray reagent and a prolonged heating period were required in order to detect the fatty acid portion of the drug. Although less than 50-μg quantities could be detected on the plate, 50 μg amounts gave well defined, reproducible spots.

The use of this proposed TLC identification method enables fast differentiation of erythromycin stearate from erythromycin base and other erythromycin derivatives —ethyl succinate, estolate, gluceptate and lactobionate.

REFERENCES

1 *British Pharmacopoeia*, Her Majesty's Stationary Office, London, 1973.
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