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CHROMATOGRAPHIC DIFFERENTIATION OF ERYTHROMYCIN AND ITS ESTERS

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SUMMARY

A rapid identification method for the detection and quality control of the erythromycin antibiotics is described. Thin-layer chromatography on sodium acetate-buffered silica gel plates enabled the differentiation of erythromycin, erythromycin estolate and erythromycin ethyl succinate from some degradation products and pharmaceutical excipients. The comparative behavior of twenty other antibiotics under these conditions is also presented.

INTRODUCTION

The official method in Canada for assaying the broad spectrum antibiotic erythromycin (Fig. 1) is microbiological[1,2]. A spectrophotometric method, based on mild alkaline hydrolysis of erythromycin or its esters has been reported by KUZEL AND COFFEY[3]. Neither method differentiates among the various active components of the erythromycin complex nor between erythromycin base and its esters in current medical use. In both cases esters such as the estolate and erythromycin ethyl succinate are first hydrolyzed and the resulting erythromycin assayed.

A problem therefore exists in that hydrolytic decomposition of ester with resulting formation of erythromycin base cannot be detected in ester formulations by these methods. The BP and USP recognize this fact and rely on separate identification tests for erythromycin estolate to differentiate it from erythromycin itself — paper chromatography (PC) in the former case and infrared spectroscopy in the latter. PC, however, is time-consuming and not suitable for rapid analysis. The infrared spectra of the base and esters show very small differences[4] and, in the case of partially hydrolyzed samples, the results may be ambiguous.

In our current program in developing chemical methods for the analysis and identification of antibiotics we have successfully assayed erythromycin and its esters by direct densitometry after separation on thin-layer chromatograms. We wish to report here a thin-layer chromatographic (TLC) system that separates erythromycin base, erythromycin estolate and erythromycin ethyl succinate from some degradation products and pharmaceutical excipients and propose its use as a rapid identification method for the detection and quality control of the erythromycin antibiotics.

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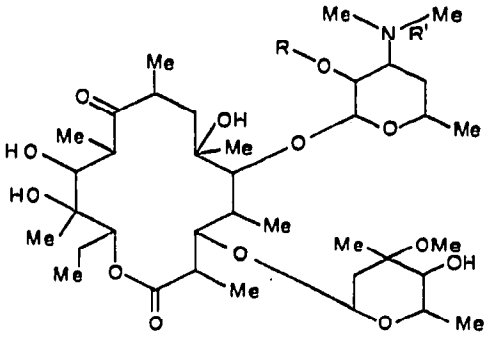

	R	R'
Erythromycin	H	
Propionyl erythromycin	CH_3CH_2CO	
Erythromycin estolate	CH_3CH_2CO	$C_{12}H_{25}OSO_3H$
Erythromycin stearate	H	$C_{17}H_{35}COOH$
Erythromycin lactobionate	H	$C_{11}H_{21}O_{10}COOH$
Erythromycin gluceptate	H	$C_6H_{13}O_6COOH$
Erythromycin ethyl succinate	$CH_3CH_2OOCCH_2CH_2CO$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

Fig. 1. Structural formulae of erythromycin derivatives.

EXPERIMENTAL

Preparation of TLC plates

TLC plates (20 × 20 cm, 0.25 mm thickness) were prepared with standard equipment using a slurry of commercial Silica Gel G (Merck) (50 g) with 0.02 *N* aqueous sodium acetate (100 ml). The plates were air-dried overnight.

Spray reagent[5]

A solution of glucose (2 g) in a mixture of 85 % phosphoric acid (10 ml), water (40 ml), ethanol (30 ml) and *n*-butanol (30 ml) was freshly prepared daily.

Solutions for spotting

A solution of each of the erythromycin compounds (Table I) was prepared in methanol (10 mg/ml). In the case of capsules containing excipients insoluble in methanol, the suspensions were centrifuged and the supernatant solutions were used for subsequent spotting. Similarly samples (10 mg) of other antibiotics were shaken with methanol (1 ml) and the supernatants were spotted.

Chromatographic procedure

Samples of the methanolic solution of each compound representing 20 μg (2 μl) were applied to the plate by means of micropipettes and the plates were inserted into a filter paper-lined chromatographic chamber which was saturated with solvent vapour for 1 h prior to use. The solvent system was methanol–0.02 *N* aqueous sodium acetate (120:30). The plates were developed to a height of 15 cm (60 min),

TABLE I

R_F VALUES AND COLOURS OF ERYTHROMYCINS ON SILICA GEL G DEVELOPED IN METHANOL–0.02 *N* SODIUM ACETATE (120:30)

Compound	*R_F value*[a]	*Colour*
Anhydroerythronolide	0.82	red
Erythromycin estolate	0.65	blue-grey
Erythromycin ethyl succinate	0.67	blue-grey
Erythromycin ethyl carbonate	0.67	blue-grey
Erythromycin	0.28	blue-grey
Erythromycin stearate	0.28	blue-grey
Erythromycin lactobionate	0.28	blue-grey
Erythromycin gluceptate	0.28	blue-grey
Anhydroerythromycin A	0.33	blue-grey

[a] Average of ten plates

removed from the tank, uniformly sprayed with the spray reagent and heated for 5 min at 150°.

The R_F values of the various erythromycin derivatives are listed in Table I together with the colour of the spot formed after spraying.

RESULTS AND DISCUSSION

Although a number of chromatographic investigations of erythromycins have previously appeared in the literature[6,7] these reports were mainly concerned with the detection of impurities and not with distinguishing between erythromycin base and its esters. BANASZEK *et al.*[7] separated erythromycin A and B from anhydroerythromycin, but in their system erythromycin estolate and erythromycin ethyl succinate ran at the solvent front and their use of formamide as a solvent left a dark background on the plate making the system unsatisfactory for quantitation by densitometry.

After examining various systems we found that the use of sodium acetate-buffered silica gel plates gave the best separation, as indicated in Fig. 2.

Using the spray reagent specified in the experimental section, all of the erythromycins appear as blue-grey spots on a white background except for anhydroerythronolide, which appears red.

In Table II are listed the R_F values and colours of other antibiotics which we tested in our system to ensure that they did not interfere. Of these twenty-three compounds only two, nystatin and amphotericin B sulphate, had R_F values similar to those of the erythromycin esters — these, however, gave bright yellow spots on spraying in contrast to the characteristic blue-grey colour of the erythromycins. Many of these other antibiotics were only slightly soluble in methanol and the majority could not be detected with the spray reagent.

Erythromycin salts such as the lactobionate, gluceptate and stearate run as the free erythromycin base in this TLC system and are readily distinguishable from the erythromycin esters and from the common degradation products anhydroerythromycin A and anhydroerythronolide. Of the three esters, erythromycin ethyl succinate

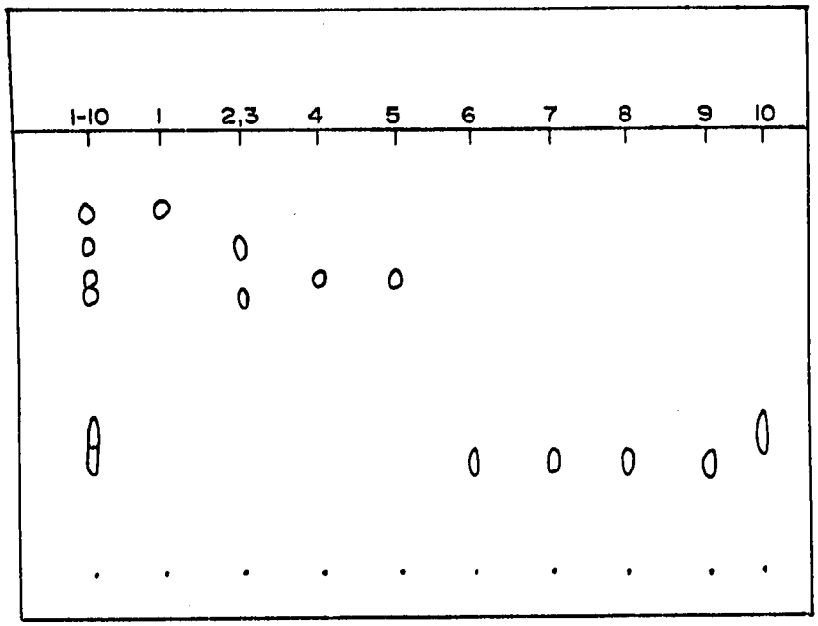

Fig. 2. Chromatogram of erythromycins on Silica Gel G. 1 = Anhydroerythronolide; 2 = sulphamerazine, sulphadiazine, sulphamethazine; 3 = erythromycin estolate; 4 = erythromycin ethyl succinate; 5 = erythromycin ethyl carbonate; 6 = erythromycin; 7 = erythromycin stearate; 8 = erythromycin lactobionate; 9 = erythromycin gluceptate; 10 = anhydroerythromycin A.

TABLE II

R_F VALUES AND COLOURS OF OTHER ANTIBIOTICS ON SILICA GEL G DEVELOPED IN METHANOL–0.02 *N* SODIUM ACETATE (120:30)

Compound	*R_F value*	*Colour*
Sulphamerazine	0.75	yellow
Sulphadiazine	0.75	yellow
Sulphamethazine	0.75	yellow
Amphotericin B sulphate	0.68	yellow
Nystatin	0.71	yellow
Tetracycline	0.10	yellow
Oxytetracycline	0.10	yellow
Demethylchlortetracycline	0.10	yellow
Penicillin G procaine	0.34	yellow
Neomycin sulphate	N.S.[a]	
Griseofulvin	N.S.	
Lincomycin·HCl	N.S.	
Streptomycin sulphate	N.S.	
Sodium novobiocin	N.S.	
Ampicillin·$3H_2O$	N.S.	
Phenoxymethyl penicillin	N.S.	
Potassium phenethicillin	N.S.	
Potassium penicillin G	N.S.	
Sodium methicillin	N.S.	
Sodium nafcillin	N.S.	
Sodium oxacillin	N.S.	
Sodium cloxacillin	N.S.	
Sodium dicloxacillin	N.S.	

[a] No visible spot.

and erythromycin ethyl carbonate run to the same height (Table I) but are distinguishable from erythromycin estolate, which has a lower R_F value. Common ingredients such as the sulpha drugs are also separated from estolate.

Any hydrolysis of the esters to form the base can easily be detected in this TLC system. Several formulations, in particular those containing erythromycin ethyl succinate, were shown to contain detectable amounts of base.

The esters are usually prescribed because of their greater absorption when administered orally, their relative tastelessness and their resistance to gastric juice when compared to erythromycin base. The use of this proposed TLC identification procedure will enable the rapid detection of decomposition and hydrolysis products in erythromycin formulations and will ensure that the base is not readily substituted for these more expensive esters.

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