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IMPROVED HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY OF ERYTHROMYCIN IN PHARMACEUTICAL SOLID DOSAGE FORMS

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SUMMARY

A reversed-phase high-performance liquid chromatography method for the assay of erythromycins in pharmaceutical preparations is descibed. Detailed experimental procedures and results are given. The major erythromycins are well separated, very good recoveries were obtained and detection limits for erythromycins B and C were estimated.

INTRODUCTION

Erythromycin is a widely used macrolide antibiotic produced by fermentation. Erythromycin A is the major component and often contains erythromycins B and C and some of its epimers and degradation products.

Several high-performance liquid chromatographic (HPLC) methods to determine erythromycin in bulk drug and solid dosage forms[1–3], fermentation extracts[1,4,5] and biological fluids[6–9] have been reported. None of these methods is fully satisfactory for routine analysis. Peak tailing and poor resolution are common; Tsuji and Kane[3] require a column temperature of 70°C to achieve satisfactory results. Better resolution can be achieved using columns of polystyrene–divinylbenzene copolymer packing materials with mobile phases of higher pH (8.0) at 60°C[10].

Instability of the baseline monitored at low wavelengths often leads to quantitation problems. Fluorometric[6] and electrochemical[7,8] detectors are highly sensitive, but are not as commonly used as UV detectors.

This report presents a simple, precise and selective HPLC method to analyze erythromycin in pharmaceutical solid dosage forms, using ambient column temperature and monitoring the column eluate at the absorption maximum near 288 nm. The method offers good peak resolution, minimal peak tailing and excellent baseline stability.

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EXPERIMENTAL

Chemicals and reagents

Erythromycin, erythromycin B, erythronolide B, macrosyl erythronolide B, and erythromycin C were obtained from Abbott Labs. Acetonitrile and methanol were HPLC grade (Fischer Scientific). Acetic acid and ammonium phosphate dibasic were ACS certified (Fischer Scientific). N,N-Dimethylcyclohexanamine was obtained from ICN Biomedical, and water was double-distilled, deionized.

Apparatus

The chromatographic system consisted of an HPLC pump (Perkin-Elmer LC Series 4 Pump), an autosampler (Perkin-Elmer LC-420 autosampler equipped with a 20-μl loop), a reversed-phase C_{18} column (Whatman Partisil 5-ODS3 RAC II, 10 cm $\times$ 4.6 mm I.D.), a variable-wavelength UV detector (Perkin-Elmer LC-95 spectrophotometer, set at 288 nm), and a computing integrator (Spectra-Physics SP4270).

Mobile phase

The mobile phase consisted of acetonitrile–methanol–N,N-dimethylcyclohexanamine–0.012 *M* dibasic ammonium phosphate (350:100:1.5:550) adjusted to pH 5.3 with acetic acid. It was filtered through a 0.45-μm Zetapor membrane, or equivalent, and degased before use. The flow-rate was 0.5 ml/min.

Preparation solvent

The preparation solvent consisted of acetonitrile–methanol–N,N-dimethylcyclohexanamine–0.012 *M* dibasic ammonium phosphate (350:100:1.5:550) adjusted to pH 8.8 with acetic acid.

Standard preparation

About 100 mg of erythromycin reference standard was accurately weighed and quantitatively transfered into a 100-ml volumetric flask. The standard was dissolved in and brought to volume with preparation solvent to produce a solution having a known concentration of about 1.0 mg/ml.

Assay preparation

The contents of 20 capsules or tablets were accurately weighed and finely powdered. An amount of the powder blend, equivalent to about 100 mg of erythromycin, was accurately weighed and quantitatively transfered into a 100-ml volumetric flask. About 70 ml of preparation solvent was added and sonicated for 10 min. The mixture was allowed to cool to room temperature, diluted to volume with the same solvent and mixed. A portion of this mixture was filtered through a 0.45-μm Zetapor membrane or equivalent, discarding the first 10 ml of the filtrate.

System suitability

Six successive injections of the standard preparation should provide a relative standard deviation (R.S.D.) of not greater than 1.0%. A plot of peak area *versus* concentration (0.4–2.8 mg/ml) should result in a straight line. The number of theoretical plates (N) should be not less than 1000. If system suitability is not obtained,

adjust the parameters, clean or replace the column, if necessary, until system suitability is obtained.

Procedure

Alternately 20-μl volumes of the standard and assay preparations were injected into the chromatographic system and the chromatograms recorded.

Calculation

Amount in mg of erythromycin per capsule or tablet = $100\,C\,(R_s/R_u)\,(W_u/W_a)$, where R_s and R_u are the peak areas obtained from the standard and assay preparations, respectively, C is the concentration, in mg/ml, of erythromycin in the standard preparation, and W_a and W_u are the average capsule or tablet weights, in mg, and the sample weight, in mg, respectively.

RESULTS AND DISCUSSION

Chromatographic detection

As the adsorption of erythromycin is very weak at 288 nm [e (molar absorbtivity) = 34.2 l/mol cm, (Fig. 1) erythromycin has been monitored at 215 nm (end absorption). During the analysis of Eryc capsules using the existing HPLC method, non-reproducible results were often experienced, which were attributed to unstable baseline, deterioration in peak shape, and bad resolution as shown in Fig. 2.

With the advent of highly sensitive UV detectors, such as the Perkin-Elmer LC-95 Spectrophotometer, it is possible now to monitor erythromycin at the maximum absorption wavelength near 288 nm, which is selective, and avoids unwanted detection of interfering substances resulting from end absorption, and provides stable and consistent baseline. This was evident by almost constant retention times and shape of the peaks throughout the testing period of more than three months.

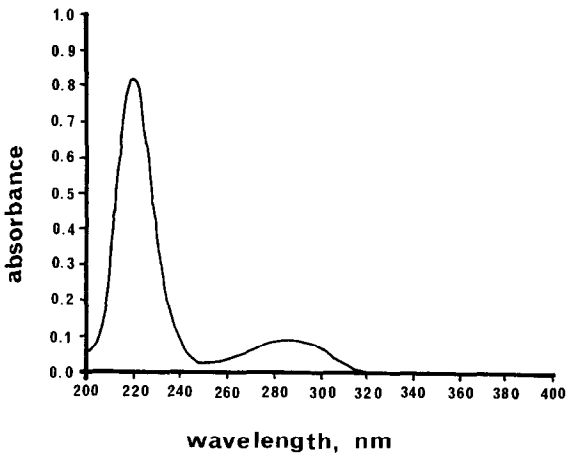

Fig. 1. UV absorption spectrum of erythromycin in mobile phase (2.0 mg/ml).

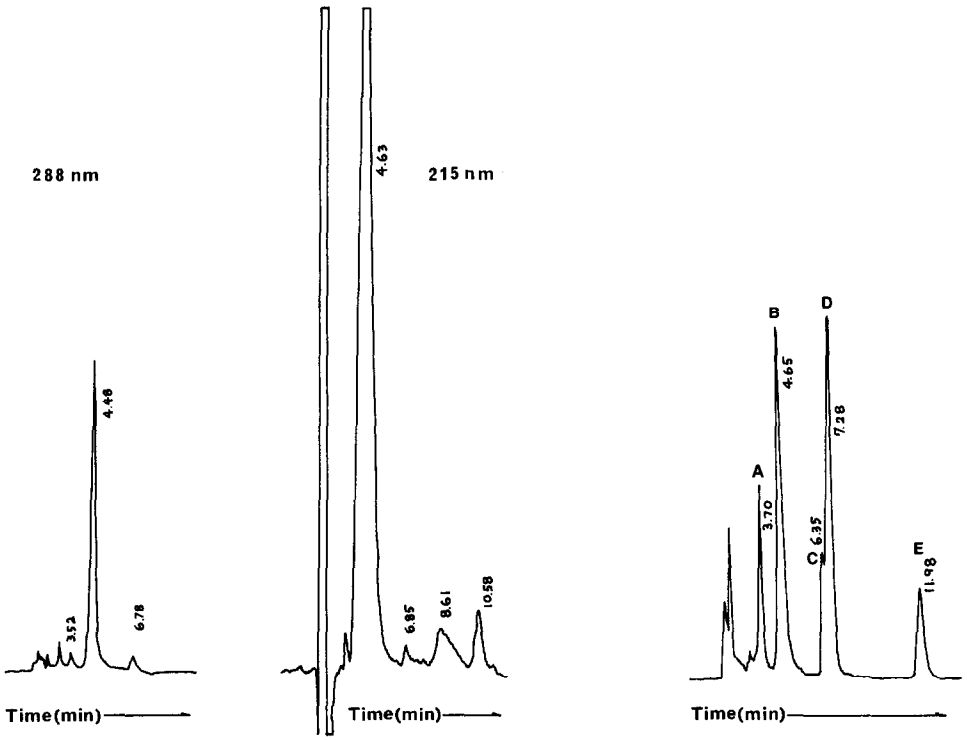

Fig. 2. Liquid chromatograms of erythromycin monitored at 215 and 288 nm. For conditions see Experimental.

Fig. 3. Liquid chromatogram of a synthetic mixture containing: (A) erythromycin C, 1.0 mg/ml; (B) erythromycin A, 2.0 mg/ml; (C) erythromycin B, 1.0 mg/ml; (D) erythronolide B, 1.0 mg/ml; (E) 3-mycarosyl erythronolide B, 0.5 mg/ml.

Chromatographic separation

Fig. 3 shows a typical chromatogram obtained from a synthetic mixture of erythromycins A, B and C and erythronolide B and macrosyl erythronolide B. Their relative retentions are given in Table I. As shown, erythromycins A, B and C are all well-resolved from each other, but erythromycin B merges with erythronolide B. Fig.

TABLE I

RELATIVE RETENTION OF VARIOUS ERYTHROMYCINS

Compound	*Relative retention*
Erythromycin A	1.0
Erythromycin B	1.4
Erythronolide B	1.6
Macrosyl erythronolide B	2.5
Erythromycin C	0.8

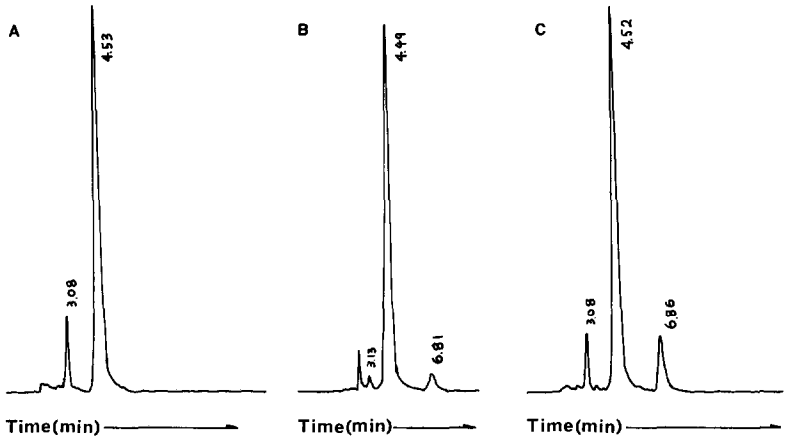

Fig. 4. Typical chromatograms obtained from: (A) USP erythromycin RS; (B) erythromycin, Abbott, Lot No. 67-189CD; (C) erythromycin, Upjohn, Lot No. 081US.

4 presents the chromatograms obtained from an USP reference standard, and two lots of bulk drug. Small amounts of erythromycins B and C were detected in each lot tested. Fig. 5 shows the chromatograms obtained from solutions of erythromycin, 5.0 mg per ml, in 0.1 *M* hydrochloric acid and 0.1 *M* sodium hydroxide containing 15% methanol, respectively, and kept at room temperature for 72 h. The samples were diluted five-fold in preparation solvent before the HPLC analysis. As shown, almost no erythromycin A remains, showing that the method is stability-indicating.

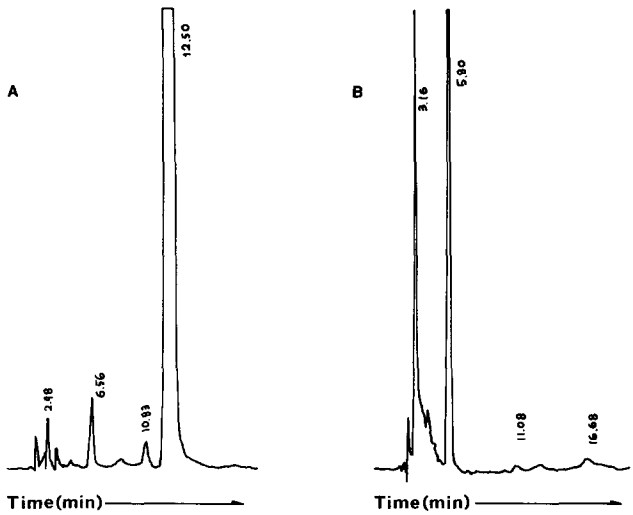

Fig. 5. Typical chromatograms obtained from erythromycin 5.0 mg/ml in 0.1 *M* hydrochloric acid (A) and 0.1 *M* sodium hydroxide containing 15% methanol (B); 72 h at room temperature.

TABLE II

RECOVERY OF ERYTHROMYCIN FROM TABLET PLACEBO

Label claim added (%)	*Recovery (%)*
80	99.8
80	99.8
100	101.5
100	99.2
100	99.0
100	99.0
100	99.8
100	98.5
Average	99.5
R.S.D. (%)	1.0
120	101.1
120	101.4

METHOD VALIDATION

Recovery and precision

Recoveries were performed by spiking erythromycin into capsule placebo at 80–120% of label claim. An average recovery of 99.5% was obtained at 100% of label claim with an R.S.D. of 1.0%. The results are summarized in Table II.

System suitability

Six replicate injections of the standard preparation gave a precision of 0.8%. A plot of peak area *versus* concentration (0.4–2.8 mg/ml) gave a straight line. The number of theoretical plates (N) and the tailing factor (T) were calculated according to USP XXI and were 1370 and 1.5, respectively. The resolution between erythromycins A and C and between erythromycins A and B were 2.7 and 3.9, respectively.

Stability of erythromycin in preparation solvent and mobile phase at room temperature

Erythomycin is not stable in acidic or alkaline solutions. Its maximum stability is between pH 6.0 and 9.5[11,12]. An aqueous alcoholic solution buffered at pH 7.0–

TABLE III

SHORT-TERM STABILITY OF ERYTHROMYCIN IN PREPARATION SOLVENT AND MOBILE PHASE

Medium	*pH*	*Initial*	*1 h*	*2 h*	*3 h*	*24 h*
Preparation solvent	8.8	100	99.5	98.2	99.0	99.7
Mobile phase	5.3	100	100.2	99.1	94.2	87.7

TABLE IV

HPLC ASSAY OF ERYTHROMYCIN SOLID DOSAGE FORMS

Sample	*mg per capsule or tablet*		
	Found	*Average*	*R.S.D. (%)*
Erythromycin 250-mg tablets, Abbott	242.5, 247.9 258.1, 240.7 251.5, 243.6	247.4	2.4
Erythrocin stearate 250-mg tablets, Abbott	249.2, 256.3 261.2, 259.7 254.2, 250.8	255.2	1.7
Erythromycin 250-mg tablets, Upjohn	264.6, 253.9 265.8, 256.7 271.4, 257.2	262.6	2.3
Erythromycin 250-mg capsules, Parke-Davis	255.8, 255.0 252.5, 252.9 258.8, 255.1	255.0	0.8

8.0 is stable for about one week under refrigeration[9]. Tsuji and Goetz[1] recommended that the pH of the solution be kept at or near 8.8. The short-term stability of erythromycin in the preparation solvent and mobile phase at room temperature was examined. The results are summarized in Table III.

Erythromycin exhibits excellent stability in preparation solvent over a period

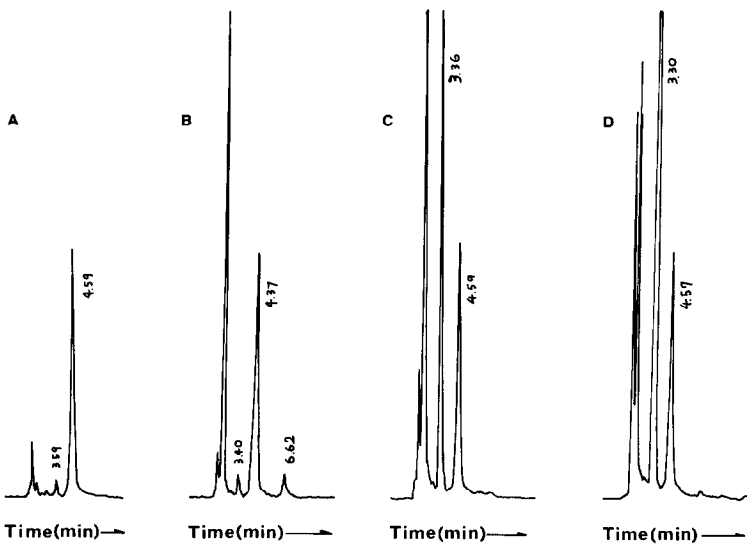

Fig. 6. Typical chromatograms obtained from dosage form analysis: (A) Eryc 250 mg capsules, WL78, 700-7, Lot 02304F, Parke-Davis; (B) E-Mycin 250 mg tablets, Lot 389RH, Upjohn; (C) Ery-Tab 250 mg, Lot 72490AF21, Abbott; (D) Erythrocin stearate filmtab 250 mg, Lot 65-348Af21, Abbott.

of 24 h. The chromatograms obtained initially and after 24 h are almost identical. Also no significant degradation of erythromycin is anticipated during the chromatographic elution as indicated from the stability in the mobile phase.

Detection limits for erythromycins B and C

Since erythromycins B and C are the major contaminants in erythromycin, the lowest detection limits were determined and found 0.5% for erythromycin B and 1.0% for erythromycin C when measured at an attenuation of 2.

Dosage form analysis

Table IV presents the assay data on four lots of commercial erythromycin solid dosage forms. Results are all within 2.4% of label claim with excellent precision. Fig. 6 shows the typical chromatograms obtained from these analyses. For comparison, microbiological assay was performed on erythromycin 250-mg capsules (Parke-Davis). An average of 255.5 mg per capsule (265.0, 245.0, 250.0, 250.0, 275.0, and 250.0) with an R.S.D. of 4.5% was obtained indicating close agreement between the HPLC and microbiological assays.

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REFERENCES

1 K. Tsuji and J. F. Goetz, *J. Chromatogr.*, 147 (1978) 359.
2 K. Tsuji and J. F. Goetz, *J. Chromatogr.*, 157 (1978) 185.
3 K. Tsuji and M. P. Kane, *J. Pharm. Sci.*, 71 (1982) 1160.
4 K. Tsuji and J. F. Goetz, *J. Antibiot.*, 31 (1978) 302.
5 G. Pellegatta, G. P. Carugati and G. Coppi, *J. Chromatogr.*, 269 (1983) 33.
6 K. Tsuji, *J. Chromatogr.*, 158 (1978) 337.
7 M.-L. Chen and W. L. Chiou, *J. Chromatogr.*, 278 (1983) 91.
8 G. S. Duthu, *J. Liq. Chromatogr.*, 7 (1984) 1023.
9 C. Stubbs, J. M. Haigh and I. Kanfer, *J. Pharm. Sci.*, 74 (1985) 1126.
10 I. O. Kibwage, E. Roets, J. Hoogmartens and H. Vanderhaeghe, *J. Chromatogr.*, 330 (1985) 275.
11 W. L. Koch, *Anal. Profiles Drug Subst.*, 8 (1979) 159.
12 F. Kavanagh, *Anal. Microbiol.*, 1 (1963) 294.