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A stability-indicating high-performance liquid chromatographic assay of erythromycin estolate in pharmaceutical dosage forms

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Summary

A high-performance liquid chromatographic (HPLC) method utilizing either ultraviolet (UV) or electrochemical detection for the analysis of erythromycin estolate in pharmaceutical dosage forms is described. Special considerations relating to the stability of the estolate moiety during sample manipulation and storage in the autosampler are discussed. The UV detector was found to be adequate for the routine analysis of dosage forms containing erythromycin estolate while the electrochemical detector provided advantages in stability studies due to its increased sensitivity and its ability to detect trace amounts of degradation products. The inclusion of oleandomycin phosphate as the internal standard greatly increased the precision of the method which was successfully employed for the analysis of individual tablet, capsule and suspension dosage forms containing varying amounts of erythromycin estolate obtained from three different manufacturers.

Introduction

Erythromycin estolate, the lauryl sulphate salt of the 2′-propionyl ester of erythromycin base, has been formulated in both liquid and solid dosage forms for oral administration. This prodrug of erythromycin is used mainly in the treatment of infections caused by gram-positive organisms and is reported to be acid-stable due to its insolubility in acidic media (Nelson, 1962). It does, however, dissociate in the less acidic environment of the small intestine to the inactive propionyl ester, in which form it is absorbed and subsequently hydrolyzed to the active base component (Tardrew et al., 1969).

Current official methods of assay for erythromycin estolate (raw material and dosage forms) involve the use of microbiological methods (BP, 1988; USP, 1990) which lack specificity and cannot differentiate between active and inactive moieties. These methods allow the determination of total erythromycin content as the erythromycin base equivalent and involve a time-consuming hydrolysis step. Since the microbiological assay is not stability-indicating, the presence of erythromycin base and any related degradation products in raw material or dosage forms of erythromycin estolate cannot be established.

Various methods have been reported for the separation of erythromycin base and estolate (Radecka et al., 1972; Richard et al., 1972; Graham

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et al., 1976; Vilim et al., 1977; Koch, 1979) and the quantitative analysis of erythromycin products in general (Tsuji and Robertson, 1971; Tsuji and Goetz, 1978; Tsuji and Kane, 1982; Geria et al., 1987). However, the quantitative analysis of dosage forms containing erythromycin estolate per se has received little attention.

This report describes a rapid, precise and stability-indicating method to analyze erythromycin estolate in raw material and in pharmaceutical dosage forms using high-performance liquid chromatography (HPLC) with either UV or electrochemical detection. The method was applied to the analysis of individual tablet, capsule and suspension dosage forms in order to determine content uniformity prior to bioavailability assessment.

Materials and Methods

Equipment

The liquid chromatograph consisted of a Waters model M6000A constant flow pump, a WISP model 710B automatic sample injector, a Kratos model 769 variable wavelength detector, a Coulochem model 5100A electrochemical detector equipped with a model 5010 analytical cell and a model 5020 guard cell, a BAS model LC-22 temperature controller and a Perkin Elmer model 56 strip-chart recorder. The mobile phase was continuously degassed with an Erma model ERC-3510 in-line solvent degasser. Other equipment included an Orion Research model 601 pH meter and an Eppendorf model 5414 micro-centrifuge.

Chemicals

All chemicals and reagents used were analytical grade. The acetonitrile and methanol were distilled-in-glass HPLC grade (Burdick and Jackson, Muskegon, MI) and the water was purified through a Milli-Q system (Millipore, Bedford, MA). Erythromycin estolate was a USP Reference Standard (Rockville, MD), oleandomycin phosphate was obtained from Pfizer Laboratories, Pietermaritzburg, RSA whilst the various dosage forms were supplied by the following companies: Ilosone tablets, capsules and suspension (Eli-Lilly, RSA); erythromycin estolate tablets and capsules (Adcock-Ingram Laboratories) and erythromycin tablets, capsules and suspensions (Lennon Laboratories, RSA).

Chromatographic conditions

Phosphate buffer (0.05 M) was prepared by adding 3.2 ml phosphoric acid to 1 l of water and adjusting the pH to 6.3 with sodium hydroxide. The mobile phase was prepared by mixing 650 ml acetonitrile with 350 ml phosphate buffer and the mixture degassed and filtered through a 0.45 μm filter (Millipore, Type HVLP). A 25 cm $\times$ 3.9 mm stainless-steel column custom-packed with Techsil C_{18}-10 μm material (HPLC Technology, U.K.) and maintained at 35°C was used together with a Waters guard-pak unit fitted with a cartridge containing 40–60 μm glass beads (Supelco, U.S.A.) The UV detector was set at a wavelength of 200 nm and was placed in-line immediately after the dual-electrode electrochemical detector which was operated in the oxidative screen mode with the upstream electrode set at +0.70 V, the downstream electrode at +0.90 V and the guard cell at +1.00 V. The mobile phase was used at a flow rate of 1.3 ml/min with a resulting back-pressure of 1500 lb/inch^2 (p.s.i.).

Internal standard

Oleandomycin phosphate (2.0 mg/ml) was dissolved in a mixture of equal parts of acetonitrile and water.

Calibration curves

Four different calibration standards were prepared as follows: Between 2.0 and 5.0 mg of erythromycin estolate was accurately weighed in a 10.0 ml volumetric flask, to which 1 ml of internal standard solution had been added. The sample was then made up to volume with acetonitrile, vortex-mixed and between 1 and 3 μl injected onto the column. Calibration curves were obtained by plotting the peak height ratios of erythromycin estolate and internal standard vs the respective calibrator concentrations.

Analysis of tablets

Intact tablets were placed in a 200 ml volumetric flask to which 100 ml deionized water had been

added. After shaking for 15 min on a mechanical shaker, 50 ml acetonitrile was added, followed by further shaking for 10 min. This process was repeated following addition of a second 50 ml acetonitrile aliquot after which the sample was made up to volume with water. The flask was then sonicated for 5 min to facilitate dissolution and allowed to equilibrate at room temperature. Then 1 ml was accurately removed using a glass pipette and transferred to a 10.0 ml volumetric flask containing 1.0 ml of the internal standard solution and made up to volume with acetonitrile. After gentle mixing, approx. 3.0 ml was transferred to a teflon centrifuge tube and spun at 12000 rpm in a high-speed micro-centrifuge for 6 min in order to precipitate any insoluble adjuvants. Sufficient sample was transferred to a WISP limited volume insert prior to injection (1–3 μl).

Six tablets from three different manufacturers were analysed in duplicate and their peak height ratios compared with those obtained for the calibration standards. These calculations yielded the amount of erythromycin estolate per tablet, which was then converted to the erythromycin base equivalent by a molecular mass correction factor of 0.6949.

Analysis of capsules

Approx. 5.0 mg of capsule content was accurately weighed and transferred to a 10.0 ml volumetric flask to which 1 ml internal standard solution and 5 ml acetonitrile had been added. After vortex-mixing for 1 min, the sample was made up to volume with acetonitrile, sonicated for 5 min and allowed to equilibrate at room temperature. Samples were centrifuged, injected and the results calculated as described for the analysis of tablets. Six capsules from three different manufacturers were analysed.

Analysis of suspensions

In order to overcome the problems associated with the transfer of small volumes of suspensions, the density of each suspension was calculated in triplicate by determining the mass of a 20.0 ml sample. Approx. 100 μl of the 125 mg/5 ml suspensions or 50 μl of the 250 mg/5 ml suspensions were transferred to a 10.0 ml volumetric flask and the mass of the transferred sample accurately weighed. Subsequently, 1 ml of internal standard solution and 4.0 ml water were added, the sample vortex-mixed (1 min) and made up to volume with acetonitrile and treated as described for the analysis of capsules. The volume of the original sample was calculated utilizing the predetermined density value and the results expressed as mg erythromycin base equivalent per 5 ml. Seven different suspension formulations were analysed in triplicate.

Validation of assay procedures

Linearity was assessed as previously described under calibration curves. Within-run precision was assessed by the repeat analysis ($n = 6$) of selected dosage forms. Sample recovery was determined using erythromycin estolate raw material (415.9, 510.0 and 637.7 mg) and proceeding as described for the analysis of tablets. Experimentally determined values were compared with theoretical values and expressed as a percentage. Sample stability in the auto-injector was assessed by re-injection of analysed samples after overnight storage in the autosampler.

Results and Discussion

Adequate separation between erythromycin estolate, erythromycin base and internal standard was obtained with no interference from dosage form adjuvants being observed. Representative chromatograms of erythromycin estolate tablets and suspensions are depicted in Fig. 1 (A–D). Adequate sensitivity was obtained by UV analysis at 200 nm allowing the same sample dilutions to be utilized for both the UV and electrochemical detectors. Utilization of a detector wavelength of 215 nm as previously described (Tsuji and Kane, 1982) resulted in a dramatic decrease in sensitivity requiring larger sample loads, thereby increasing the possibility of column overloading and interference from sample contaminants. The electrochemical detector was more sensitive than the UV detector and could be operated at lower amplifications than those required for the analysis of these compounds in biological fluids (Stubbs and

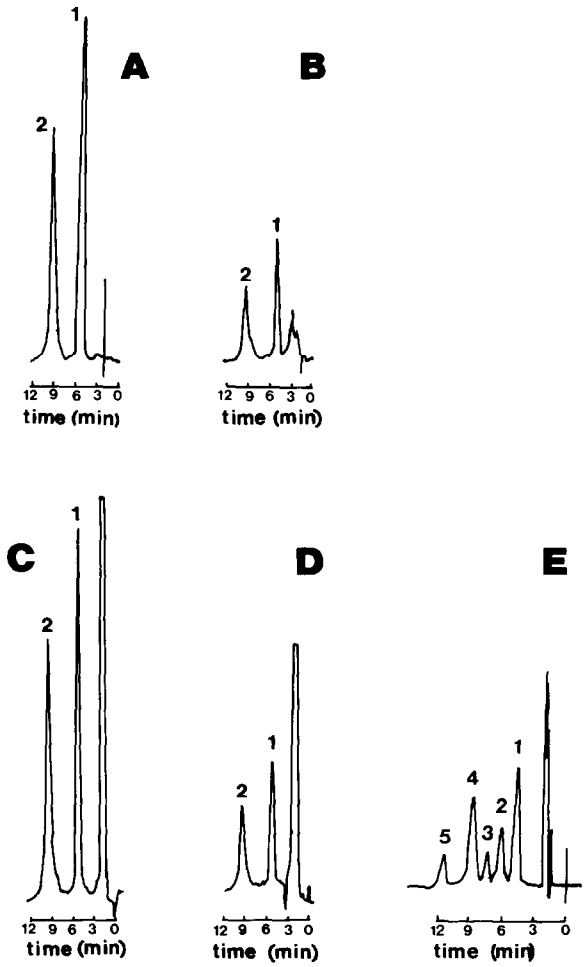

Fig. 1. High-performance liquid chromatograms of erythromycin estolate dosage forms (1, internal standard; 2, erythromycin estolate): (A) Tablet EL1 – electrochemical detection; (B) tablet EL1 – ultraviolet detection; (C) suspension EL3 – electrochemical detection; (D) suspension EL3 – ultraviolet detection; (E) sample containing oleandomycin (1), erythromycin base (2), anhydroerythromycin (3), erythromycin estolate (4), erythromycin enol ether (5) – electrochemical detection.

Kanfer, 1988). This resulted in a more stable response with an associated reduction in day-to-day sensitivity variations. Tables 1–4 summarize results obtained utilizing both detection methods which clearly indicate the applicability of either technique. The major advantage of the electrochemical detector is its increased sensitivity which enabled the detection of trace amounts of erythromycin base in the formulations while the UV detector is more suited to lengthy automated analyses especially when performed by less experienced technical staff.

TABLE 1

Tablet dosage form analysis – stability of samples in the automatic sample injector (24 h)

Product	Electrochemical		Ultraviolet	
	Original analysis (mg)	Repeat analysis (mg)	Original analysis (mg)	Repeat analysis (mg)
EL1	547.6	552.7	540.8	549.3
AI1	508.5	501.1	496.8	497.6
LL1	482.9	470.0	483.0	463.2

The formation of acid degradation products on storage and particularly during dissolution studies is highly possible. The electrochemical detector thus has an additional advantage particularly in the detection of anhydroerythromycin derivatives which are major acid degradation products (Atkins et al., 1986), and which are associated with poor UV absorption characteristics. They are, however readily detected by electrochemical methods (Stubbs et al., 1987). The use of highly end-capped

TABLE 2

Dosage form analysis – precision data

Product	*n*	Electrochemical		Ultraviolet	
		Mean contents (mg) [a]	R.S.D. (%)	Mean contents (mg) [a]	R.S.D. (%)
Tablets					
EL1	6	547.6	1.8	540.8	1.4
AI1	6	508.5	0.7	496.8	1.9
LL1	6	482.9	2.3	483.0	1.8
Capsules					
EL2	6	287.3	1.3	281.5	2.2
LL2	6	273.0	0.8	278.2	1.8
AL2	6	234.2	1.5	230.7	2.5
Suspensions					
EL3	6	132.3	1.1	131.9	4.8
LL4	6	132.4	1.4	131.3	2.5

[a] Mean contents = erythromycin base equivalent per tablet, capsule or 5 ml of suspension.

TABLE 3

Dosage form analysis – recovery data (tablet analysis)

Sample no.	Estolate added (mg)	Electrochemical		Ultraviolet	
		Estolate found (mg)	Recovery (%)	Estolate found (mg)	Recovery (%)
1	415.9	407.6	98.2	402.8	97.0
2	415.9	396.0	95.4	402.8	97.0
3	415.9	407.6	98.2	422.2	102.0
4	521.8	512.5	98.2	481.3	92.2
5	521.8	512.5	98.2	501.0	96.0
6	521.8	512.5	98.2	501.0	96.0
7	637.7	675.5	106.0	628.7	98.6
8	637.7	628.9	98.6	628.7	98.6
9	637.7	652.2	102.3	638.5	100.1
Overall recovery			99.3 ± 2.8		97.5 ± 2.8

C_{18} columns for dosage form analysis is, however, not recommended since erythromycin estolate tends to be strongly retained on such stationary supports resulting in long run times under the optimum conditions required for the separation of erythromycin base and the internal standard. During initial developmental studies, methanol was used as the organic solvent for sample dilutions as described in the official compendia (BP, 1988; USP, 1990). Inconsistent results were obtained with spurious peaks being detected by the electrochemical detector. Further studies are being performed at present to confirm and possibly explain these findings in more detail. Substitution of acetonitrile for methanol alleviated these problems completely with samples stored in the autosampler overnight yielding highly similar results on reanalysis (Table 1).

TABLE 4

Chromatographic responses of erythromycin estolate raw materials

	Supplier	Estolate peak height (mm)	Mass powder [a] (mg)	Relative response (mm/mg)
Batch 1	USP	95.5	10.5	9.1
	AI	94.5	11.3	8.4
	LL	92.0	11.0	8.4
	EL	88.0	9.8	9.0
Batch 2	USP	48.0	7.4	6.5
	AI	62.0	10.6	5.9
	LL	67.5	10.4	6.5
	EL	70.5	10.6	6.6

[a] Mass powder = mass of erythromycin estolate powder dissolved in 10 ml acetonitrile.

Assay validation

Calibration curves prepared from standards containing 2.11, 2.60, 3.21 and 4.07 mg/10 ml were linear with a slope of 0.17, a y-intercept of zero and a correlation coefficient of 0.994.

Precision data for the analysis of erythromycin estolate tablets, capsules and suspensions utilizing both UV and electrochemical detection are summarized in Table 2.

For the tablet and capsule analyses precision was excellent with relative standard deviations being less than 2.5%. The advantage of incorporating an internal standard is clearly evident, since sample volume variations during centrifugation and storage in the sample injector are of no consequence. The technique utilized in the tablet assay allowed for tablet disintegration and de-aggregation in water prior to dissolution of the poorly water-soluble drug in acetonitrile. This procedure proved highly successful and reduced the possibility of drug entrapment within intact granules which may occur if organic solvents are added directly to crushed tablet matrices.

As expected, the recovery study indicated minimal sample losses (Table 3) since no complex sample manipulations were performed. Differences in chromatographic response amongst the various batches of erythromycin estolate raw material were observed when compared with the USP reference standard. Comparison of these responses (Table 4) indicated some slight variations between samples, with the Adcock-Ingram sample exhibiting the lowest response in both sets of standards.

Dosage form analysis

Results obtained from the analysis of erythromycin estolate tablets, capsules and suspensions utilizing both UV and electrochemical detection are summarized in Table 5.

TABLE 5

Analysis of (A) tablets and capsules, and (B) suspensions

Product	n [a]	Electrochemical			Ultraviolet		
		Mean contents [b] (mg)	% Label claim	R.S.D. (%)	Mean contents [b] (mg)	% Label claim	R.S.D. (%)
(A) *Tablets and capsules*							
Tablets							
EL1	6	547.6	109.5	3.1	540.8	108.2	4.8
AI1	6	508.5	101.7	2.5	496.8	99.4	3.4
LL1	6	482.9	96.6	1.6	483.0	96.6	1.4
Capsules							
EL2	6	287.3	114.9	2.3	281.5	112.6	3.0
LL2	6	273.3	109.2	3.3	278.2	111.3	2.7
AL2	6	234.2	93.6	10.5	230.7	92.3	10.3
(B) *Suspensions*							
		Electrochemical					
		Mean contents [b] (mg/5 ml)	% Label claim	R.S.D. (%)			
EL3	1	132.3	105.8	3.3			
LL4	1	132.4	106.0	4.2			
LL5	1	123.9	99.1	6.4			
LL6	1	123.0	98.4	6.1			
LL7	1	266.8	106.7	5.9			
LL8	1	232.2	93.3	6.2			
LL9	1	258.5	103.4	6.0			

[a] n, number of each product tested (suspensions analysed in triplicate).
[b] Mean contents = erythromycin base equivalent.

The content uniformity of all three tablet products were found to comply with specifications with relative standard deviations obtained from the analysis of six tablets per batch being less than 3.5% in all cases. Mean values obtained for the amount of drug per tablet, expressed as a percentage of the label claim, show that the three different products fell within the permissible range of 90–120% (USP, 1990).

The content uniformity of capsules is highly dependent on the method of capsule filling, and is reflected in the uniformity of the capsule mass. Products EL2 and LL2 had capsule mass variations in the order of 1–3% with similar values for variations in drug contents. However, two capsules from the product AI2 appeared to have been over-filled making the removal of the contents extremely difficult which resulted in 10% variations in both capsule mass and drug content. The mean drug content of capsules EL1 and LL2 were almost identical (110%) which was within the pharmacopoeial limits (USP, 1990) for erythromycin estolate capsules (90–115%).

The product AI2 had a mean content of 94% if the results of all six capsules were incorporated. However, if the results of the two overfilled capsules were excluded, the mean drug content was only 86% of label claim. The wide variations in capsule contents reported for product AI2 would make it an unsuitable product for pharmacokinetic studies and would certainly complicate the performance of comparative bioavailability studies unless capsules were pre-weighed prior to administration.

It is possible that the lower drug content values obtained for the tablet AI1 and the capsule AI2

may be related to the previously mentioned discrepancy regarding the decreased chromatographic response of the AI raw material compared to the USP standard (Table 4).

Sampling from suspensions is difficult, either due to their "syrupy" nature or their settling rate. In order to facilitate the transfer of small volumes of these suspensions, disposable pipette tips were modified by removing 5 mm from their ends, thereby increasing their bore considerably. Each of the seven different erythromycin estolate suspensions analysed, fell within the compendial limits of 90–115% of label claim (USP, 1990). The products LL6 and LL9 were manufactured 3 years prior to analysis yet showed no signs of excessive estolate hydrolysis or formation of any other degradative compounds with only trace amounts of erythromycin base being detected by the electrochemical detector.

In summary, the HPLC method described, utilizing either UV or electrochemical detection is ideally suited for the analysis of erythromycin estolate dosage forms. It is rapid, precise and stability-indicating offering distinct advantages over the compendial microbiological assays which are relatively imprecise, time-consuming and tedious to perform.

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