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Simple and Rapid Spectrophotometric Method for the Analysis of Erythromycin in Pharmaceutical Dosage Forms

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ABSTRACT

This work aimed to develop a simple and rapid spectrophotometric method for the analysis of erythromycin in pharmaceutical dosage forms. Direct UV and first derivative measurements at the wavelengths of 285 and 300 nm, respectively, in combination with standard addition method gave promising results. In both techniques, methanol was used as a solvent and dibasic potassium phosphate buffer (pH 8) was used to hydrolyze erythromycin stearate to erythromycin. Both the direct UV and first derivative measurements using standard addition method illustrated excellent linearity in the concentration range of 3-15 mg/mL (r^2 > 0.98 and > 0.99, respectively) with good precision (%RSD < 0.65%). The limits of detection (LOD) of direct UV and first derivative measurements were 0.08 and 1.37 mg/mL, respectively, and the limits of quantitation (LOQ) were 0.24 and 4.17 mg/mL, respectively. However, the first derivative measurement showed better % mean recovery (97.6% and 106.5% for brand A and B, respectively, %RSD < 3.34%) than the direct UV measurement (66.03% and 43.80% for brand A and B, respectively, %RSD up to 47.39%). Thus, the first derivative measurement using standard addition method was valuable for analyzing erythromycin in dosage forms, which excipients strongly interfere the UV absorbance of the drug.

Key words: erythromycin, spectrophotometric method, direct UV measurement, first derivative measurement

INTRODUCTION

Erythromycin, produced by *Saccharopolyspora erythreas* (formerly known as Streptomyces erythraeus)[(1)], is a macrolide antibiotic consisting of a 14 member ring, a ketone group, two glycosidic bonds and a dimethylamino group (Figure 1)[(2-4)]. The drug targets at the ribosome and inhibits the protein synthesis of Gram positive bacteria such as Mycoplasma and Chlamydia[(5-6)]. Erythromycin is used for treatment of several infection diseases and in patients allergic to the penicillins. Erythromycin easily degrades in acidic conditions giving inactive compounds, 8,9-anhydro-6,9-hemiketal and erythromycin-6,9,12-spiroketal[(7)]. To increase its acid stability and bioavailability, erythromycin is available in several forms including estolate, ethysuccinate and stearate.

Several methods have been proposed for the analysis of erythromycin. Dehouck *et al.*[(4)] reported the HPLC analysis of erythromycin and benzoylperoxide in acne gel on a Xterra RP18 column using acetonitrile, 0.2 M dipotassium hydrogen phosphate and water (35:5:60, v/v) as a mobile phase and a detection wavelength at 215 nm. Leal *et al.*[(8)] analyzed erythromycin and other six macrolide antibiotics by HPLC using a C18 column, a mobile phase consisting of phosphate buffer (pH 2.5) and acetonitrile and monitored the wavelengths in a range of 204-287 nm. HPLC-MS (mass spectrometry) was also employed for the analysis of seven macrolide antibiotic residues in fish with a detection limit of 0.01 μg/mL[(9)]. Hilton and co-workers[(10)] used HPLC-electrospray MS in combination with solid phase extraction (SPE) for the detection of several antibiotics contaminated in water including erythromycin. Flurer *et al.*[(11)] proposed micellar eletro-

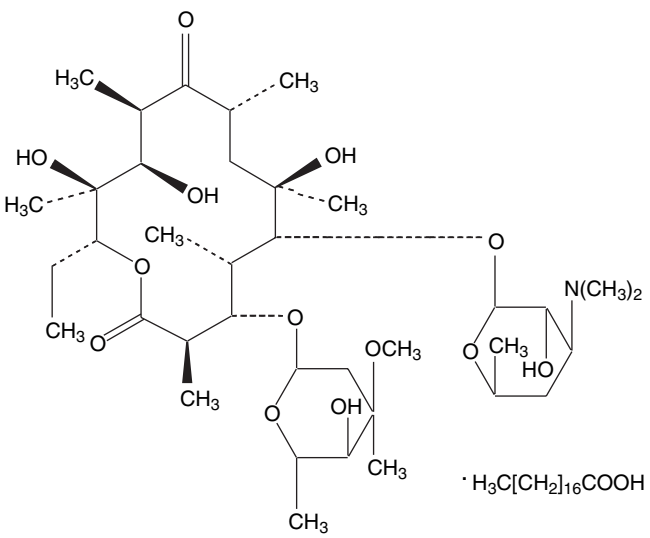

Figure 1. Structure of erythromycin stearate.

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kinetic chromatography (MEKC) for the determination of β-lactam antibiotics, aminoglycoside, clindamycin phosphate and erythromycin stearate using borate buffer containing sodium dodecyl sulfate as a background electrolyte. Spectrofluorometry[12] and spectrophotometry using complex formation[13-14] were also proposed for the analysis of erythromycin in formulations.

The United States Pharmacopoeia (USP)[15] and the British Pharmacopoeia (BP)[16] recommend an HPLC method and microbial assay for the analysis of erythromycin content in raw material and finished products, respectively. Although HPLC is suggested, the technique requires highly skillful operator and the instrument is expensive and not available for most local manufacturers. Whereas, the microbial assay is time-consuming. The aim of this work was to develop a simple and rapid spectrophotometric method for the analysis of erythromycin in pharmaceutical preparations. Both the direct ultraviolet (UV) and first derivative (D_1) measurements with external standard (ESM) and standard addition methods (SAM) were performed. The D_1 measurement using the standard addition method was investigated to evaluate whether matrices from tablets would interfere the analysis. The proposed method can be applicable to various laboratories because of its simplicity, low cost and the availability of the spectrophotometer, which is common to most manufacturers. The method serves as an alternative to the methods described in pharmacopoeias.

MATERIALS AND METHODS

I. *Chemicals*

Analytical grade reagents and solvents were used in all experiments. Standard erythromycin stearate was purchased from Sun Pharma (Mumbai, India), dibasic potassium phosphate was from Hopkin & Williams (London, England) and phosphoric acid was from BDH Lab (Dorest, England). Erythromycin stearate tablets (brand A) and capsules (brand B) (equivalent to 250 mg erythromycin per tablet or capsule) were from SeaPharm Manufacturing (Ayudhaya, Thailand) and from a local drugstore, respectively. Water was double distilled.

Standard solutions were prepared by dissolving appropriate amount of erythromycin stearate in methanol and dibasic phosphate buffer pH 8.0 (1:1) to obtain the final concentrations of 3, 6, 9, 12 and 15 mg/mL.

Twenty tablets of brand A were weighed, finely ground and mixed. In case of brand B, powder from 20 capsules was emptied from the shells and mixed. Sample solutions were prepared in triplicate by weighing appropriate amount of erythromycin, dissolving in methanol and dibasic phosphate buffer pH 8.0 (1:1) to obtain the final concentration of 12 mg/mL (for ESM experiments), and filtering through a Whatman paper no. 1

Standard addition solutions were prepared by transferring 10 mL of various standard solutions (3, 6, 9, 12 and 15 mg/mL), adding 5 mL of sample solutions (20 and 25 mg/mL for brands A and B, respectively) and adjusting to 25 mL with methanol and dibasic phosphate buffer pH 8.0 (1:1). Standard addition solutions were prepared as described in Table 1.

II. *Instrumentation*

The pH measurements were carried out with a Consort C830 pH meter equipped with a glass combined electrode (Turnhout, Belgium). UV absorbance and spectra were obtained from a UV-160A Shidmadzu spectrophotometer (Kyoto, Japan). The direct UV and D_1 measurements were performed at 285 and 300 nm, respectively.

III. *Analytical Performance Characteristics*

Analytical performance characteristics including linearity, precision, accuracy, limit of detection (LOD) and limit of quantitation (LOQ) for the analysis of erythromycin by spectrophotometry using direct UV and D_1 measurements were evaluated. Standard curves were obtained by measurement the absorbance of the standard solutions of erythromycin in a range of 3-15 mg/mL for ESM and in a range of 25-120% of the nominal concentration (5 mg/mL) for SAM. Linear regression and correlation coefficient (r^2) were calculated using Microsoft Excel® program. Precision of the method was determined by repetitive measurements (n = 3) of the absorbance of standard solutions at the top, middle and bottom points of the standard curve and percent relative standard deviations (%RSDs) were calculated. Recoveries (%R) of the method were determined from SAM by spiking five different concentrations of standard solutions in a range of 25-120% of the nominal sample concentration (5 mg/mL) into the sample solutions as described in Table 1. Recovery experiments were performed in triplicate for both brands A and B and %R was calculated using Eq (1).

$$\%R = \frac{\text{amount found}}{\text{amount added}} \times 100 \quad (1)$$

LOD and LOQ are defined as the lowest amount that can be detected and that can be accurately quantified,

Table 1. Standard addition solutions

Flask no.	1	2	3	4	5	6
Std. 3 mg/mL (mL)	10	-	-	-	-	-
Std. 6 mg/mL (mL)	-	10	-	-	-	-
Std. 9 mg/mL (mL)	-	-	10	-	-	-
Std. 12 mg/mL (mL)	-	-	-	10	-	-
Std. 15 mg/mL (mL)	-	-	-	-	10	-
Sample (mL)	5	5	5	5	5	5
Methanol: buffer pH 8.0 (1:1) q.s. to (mL)	25	25	25	25	25	25

respectively. LOD and LOQ were calculated using Eq (2) and (3), respectively, where SD is the standard deviation of the blank and S is the slope of the standard curve.

$$LOD = \frac{3.3\ SD}{S} \quad (2)$$

$$LOD = \frac{10\ SD}{S} \quad (3)$$

RESULTS AND DISCUSSION

I. *Direct and UV D_1 Measurements Using External Standard Method*

Standard erythromycin solutions gave typical direct UV and D_1 spectra in methanol and dibasic phosphate buffer pH 8.0 (1:1) as shown in Figures 2 and 3, respectively. The maximum direct UV absorbance of erythromycin was obtained at the wavelength of 285 nm and the D_1 absorption was measured at 300 nm. Preliminary experiments, using ESM, showed that both measurements provided good linearity ($r^2 > 0.999$, in a range of 3-15 mg/mL) and good precision (%RSD < 1.79%). Both techniques were initially used for assay of erythromycin content in brands A and B (Table 2). The high %label amount (120-154%, %RSD = 3.47-6.03%) with varied and low % recovery indicated that matrices in samples might interfere with the measurements and SAM should be employed to overcome this problem. Thus, the direct UV and D_1 measurements in combination with SAM were further investigated in both brands A and B for the remaining of the study.

II. *Analytical Performance Characteristics of Direct UV and D_1 Measurements Using Standard Addition Method*

For the standard addition experiments, sample soultions of brand A and B were prepared at 20 and 25 mg/mL, respectively, and 5 mL of these solutions was added into the standard solution as described in MATERIALS AND METHODS. The total concentration in the standard addition experiments provided reasonable absorbencies in a range of 0.5-0.7. Brand A was prepared at the lower concentration (20 mg/mL) and than brand B (25 mg/mL) in the standard addition experiments, since matrices in brand A interfered with the UV absorption and gave the absorbance, which was out of the linearity range.

Spectrophotometric characteristics in terms of absorptivity and Sandell's sensitivity are presented in Table 3. For both the direct UV and D_1 measurements, the absorptivity and Sandell's sensitivity values from brand A and B were similar. It is evident that the direct UV measurement was approximately 20 times more sensitive than the D_1 measurement. The molar absorptivity of the direct UV measurement was higher than that from the D_1 measurement, whereas the Sandell's sensi-

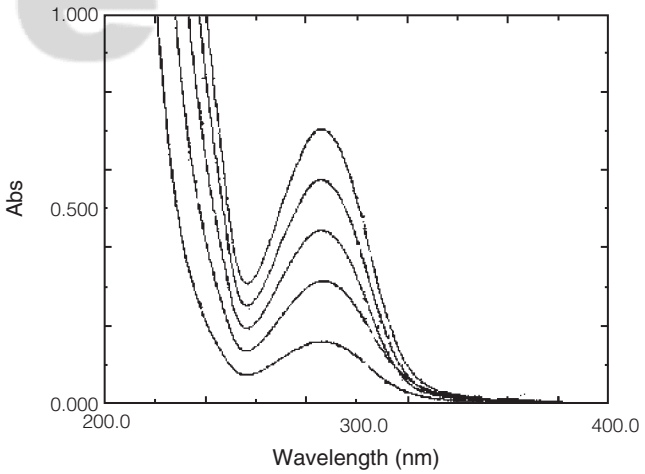

Figure 2. Typical direct UV spectra of various concentration of standard erythromycin solutions from 3 (bottom) 6, 9, 12 and 15 (top) mg/mL in methanol and dibasic phosphate buffer pH 8.0 (1:1).

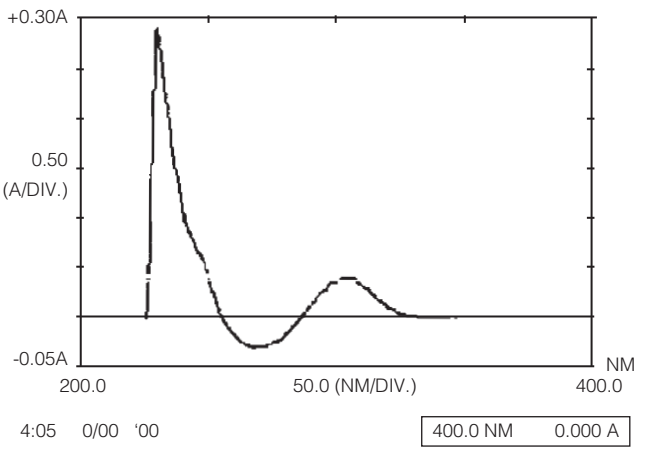

Figure 3. A typical first derivative measurement spectrum of a standard erythromycin solution (12 mg/mL in methanol and dibasic phosphate buffer pH 8.0 (1:1).

Table 2. Assay data (% label amount) from the direct UV and D_1 measurements using external standard and standard addition methods (n = 3)

	Direct UV		D_1	
	ESM[a]	SAM[b]	ESM	SAM
Brand A	154.13	154.61	139.08	134.98
Brand B	126.19	137.02	120.19	99.85

[a]ESM = external standard method.
[b]SAM = standard addition method.

Table 3. Molar absorptivity (ε) and Sandell's sensitivity (s) data

	Direct UV		D_1	
	ε (L/mol cm)	s (mg/cm^2)	ε (L/mol cm)	s (mg/cm^2)
Brand A	37.43	0.020	1.89	0.389
Brand B	44.03	0.017	1.98	0.373

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tivity values were vice versa. This data indicated that the direct UV measurement was more sensitive than the D_1 measurement i.e. the small change in concentration caused a large change in the direct UV measurement, but only a small change in the D_1 measurement. This remark was later confirmed by the slope of the linear regression, LOD and LOQ.

The linearity, precision, LOD and LOQ data of the direct UV and D_1 measurements using SAM was shown in Table 4. Both direct UV and D_1 measurements gave good linearity ($r^2 > 0.98$), precision (%RSD < 0.65%) with acceptable LOD (0.236 mg/mL) and LOQ (4.17 mg/mL) for a spectrophotometric method. Slopes from the direct UV measurement were approximately 16-18 times higher than those of D_1 measurement, which indicated that the former method was more sensitive than the latter. This data was also in agreement with the absorptivity, Sandell's sensitivity (Table 3), LOD and LOQ values (Table 4). Recoveries of the methods for brands A and B were shown in Table 5. The recovery data from the direct UV measurement revealed very poor and varied %recoveries (14.89-81.60%) with high %RSD (up to 47.39%). Although SAM was used, the low recoveries were found in case of direct UV measurement. These results indicated that the direct UV measurement was not suitable for the analysis of erythromycin in these samples. Whereas, D_1 measurements offered good recoveries within 94.92-109.32% with the %RSD of less than 3.34%. We reasoned that matrices from samples might interfere with the direct UV absorption of erythromycin in the samples. In order to completely eliminate the matrix effect, the D_1 measurement with SAM is recommended. These results strongly indicated that D_1 measurement was superior to the direct UV measurement in term of accuracy.

III. *Applications*

Table 2 compares the assay data from direct UV and D1 measurements using ESM and SAM. The analytical performance characteristic data from the previous section indicated that the D_1 measurement with SAM provided more reliable results than the direct UV measurement. This conclusion was made based on the analytical performance characteristic data in Table 4 and 5. D_1 measurement with SAM provided better linearity precision and accuracy than direct UV. Thus, the D_1 measurement with SAM was employed to evaluate the erythromycin content in the samples. In the current work, one sample complied with the USP standard (90.0-120.0%), whereas the other failed to meet the requirement (Table 2).

Table 4. Linearity, precision, LOD and LOQ of the direct UV and D_1 measurements using standard addition method

	Linearity		Precision (%RSD) (n = 3)		LOD (mg/mL)		LOQ (mg/mL)	
	Direct UV	D_1	Direct UV	D_1	Direct UV	D_1	Direct UV	D_1
Brand A	y = 0.0434x + 0.2736 (r^2 = 0.9892)	y = 0.0024x + 0.0132 (r^2 = 0.9967)	0.48	0.00	0.076	1.37	0.230	4.17
Brand B	y = 0.0424x + 0.2843 (r^2 = 0.9836)	y = 0.0026x + 0.0127 (r^2 = 0.9917)	0.65	0.00	0.078	1.27	0.236	3.85

Table 5. Recovery data of the direct UV and D_1 measurements using standard addition method

	Brand A				Brand B			
	Direct UV		D_1		Direct UV		D_1	
Amount added (mg/mL)	Amount found (mg/mL)	%R	Amount found (mg/mL)	%R	Amount found (mg/mL)	%R	Amount found (mg/mL)	%R
1.18	0.40	33.90	1.12	94.92	0.46	38.98	1.29	109.32
2.35	1.45	61.70	2.32	98.72	0.35	14.89	2.54	108.09
3.53	2.59	73.37	3.52	99.72	1.32	37.39	3.80	107.65
4.7	3.74	79.57	4.72	100.43	2.89	61.49	5.05	107.45
5.87	4.79	81.60	5.52	94.04	3.89	66.27	5.89	100.34
Average		66.03		97.56		43.80		106.57
SD		19.56		2.90		20.75		3.56
%RSD		29.63		2.97		47.39		3.34

CONCLUSIONS

A spectrophotometric method, without the use of complex formation or derivatization, for the determination of erythromycin in dosage forms was developed. Erythromycin can be determined by direct measuring the absorbances at the wavelength of 285 nm or by first derivative measurement at the wavelength of 300 nm using standard addition method. Both methods provided good linearity, precision, LOD and LOQ. However, the D_1 measurement showed superior recoveries to the direct UV measurement. The standard addition method was also required for the analysis of erythromycin in samples in order to minimize the interference from matrices. Thus, the first derivative measurement using the standard addition method was recommended for the analysis of erythromycin in dosage forms. Unlike other researchers, which reported the use of advanced techniques (e.g. HPLC, HPTLC and CE), we described a simple spectrophotometric method for the determination of erythromycin. The proposed method will not replace the methods recommended in USP or BP or other published methods. But, it will serve as a rapid, convenient and inexpensive alternative, which can be applicable for most routine quality control in laboratories.

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REFERENCES

1. Labeda, D. P. 1987. Transfer of the type strain of Streptomyces erythraeus (Waksman 1923) Waksman and Henrici 1984 to the genus Saccharopolyspora Lacey and Goodfellow 1975 as *Saccharopolyspora erythraea* sp. nov., and designation of a neotype strain for *Streptomyces erythraeus*. Int. J. syst. Bacteriol. 37: 19-22.
2. O'Neil, M. J., Smith, A., Heckelman, P. E. and Budavari, S. 2001. The Merck Index – An Encyclopedia of Chemicals, Drugs and Bilogicals. 13th ed. pp. 654-655 Merck & Co., Inc. Whitehouse Station, New Jersey, U. S. A.
3. Chitneni, S. K., Govaerts, C., Adams, E., Schepdael, A. V. and Hoogmartens, J. 2004. Identification of impurities in erythromycin by liquid chromatography-mass spectrometric detection. J. Chromatogr. A 1056: 111-120.
4. Dehouck, P., Van Looy, E., Haghedooren, E., Deckers, K., Vander Heyden, Y., Adams, E., Roets, E. and Hoogmartens, J. 2003. Analysis of erythromycin and benzolperoxide in topical gels by liquid chromatography. J. Chromatogr. B 794: 293-302.
5. Omura, S. and Tanaka, Y. 1986. Macrolide antibiotics. In "Biotechnology". p. 359. Verlag Chemie. Weinheim, Germany.
6. Omura, S. (ed) 1984. Macrolide Antibiotics: Chemistry, Biology and Practice. p. 26. Academic Press. Orlando, U. S. A.
7. Horie, M., Takegami, H., Toya, K. and Nakazawa, H. 2003. Determination of macrolide antibiotics in meat and fish by liquid chromatography-electrospray mass spectrometry. Anal. Chim. Acta 492: 187-197.
8. Koch, W. L. 1979. Erythromycin. In "Analytical Profiles of Drug Substances". Vol. 8. pp. 159-177. Florey, K., ed. Academic Press. New York, U. S. A.
9. Leal, C., Codony, R., Compano, R., Granados, M. and Dolars Part, M. 2001. Determination of macrolide antibiotics by liquid chromatography. J. Chromatogr. A 910: 285-290.
10. Hilton, M. J. and Thomas, K. V. 2003. Determination of selected human pharmaceutical compounds in effluent and surface water samples by high-performance liquid chromatography-electrospray tandem mass spectrometry. J. Chromatogr. A 1015: 129-141.
11. Flurer, C. L. and Wolnik, K. A. 1994. Chemical profiling of pharmaceuticals by capillary electrophoresis in the determination of drug origin. J. Chromatogr. A 674: 153-163.
12. Khashaba, P. Y. 2002. Spectrofluorimetric analysis of certain macrolide antibiotics in bulk and pharmaceutical formulations. J. Pharm. Biomed. Anal. 27: 923-932.
13. Amin, A. S. and Issa, Y. M. 1996. Selective spectrophotometric method for the determination of erythromycin and its esters in pharmaceutical formulations using gentiana violet. J. Pharm. Biomed. Anal. 14: 1625-1629.
14. Gallagher, P. A. and Danielson, N. D. 1995. Colorimetric determination of macrolide antibiotics using ferric ion. Talanta 42: 1425-1432.
15. The United States Pharmacopeia (USP 26), the National Formulary (NF 21). 2003. pp. 727-741. United State Pharmacopeial Convention Inc. Rockville, U. S. A.
16. British Pharmacopoeia (BP) 2001. pp. 650-659. The Stationery Office. London, U. K.