March 2009 Volume 8 Issue 1

Trade Science Inc.

Analytical CHEMISTRY
An Indian Journal

Full Paper

ACAIJ, 8(1) 2009 [21-25]

Simple and rapid spectrophotometric method for the estimation of erythromycin esters in pharmaceutical formulations

A.K.Shukla[1], Priti Mehta*[2], R.V.Shah[3], R.C.Patel[3], Foram Makadia[3], Viral Siddhpura[3], Hiral Rathod[3]

[1]Suvik Pharmaceutical Laboratories, Sector 29, Gandhinagar, 382029, (INDIA)

[2]Institute of Pharmacy, Nirma Institute of Science and Technology, Ahmedabad, 382481, (INDIA)

[3]K.B. Institute of Pharmaceutical Education and Research, Sector 23, Gandhinagar, 382023, (INDIA)

Tel : 9898335567

E-mail : drpritimehta@nirmauni.co.in

Received: 15th December, 2008 ; Accepted: 30th December, 2008

ABSTRACT

A simple and rapid method has been developed for the quantitative estimation of erythromycin esters in bulk and in pharmaceutical formulations. Erythromycin esters were found to react with o-nitro benzaldehyde in presence of acetic acid-hydrochloric acid mixture to form a colored product having useful absorption band at 486nm. Different variables affecting the color development were studied and optimized. The method was used to determine 10-50μg/ml erythromycin in final measured solution. The simplicity of the method permitted rapid analysis and it was suitable for routine control. In order to establish the validity of proposed procedure, commercially available Pharmaceutical formulations were analyzed. The reliability of the method was estimated by parallel determination against the reported method.

KEYWORDS

Erythromycin esters;
Spectrophotometric analysis;
Formulation study.

INTRODUCTION

Erythromycin is a macrolide antibiotic produced by a strain of steromysis ertrus that is used primarily against gram positive bacteria. Although first used in 1952 it is still one of commonly used antibiotics and it has proved to be safe and effective in infections for a number of common infections.

Erythromycin is incompletely but adequately absorbed from the upper part of the small intestine, it is rendered inactive by gastric juices and the drug is therefore administered as protected tablets or capsules containing pellets that dissolved in the duodenum.

Various esters of erythromycin like stearate, estolate, succinate have been prepared in an attempt to improve stability and facilitated absorption.

Microbiological assay is the official method for the estimation of dosage forms of erythromycin esters in Indian Pharmacopoeia[1] (IP) and United state Pharmacopoeia[2] (USP). Microbiological methods, which involve the growth of the probable microorganism in the medium containing the antibiotic, suffer from a variety of disadvantages including the lengthy incubation period needed and lack of the sensitivity towards the antibiotics. In USP[2] and BP[3] HPLC method is given for the estimation using methanol and acetonitrile. Terespolsky SA et al[4] have reported instability of different erythromycin esters in both the solvents.

A UV-VIS spectroscopic method was reported for determination of erythromycin after alkaline hydrolysis[5]. Colorimetric methods were developed in concentrated sulphuric acid for estimation of Erythromycin

Full Paper

at 470nm[6,7]. Ford et al.[8] have developed a colorimetric method in which Erythromycin form a dark blue complex with tetrazolium blue. Variety of dyes such as thymol blue[9], bromophenol blue[9] and bromocresol purple[8] which undergo ion pair formation with erythromycin were utilized for the measurement of erythromycin solutions at low concentration. Napthotriazole disulfonate[10,11] and erythrosine B[12] were also used for spectrofluorimetric assay. A chemilumenance method for erythromycin was also an option[13]. However all of these reported methods require extraction with organic solvents or having lengthy sample preparation with elevated temperature. The purpose of this work was to develop simple, rapid and useful method for the determination of erythromycin esters in bulk and in its different formulations for routine quality control.

METARIALS AND METHOD

Instruments

A Jasco V-530 double bean UV visible spectrophotometer (Japan/India) with matched 10 mm quarts cells was used for all absorbance measurements under the following operating conditions: scan speed medium (400nm/min), scan range 350-800nm and slit width 2nm. Spectra were automatically obtained by Jasco system software. All the spectrophotometric measurements were performed at 25^0C.

Reagents

Analytical grade reagents were used throughout the study. Erythromycin estolate (63.27% purity on basis of erythromycin) was gifted by Suvik Pharmaceuticals Laboratories (Gandhinagar/India).Erythromycin stearate and erythromycin succinate were gifted by sun Pharmaceutical Ltd (Baroda/India). Glacial acetic acid was purchased from Rankem, RFCL Limited (New Delhi/ India) and hydrochloric acid was purchased from Suvithinath laboratory (Baroda/India). o-Nitrobenzal dehyde was obtained from Loba chemie Pvt. Limited (Ahmadabad /India). Different Formulations of erythromycin esters were procured from the market.

Standard solution preparation

Sufficient amount of standard Erythromycin esters (~63.5 mg of free base) was weighed and transferred in 25 ml volumetric flask, individually. Dissolved each in 15 ml glacial acetic acid and made up the volume with the same. Further 5 ml of above solution was diluted to 25 ml with glacial acetic acid to prepare erythromycin stock solution. Erythromycin stock solution remains stable for 15 days if kept refrigerated. Working solution of lower concentration was prepared freshly by appropriate dilution of the stock solution with glacial acetic acid.

A 0.4%w/v o-nitrobenzaldehyde solution was prepared freshly at time of use by dissolving the required amount in glacial acetic acid.

Preparation of linearity curve

Aliquots of 0.5, 1.0, 1.5, 2, 2.5 and 3.0 ml of erythromycin stock solution were transferred to series of 25 ml volumetric flask. Add 2 ml of freshly prepared o-nitrobenzaldehyde solution (0.3% w/v) followed by 3 ml hydrochloric acid in each flask. Leave the reaction mixture to room temperature for 20 minutes. Make up the volume with glacial acetic acid. Mix the contents and measure the absorbance at 486 nm against the blank solution prepared in same manner simultaneously.

Formulation studies

For tablets

Weigh and powder twenty tablets. A tablet powder equivalent to 150 mg erythromycin base was extracted with 40 ml glacial acetic acid and filter through whatmann filter paper. Volume was made up to 50 ml with glacial acetic acid. The filtrate was further diluted to obtain a nominal 48μg/ml and analyzed as above.

For suspension

Marketed erythromycin ester suspension was shaken properly and 6 ml of suspension was accurately transferred in 50 ml volumetric flask. Erythromycin was extracted with 40 ml glacial acetic acid, filtered through whatmann filter paper and made up the volume up to 50 ml with glacial acetic acid. The filtrate was further diluted to obtain a nominal 48μg/ml and analyzed as above.

For dry syrup

Marketed dry syrup was reconstituted with the help of distilled water, mixed and 6 ml was further analyzed as given in 'for suspension'.

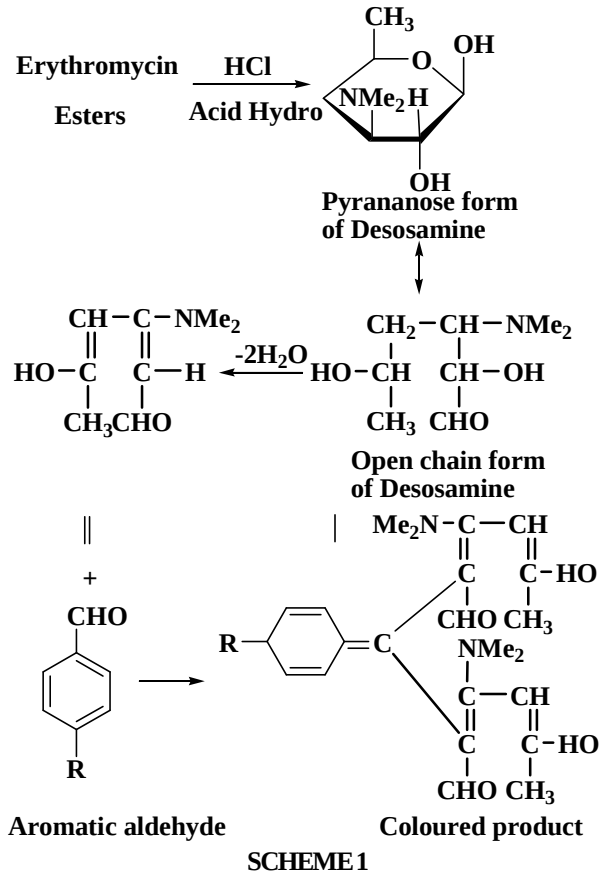
RESULTS AND DISCUSSION

Proposed reaction mechanism

In presence of hydrochloric acid and glacial acetic acid medium, Erythromycin undergoes hydrolysis lead to the formation of desosamine[14] and subsequently sugar dehydration reaction proceed by elimination of water from the open chain form (I) giving the unsaturated B-dimethylamino aldehyde (II). The active position at C_2 in II may be involved in condensation reactions with aromatic aldehyde such as o-nitrobenzal dehyde, furnishing the corresponding colored product (SCHEME 1).

Reaction condition

Investigation was carried out to elucidate the most favorable condition for reaction between erythromycin esters and o-nitrobenzaldehyde to achieve maximum color development. The absorption spectra ($\lambda_{max,}$ 286nm) of the colored product of Erythromycin estolate and o-nitro benzaldehyde in presence of acetic acid and hydrochloric acid is shown in figure1.



SCHEME 1

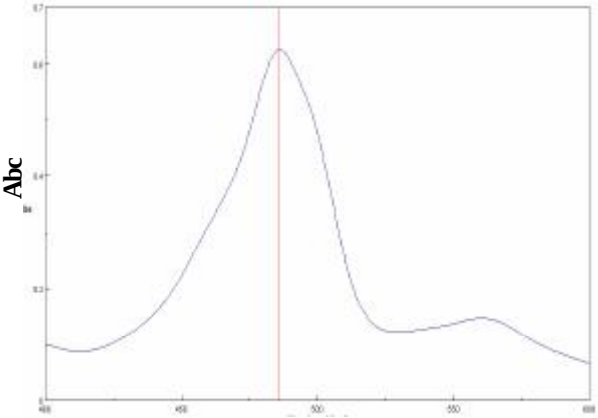


Figure 1: Absorption spectra of Erythromycin estolate colored product in presence of acetic acid and hydrochloric acid mixture (λ_{max}, 486nm)

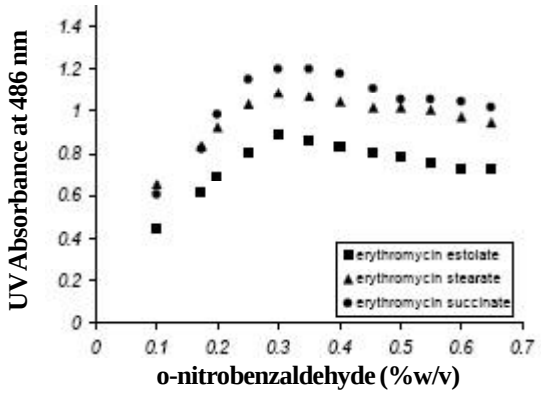


Figure 2: Optimization of the absorbance of Erythromycin solution as a function of o-nitrobenzaldehyde concentration. The reaction was followed at 25^{0}C

The optimum conditions for the developed procedure was established by varying the parameters one at a time and observed the effect produced on the absorbance of the color species.

Effect of reagent concentration: the optimum concentration of o-nitrobenzaldehyde for maximum color formation was found to be 2 ml of 0.3%w/v per 25 ml of the reaction mixture (Figure 2). Optimum volume of concentrated hydrochloric acid was found to be 3.0 ml.

Effect of time and temperature: maximum color intensity was attained within 20 min of the addition of o-nitro benzaldehyde followed by hydrochloric acid at 25±2^{0}C and colored species were stable for 30 min (Figure 3).

Changing the order of addition of reagents resulted

Full Paper

in low sensitivity and slow color development. The absorbance of developed color was decreased when water used instead of glacial acetic acid for final dilution.

Analytical data

Under the proposed experimental conditions, absorbance was linearly proportional to the concentration in the range of 5-50μg/ml of erythromycin as in TABLE 1. The linear regression equation was derived using least square method. The molar absorbtivity and regression results are summaries in TABLE 1.

The mean of six replicate analysis of a solution of erythromycin at a concentration of 20μg /ml assayed as its prepared value gave a relative standard deviation of 1.54%. This level of precision is adequate for the quality control analysis of Pharmaceutical preparations. The accuracy of the method was tested by applying the recommended method using gentiana violet[15].

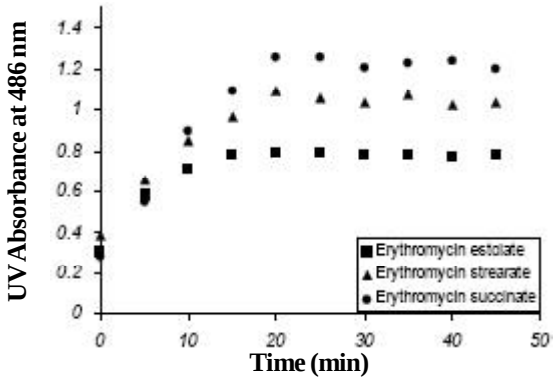


Figure 3: Absorbance of colored product of Erythromycin esters as a function of time at 25^{0}C

Interference

The influence of concomitant compounds was studied. Solutions of erythromycin esters and each compound tested were mixed to obtain samples containing 20μg/ml erythromycin and various concentrations of the foreign compounds. The tolerance ratio of each foreign compound was taken as the largest amount yielding an error of ≥± 2.5% in the absorbance of the formed complex. Glucose, sucrose, lactose, galactose and saccharin were tolerated in large amounts (a 500-fold excess was the maximum molar ratio tested) and 100-fold excesses of starch and citric acid were also tolerated.

Analysis of pharmaceutical formulations

In order to establish the validity of proposed procedure, commercially available pharmaceutical formulations were analyzed. The data in TABLE 2 show that erythromycin content as measure by the proposed method was in excellent agreement with those obtain

TABLE 1: Optical characteristics, precision and accuracy

Parameters	Ery estolate	Ery succinate	Ery stearate
Linear range (μg/ml)	5-50	5-50	5-50
Molar absorptivity (L mol^{-1} cm^{-1})	14,383	19,567	16,879
Linear regression equation[a]	A = 0.020c - 0.044	A = 0.0269c - 0.075	A = 0.0336c - 0.998
Correlation coefficient	0.998	0.999	0.998
Relative standard deviation[b] (%)	1.54	1.32	1.58

[a]c is the concentration in μg/ml, [b]Six replicate samples (concentration 20 μg/ml)

TABLE 2: Analysis of Pharmaceutical formulations of erythromycin Esters by proposed and reference methods

Marketed formulations	Sample	Nominal value (mg)	Amount found (mg)[f] ± S.D.		Recovery (%)
			Reference method	Proposed method	
Tablet	Althrocin[a]	250	245.4±2.4	248.35±1.37	99.34
	Rymycin[b]		252.1±2.6	254.57±1.54	101.83
	Erythrocin[c]		253.5±2.2	251.40±1.19	100.56
Kid Tablet	Althrocin[a]	125	116.4±2.4	119.63±1.04	95.71
	Rymycin[b]		121.9±2.4	124.45±0.98	99.56
	Eltocin[d]		124.3±2.1	122.48±1.10	97.98
Suspension	Rymycin[b]	125/5ml	126.1±1.9	128.30±1.44	102.64
	Erythrocin[c]		120.9±2.2	122.53±1.13	98.03
	Althrocin[a]		119.2±2.3	117.71±1.54	94.17
Dry syrup	E.Mycin[e]	100/5ml	94.9±2.1	92.87±1.86	92.87

[a]Manufactured by Alembic Pharmaceutical Ltd., Baroda, India; [b]Manufactured by Suvic Pharmaceutical Laboratories, Gandhinagar, India; [c]Manufactured by Pfizer Pharmaceuticals Ltd., Mumbai, India; [d]Manufactured by IPCA laboratories Ltd., Mumbai, India; [e]Manufactured by Themis Labs, Valsad, India; [f]Average of six determinations

by reference method[15]. The accuracy of the procedure was further confirmed by recovery experiment. Interference from tablet excipients or oral suspension matrices was not a problem.

CONCLUSION

Results indicate that erythromycin esters can react with o-nitro benzaldehyde in presence of glacial acetic acid- hydrochloric acid mixture to form a colored product which can be analyzed at 486 nm. The spectrophotometric procedure developed for erythromycin esters allow its determination in pharmaceutical preparation without interference. Compared with the reference method, the recommended procedure offers considerable economy as regards reagent consumption and time requirement for the analysis without any loss of precision. The proposed procedure is useful for routine quality control of erythromycin esters in pharmaceutical dosage from.

REFERENCES

[1] 'Indian Pharmacopoeia', 1st Eds., New Delhi, the Controller of Publication, **(1996)**.

[2] United State Pharmacopoeia, 2nd Eds. US Pharmacopeial Convention, Twin brook Rockway, Rockville, MD., **(2008)**.

[3] 'British Pharmacopoeia', London, H.M.Stationary office Limited, **1,2**, **(2005)**.

[4] S.A.Terespolsky, I.Kanfer; Int.J.Pharm., **115**, 123 **(1995)**.

[5] J.B.Tepe, L.V.St.John; Anal.Chem., **27**, 744 **(1955)**.

[6] N.D.Danielson, J.A.Holeman, D.C.Bristol, D.H. Kirnzer; J.Pharm.Biomed.Anal., **11**, 121 **(1993)**.

[7] P.A.Gallapher, N.D.Danielson; Talanta, **42**, 1425 **(1995)**.

[8] J.H.Ford, G.C.Prescott, J.W.Hinman, E.V.Caron; Anal.Chem., **25**, 1195 **(1953)**.

[9] D.Dabrowsha, A.Regosz, R.Pikos, M.Mierzwa, B.Paurch; Microchem J., **41**, 210 **(1990)**.

[10] K.Y.Tserng, J.W.Wagner; Anal.Chem., **48**, 348 **(1976)**.

[11] A.Sanz, A.Tomas, M.Martinez-Lozano, T.Perzruiz; Analyst, **118**, 567 **(1993)**.

[12] P.Y.Khashaba; J.Pharm Biomed Anal; **27**, 923 **(2002)**.

[13] N.D.Danielason, L.He, L.B.Noffsinger, L.Trelli; J. Pharm.Biomed.Anal., **7**, 1281 **(1989)**.

[14] C.S.P.Sastry, K.Rama Rao, D.S.Prasad; Micro chimica Acta, **122**, 53 **(1996)**.

[15] A.S.Amin, Y.M.Issa; J.Pharm.Biomed.Anal., **14**, 1625 **(1996)**.