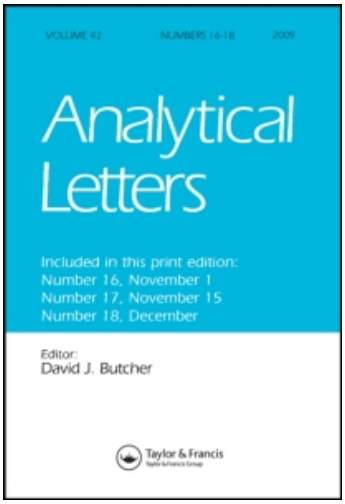

Analytical Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597227

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Online publication date: 30 April 2001

To cite this Article Issa, Y. M. and Amin, A. S.(2001) 'EXTRACTION-COLORIMETRIC METHOD FOR THE DETERMINATION OF ERYTHROMYCIN AND ITS ESTERS IN DOSAGE FORMS USING CHROMOTROPIC ACID AZO DYES', Analytical Letters, 34: 7, 1163 — 1173
To link to this Article: DOI: 10.1081/AL-100104962
URL: http://dx.doi.org/10.1081/AL-100104962

ANALYTICAL LETTERS, 34(7), 1163–1173 (2001)

PHARMACEUTICAL ANALYSIS

EXTRACTION-COLORIMETRIC METHOD FOR THE DETERMINATION OF ERYTHROMYCIN AND ITS ESTERS IN DOSAGE FORMS USING CHROMOTROPIC ACID AZO DYES

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ABSTRACT

Accurate, rapid and sensitive extraction-colorimetric method for the determination of erythromycin and its esters, depending on the formation of an ion-pairs with chromotropic acid (I), chromotrope 2B (II), chromotrope 2R (III), arsenazo I (IV), arsenazo III (V), benzocaprol red (VI) and acid ethyl blue (VII) is described. The calibration curves resulting from the measurements of absorbance-concentration relations (at the optimum reaction conditions) of the extracted ion-pairs are linear over the concentration range 0.4–56 $\mu g\,ml^{-1}$ with a relative standard deviation (RSD) of 1.3% for 25 $\mu g\,ml^{-1}$ erythromycin. The detection limit, quantification limit, the molar absorptivity and Sandell sensitivity for erythromycin ion-pairs were evaluated. The interference from excipients commonly present in dosage forms and common degradation product was studied. The method is

highly specific for the determination of stearate and succinate esters in dosage forms. The method has been compared to the official method and found to be simple, accurate (t-test) and reproducible (F-test). The developed method was applied for bulk drug, esters and some of their dosage forms without interferences from additives and excipients.

Key Words: Chromotropic acid azo dyes; Erythromycin and its esters determination; Extraction; Colorimetry; Dosage forms analysis.

INTRODUCTION

Erythromycin is a mixture of macrolide antibiotics consisting largely of erythromycin A, (2R, 3S, 4S, 5R, 6R, 8R, 10R, 11R, 12S, 13R)-5-(3-amino-3,4,6-trideoxy-N,N-dimethyl-β-D-xylo-hexopyranosyloxy)-3-(2,6-dideoxy-3-C,3-O-dimethyl-α-L-ribo-hexyopyranosyloxy)-13-ethyl-6,11,12-trihydroxy-2,4,6,8,10,12-hexamethyl-9-oxotridecan-13-olide[114-07-8]. It is produced by the growth of a strain of Streptomyces Eryhreus that is used primarily against gram-positive bacteria. It has four ester forms, estolate [3521-62-8], ethyl succinate [41342-53-4], lactobionate [3847-29-8] and stearate [643-22-1]. Although first used in 1952, it is currently one of the most commonly used antibiotics and it has proved to be a safe and effective therapy for a number of common infections. It 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 entericoated pellets that dissolve in the duodenum. Various esters of erythromycin have been reported in an attempt to improve stability and facilitate absorption. Most derivatives of erythromycin have special properties, which are adopted for specific pharmaceutical uses.

Microbiological methods, which involve the growth of a probe microorganism on a medium containing the antibiotic, suffer from a variety of disadvantages including the lengthy incubation periods required and the lack of selectivity towards other antibiotics. Various chemical methods based on gas chromatography (1,2), liquid chromatography (3–5), adsorptive stripping voltammetry (6,7) and ultraviolet/visible spectrophotometry (8,9) have been used for the determination of this drug.

The formation of ion pair complex provides a simple and rapid convenient method for the measurement of erythromycin solutions at low

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concentrations. The acidic dyes bromocresol purple (10), bromophenol blue(11), bromothymol blue (11,12), methylthymol blue (12), thymol blue (12), orange IV (11), methyl orange (11), erythrosine B (13) and gentiana violet (14) have been used for spectrophotometric determinations of the studied drug.

The purpose of this work was to investigate systematically the formation and extraction behaviour of erythromycin ion pair using chromotropic acid azo dyes as reagents in order to develop useful colorimetric method for determination of it and its esters in pure and in dosage forms. The proposed method has additional advantages of avoiding time-consuming and selectivity of the extracted form in the presence of common additives and degradate products. The proposed method has been success-fully applied to determine erythromycin base and its esters in dosage forms.

EXPERIMENTAL

Reagents

Analytical-reagent grade chemicals and doubly distilled water were used throughout.

Erythromycin (98% purity) was obtained from sigma (St. Louis, MO, USA) and used as received. A standard 2×10^{-3} M solution was prepared by dissolving 0.1468 g of the antibiotic in 100 ml of a 25% (v/v) ethanol-water mixture; this solution remained stable for one month if kept refrigerated. Further dilutions were made to prepare standard solutions containing 10–200 $\mu g\,ml^{-1}$.

A 2×10^{-3} M solutions of chromotropic acid (I), chromotrope 2B (II), chromotrope 2R (III), arsenazo I (IV), arsenazo (III) (V) (Aldrich products), benzocaprol red (VI) and acid ethyl blue (VII) (prepared as recommended previously) (15) were prepared by dissolving the required amount of each reagent in water.

Equipments

A Perkin-Elmer (Norwalk, CT, USA) λ3B UV/VIS spectrophotometer and Jasco 530 V (Tokyo, Japan) spectrophotometer, both with matched 10 mm quartz cells, were used. The pH of solutions was checked using an Orion research Model 601A/digital ionalyzer.

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General Procedures

Using Reagents I–III

Into 50 ml separating funnels aliquots containing up to 500 μg ml^{-1} erythromycin solution were pipetted, 5.0 ml of 2×10^{-3} M reagent I, II or III and 15 ml of 0.2 M HCl. The solution was diluted to 25 ml with water and 10 ml of chloroform were added. After shaking for 1.0 min, the mixture was centrifuged for 1.0 min at 2000 rev min^{-1}. After separating the two layers, the absorbance of the chloroform extracts was measured at 547, 542 and 558 nm on using reagents I, II and III, respectively, against a reagent blank prepared by the same treatment.

Using Reagents IV–VII

Into 50 ml separating funnels, aliquots containing up to 560 μg ml^{-1} of erythromycin solution, 3.0 ml of 2×10^{-3} M reagent solution (IV–VII) and 10 ml of 0.2 M H_2SO_4 were pipetted. The solution was diluted to 25 ml with water, and 10 ml of benzene was added. After shaking for 1.0 min, the mixture was centrifuged for 1.0 min at 2000 rev min^{-1}. The two layers was separated and the absorbance of the benzene extracts was measured at 536, 637, 585 and 675 nm using reagents IV, V, VI and VII, respectively, against a reagent blank containing reagent treated in the same manner.

Determination of Erythromycin in Pharmaceutical Preparations

The required amount of pharmaceuticals to contain 50 mg of erythromycin base was dissolved in 25% (v/v) ethanol-water and any remaining residue was filtered. The clear solution was made up to 100 ml in a calibrated flask. A suitable aliquot was analysed as recommended in the general procedure (A or B).

RESULTS AND DISCUSSION

Erythromycin contains one secondary amine in the desosamine moiety and can be readily protonated. Chromotropic acid and its azo derivatives have been used as ion-pairing agents for the spectrophotometric and extraction-spectrophotometric determination of some pharmaceutical drugs (15–18). The formation of associates between erythromycin and chromotropic dyes is of potential application in the extraction-colorimetric determination of this antibiotic.

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Erythromycin can be transferred from the aqueous phase into organic phase in the form of an ion pair with the anion of these dyes. The following chemical reaction takes place.

$$E^+(aq) + D^-(aq) \leftrightarrows E^+D^-(aq) \leftrightarrows E^+D^-(org)$$

Where E^+ and D^- represent the protonated erythromycin and the anion of the dye, respectively.

To optimize conditions for the complete formation of ion-pair complexes, we have investigated a number of parameters such as acidity, time and temperature, reagent concentration, extracted solvent and solvent ratio. The influence of those variables on the reaction has been tested.

Effect of Acidity

The effect of acidity was investigated by using hydrochloric, sulphuric, nitric, acecitric acids. The results indicate that the ion pair complex is best formed in 0.2 M hydrochloric acid medium on using reagents I–III, whereas 0.2 M sulphuric acid is the optimum media on using reagents IV–VII. The colour intensity of the extracted form reached a maximum on adding 15 ml of 0.2 M HCl (for I–III) or 10 ml of 0.2 M H_2SO_4 (for IV–VII) to 25 ml aqueous phase. This was chosen for all further investigation.

Effect of Time and Temperature

Chromotropic reagents form coloured ion pairs rapidly with erythromycin at $25 \pm 2^{\circ}C$. The extracted coloured form attained maximum intensity instantaneously after shaking and centrifuged for 1.0 min. The coloured ion-pairs were stable for more than 8.0 hrs. Raising the temperature up to $50^{\circ}C$ has no change on the absorbance of the ion pairs, while boiling decreases the colour intensity with blue shift in λ_{max} of the formed ion pairs.

Effect of Reagent Concentration

The optimum concentration of the studied reagents (I–VII) was investigated, for maximum colour formation. It was found that 5.0 ml of 2×10^{-3} M of reagents (I–III) was sufficient for maximum colour intensity, whereas 3.0 ml of 2×10^{-3} M of other reagents (IV–VII) was required per 25 ml of aqueous phase.

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Effect of Extracted Solvent

The polarity of solvent employed affects both wavelength and intensity of the maximum absorption. The results using different extraction solvents applying reagents (I–VII) showed that chloroform and 1,2-dichloro-ethane were the best solvents to extract the ion-pairs of reagents (I–III). The former was selected because of its slightly higher sensitivity and considerably lower extraction ability for the reagent. For other reagents (IV–VII), benzene was found to be the selected solvent for their ion pairs with erythromycin due to highly coloured extracted products, in addition to the lower extraction ability for reagents (IV–VII) in the benzene layer. Reproducible absorbance readings were obtained after a single extraction with 10 ml of chloroform on using reagents (I–III) and/or benzene using reagents (IV–VII) as optimum volume for complete extraction process. The over-all extraction efficiency was 99.7%. Repeated extraction did not show an increase in the recovery percent results.

Nature of Ion Pair

Job's method of continuous variations and the molar ratio methods using both a variable reagent concentration and a variable erythromycin concentration established the composition of the ion pair. The results obtained with these methods showed that the composition of the associate was equimolar (1:1) and the shapes of the curves indicated that the ion pair was labile. Hence, a large excess of reagent must be used to enhance the stability of the ion pair.

Calibration Range, Sensitivity and Precision

The calibration graph was prepared by the general procedure A or B. The relationship between the absorbance of the extracted layer and erythromycin base concentration in the aqueous phase was linear over the range 0.4–56 $\mu g\,ml^{-1}$ ($r = 0.9996$). The optimum concentration range for the effective colorimetric determination of erythromycin base as evaluated from Ringbom's plot is 1.2–53.5 $\mu g\,ml^{-1}$. The detection limits, calculated as the value corresponding to three times the standard deviation of the blank, was 0.08 $\mu g\,ml^{-1}$ of erythromycin base. The above results were collected in Table 1. A statistical study performed on a series of ten samples at 25 $\mu g\,ml^{-1}$ level of erythromycin base, erythromycin ethyl succinate, erythromycin stearate and erythromycin propionate yielded relative standard

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deviations of 1.3, 1.5, 1.7 and 2.0%, respectively. The linear regression equation derived using the least-squares method was also calculated and recorded in Table 1. The apparent molar absorptivity and Sandell sensitivity for each system were calculated and recorded in Table 1.

The performance of the proposed method was judged by calculation of t- and F-values compared with the official method (19) [depending on biological assay procedure] at 95% confidence level for five degrees of freedom (20) and the results showed that the calculated t- and F-values did not exceed the theoretical values indicating that there is no significant different between the proposed and official methods.

Interferences

The influence of concomitant compounds was studied. Solutions of erythromycin and each compound tested were mixed to obtain samples containing $20\,\mu g\,ml^{-1}$ and up to $500\,\mu g\,ml^{-1}$ of the foreign compound. The tolerance ratio of each foreign compound was taken as the largest amount yielding an error of less than $\pm 3.0\%$ in the absorbance of erythromycin ion-pair. Galactose, glucose, lactose, sucrose and saccharin were tolerated in large amounts (500-fold excesses were the maximum molar ratio tested) and 350-fold excess of starch, citric acid and acetylsalicyclic acid were also tolerated. Succinate, ethyl succinate, stearate, propionate do not interfere up to 75-fold excess. For this reason, the determination of erythromycin in its esters can occur without hydrolysis to yield the base form.

Analytical Applications

Determining erythromycin base and its esters in their dosage forms checked the validity of the proposed method. Commercially available pharmaceutical dosage forms were analysed using reagents (I–IV). Interference from tablet, oral suspension or drop matrics or the dyes present in the granules was not problem. The data in Table 2 show that the erythromycin contents as measured by the proposed method were in a good agreement with those obtained by the official one (19).

The results were also evaluated statistically using Student t- and F-values. Mean values were obtained at 95% confidence limits for five degrees of freedom, and the results showed that the calculated t- and F-value did not exceed the theoretical values indicating that the differences between the proposed and official method is not significant. Low standard deviations of the proposed method indicate that the method to be

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Table 1. Quantitative Parameters for the Erythromycin On-Pair Complex Formation Using Reagents (I–VII)

Parameters	Reagent						
	I	II	III	IV	V	VI	VII
Extracted solvent	Chloroform	Chloroform	Chloroform	Benzene	Benzene	Benzene	Benzene
λ_{max} (nm)	547	542	558	536	637	585	675
Beer's law limits $\mu g\,ml^{-1}$	0.4–43.0	0.4–50.0	0.4–47.0	0.4–52.0	0.4–49.0	0.4–56.0	0.4–54.0
Ringbom optimum range $\mu g\,ml^{-1}$	1.2–40.4	1.0–47.2	1.2–44.2	1.0–50.0	1.2–46.6	1.2–53.2	1.0–51.6
Molar absorptivity $1\,mol^{-1}\,cm^{-1}$	1.50×10^4	1.25×10^4	1.17×10^4	1.33×10^4	1.39×10^4	1.02×10^4	1.10×10^4
Sandell sensitivity $\mu g\,cm^{-2}$	0.049	0.059	0.063	0.055	0.053	0.072	0.067
Detection limits	0.08	0.08	0.08	0.06	0.08	0.08	0.06
Regression equation*							
Slope (b)	0.0215	0.017	0.016	0.018	0.019	0.014	0.015
Intercept (a)	− 0.016	0.011	− 0.09	0.13	0.08	− 0.07	− 0.010
Correlation coefficient (r)	0.9996	0.9993	0.9995	0.9992	0.9990	0.9996	0.9994
Range of error (%)	± 1.3	± 1.0	± 1.5	± 0.9	± 1.7	± 1.2	± 1.6
Standard deviation (%)	0.46	0.73	0.67	0.58	0.49	0.38	0.54
Calculated t-value (2.57)**	1.24	1.07	1.36	1.18	1.43	0.92	1.11
Calculated F-value (5.05)**	2.38	2.11	2.68	2.30	2.76	2.05	2.25

*$A = a + bC$ where C is the concentration in $\mu g\,ml^{-1}$.
**Values in parenthesis are the theoretical t- and F-values for five degrees of freedom and 95% confidence limit.

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Table 2. Determination of Erythromycin and Its Esters in Pharmaceutical Dosage Forms

Sample	Supplier	Nominal Value (mg)	Found[a] ± (SD) I	II	III	IV	V	VI	VII	Official Method
Tablets										
Erythrocin[b]	Kahira Pharm. & Chem. Ind. Co. Egypt.	250	247 (0.63)	252 (0.51)	251 (0.37)	248 (0.71)	250 (0.33)	249 (0.82)	248 (0.63)	245 (1.11)
		500	504 (0.82)	498 (0.47)	497 (0.53)	505 (0.88)	496 (0.71)	504 (0.55)	502 (0.41)	490 (1.36)
Erythrin[c]	Misr. Co. for Pharm. Ind. Egypt	250	251 (0.44)	248 (0.79)	249 (0.83)	252 (0.50)	251 (0.55)	248 (0.42)	252 (0.82)	246 (1.40)
Granules										
Erythrocin[b]	Kahira Pharm. & Chem. Ind. Co. Egypt.	200	199 (0.53)	200 (0.35)	202 (0.72)	198 (0.67)	199 (0.48)	201 (0.66)	198 (0.57)	196 (1.51)
Suspension										
Erysthrocid[c]	Chem. Ind. Development Egypt.	200/5 ml	202 (0.77)	199 (0.64)	201 (0.46)	200 (0.36)	201 (0.64)	199 (0.39)	201 (0.74)	205 (1.83)
Erythrin[c]	Misr. Co. for Pharm. Ind. Egypt.	200/5 ml	200 (0.34)	202 (0.84)	199 (0.61)	198 (0.46)	202 (0.85)	201 (0.72)	198 (0.63)	204 (1.73)
Drops										
Erythrin[c]	Misr. Co. for Pharm. Ind. Egypt	200/5 ml	201 (0.57)	198 (0.70)	200 (0.32)	202 (0.83)	199 (0.54)	198 (0.69)	202 (0.59)	195 (1.92)
Erythrocin[b]	Kahira Pharm. & Chem. Ind. Co. Egypt.	200/5 ml	198 (0.75)	201 (0.56)	198 (0.61)	199 (0.42)	198 (0.82)	200 (0.34)	199 (0.49)	203 (1.58)

[a]Average of six determinations.
[b]Erythromycin stearate.
[c]Erythromycin ethylsuccinate.

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accurate, precise and can be used simply for routine work in drug control laboratories.

CONCLUSIONS

The proposed method reports a new example for ion-pair complexes in drug analysis. The method was successfully utilized for determining erythromycin and its esters in pure form as well as in dosage forms and proved to be highly sensitive, accurate, precise, simple in handling and with higher tolerance limits. Student t- and F-values gave lower values relative to the theoretical ones indicating high accuracy and precision with no significant differences compared to the official one (19). Therefore these reagents can be safely used for quality control of erythromycin and its esters in pure and in its dosage forms.

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Received May 25, 2000
Accepted January 18, 2001

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