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Short communication

Selective spectrophotometric method for the determination of erythromycin and its esters in pharmaceutical formulations using gentiana violet

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Abstract

A simple and selective method for the determination of erythromycin and its stearate and succinate esters in their pure forms and in pharmaceutical formulations is described. The procedure is based on the formation of a blue-coloured ($\lambda_{max} = 633$ nm) complex with gentiana violet in alkaline medium. Different variables affecting the colour development were studied and optimized. The method was used to determine 2.5–25 $\mu g\ ml^{-1}$ of erythromycin in the final measured solution. The simplicity of the method permits rapid analysis and it is thus suitable for routine control. The method is highly specific for the determination of stearate and succinate esters in pharmaceutical formulations. The reliability of the method was established by parallel determinations against the official British Pharmacopoeial method.

Keywords: Erythromycin and its esters; Gentiana violet; Pharmaceutical analysis; Spectrophotometric determination

1. Introduction

Erythromycin is a macrolide antibiotic produced by a strain of *Streptomyces erythreus* that is used primarily against gram-positive bacteria. Although first used in 1952, it is still one of the most commonly-used antibiotics and it has proved to be a safe and effective treatment 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 enteric-coated pellets that dissolve in the duodenum.

Various esters of erythromycin have been prepared in an attempt to improve stability and facilitate absorption. Most derivatives of erythromycin have special properties and are adopted for specific pharmaceutical uses. Erythromycin estolate is more acid stable than ery-

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thromycin and thus gives higher levels in the blood when taken with meals than erythromycin. The water-soluble glucoheptonate and lactobionate salts are best suited for the preparation of parenteral products. Erythromycin and its derivatives are available in a wide variety of oral, topical and parenteral products.

Microbiological methods, which involve the growth of a probe micro-organism on a medium containing the antibiotic, suffer from a variety of disadvantages including the lengthy incubation periods needed and lack of selectivity towards other antibiotics. Chemical methods based on ultraviolet/visible spectrophotometry [1,2], gas chromatography [3,4], liquid chromatography [5–7], absorptive stripping voltammetry [8] and computerized flow constant-current stripping analysis [9] have been used for the determination of erythromycin.

Ion-pair formation provides a rapid and convenient method for the measurement of erythromycin solutions at low concentration. The acidic dyes Orange IV [10], Methyl Orange [10], Thymol blue [11], Methylthymol Blue [11], Bromothymol Blue [10,11], Bromophenol Blue [11], and Bromocresol Purple [12] have been used for spectrophotometric determination. Naphthotriazole disulfonate [13] and Erythrosine B [14] and ^{35}S-labelled Methyl Orange [15] have been used for spectrofluorometric and radiochemical assays respectively.

The purpose of this work is to investigate the optimum conditions for ion-pair complex formation to develop a useful spectrophotometric method for the determination of erythromycin and its stearate and succinate esters in their pure forms and in pharmaceutical formulations.

2. Experimental

The absorbance measurements were recorded using a Perkin–Elmer Model Lambda 3B double beam UV–visible spectrophotometer fitted with matched 10 mm quartz cells. pH values were measured with an Orion Research Model 601 A/Digital Ionalyzer pH meter. The pH meter was calibrated regularly before use with standard buffer solutions. All the spectrophotometric measurements were performed in 40% (v/v) methanol–water at 25°C. The pH meter readings in 40% (v/v) methanol were corrected as described by Douheret [16].

2.1. Reagents

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

Erythromycin (98% purity) was obtained from Sigma (St. Louis, MO) and used as received. A standard solution of 1×10^{-3} mol dm^{-3} in 100 ml of 40% (v/v) methanol–water was prepared; this solution remains stable for 1 month if kept refrigerated. Working solutions of lower concentrations were freshly prepared by appropriate dilution of the standard solution.

A 2.0×10^{-3} M gentiana violet (MW = 407.99; Aldrich) stock solution was prepared by dissolving the required amount in methanol.

Borate buffer solutions of pH 5–11 were prepared as recommended [17].

2.2. General procedure

Pipette 2.5 ml of 2×10^{-3} M gentiana violet into a 25 ml measuring flask. Add 12.5 ml borate buffer solution (pH 8.5) and 7.5 ml methanol, a volume containing 625 μg of erythromycin, and make up to the mark with water. Leave the reaction mixture to stand at room temperature (24 ± 2°C) for 5 min. Measure the absorbance at 633 nm against a blank solution prepared in the same manner.

Erythromycin derivatives (esters and salts) have to be hydrolysed to yield free erythromycin base prior to assay. For this purpose, sufficient sample ($\equiv$ 62.5 mg of free base) was placed in a 100 ml measuring flask, dissolved in 40 ml methanol and diluted to about 90 ml with pH 8 phosphate buffer. After incubating at 60°C for 3 h, the solution was diluted to 100 ml with doubly-distilled water and analysed by the procedure described for erythromycin base.

3. Results and discussion

Investigations were carried out to elucidate the most favourable conditions for ion-pair formation between erythromycin and gentiana violet to achieve maximum colour development in the determination of erythromycin. The influence of different variables on the reaction has been tested.

3.1. Effect of solvent

The type of solvent employed affects both wavelength and intensity of the maximum absorption. The effect of methanol, ethanol, propan-l-ol, acetone and dioxane was investigated. The results showed that methanol gives the highest absorbance value and longest wavelength. Moreover, the effect of the percentage of methanol was also investigated and it was found that 40% (v/v) gives the highest absorbance and prevents the formation of precipitates.

3.2. Effect of pH

The effect of pH was investigated by using borate buffer solutions of pH 5–11. The results indicate that the complex is best formed in alkaline media with pH 7.5–9.5 and a value of 8.5 was selected for further study, since the results are highly concordant at such a pH value. 12.5 ml buffer solution in a total volume of 25 ml gives the maximum absorbance value.

3.3. Effect of reagent concentration

The optimum concentration of gentiana violet in the final solution, for maximum colour formation, was found to be 2.5 ml of 2×10^{-3} M reagent solution per 25 ml of the reaction mixture (Fig. 1).

3.4. Effect of time and temperature

Gentiana violet forms a coloured complex rapidly with erythromycin at 24 ± 2°C and the colour attains maximum intensity after 5 min. The blue-coloured complex was stable for more that 12 h. Raising the temperature to 50°C has no effect on the absorbance of the complex while boiling destroys the coloured complex formed.

3.5. Nature of complex

The stoichiometry of the complex formed between gentiana violet and erythromycin was investigated at pH 8.5 by the molar ratio and continuous variation methods. The results indicate that the coloured species are formed by the reaction of the two compounds in a molecular ratio of 1:1 (Fig. 2). The reaction of gentiana violet with erythromycin occurs through the formation of an ion-pair complex.

3.6. Quantification

A linear correlation was found between absorbance and concentration in the range 2.5–25 μg ml^{-1} of erythromycin. The linear regression equation derived using the least-squares method [18] is $A = 0.0073 + 0.0216C$ (C is concentration (μg ml^{-1}); $r = 0.9999$). The validity of the derived regression equation was assessed in the determination of the drug in tablets, granules, suspensions and drops. The apparent molar absorptivity of the resulting coloured product was 1.59×10^4 l mol^{-1} cm^{-1} whereas the Sandell sensitivity amounts to 4.63 ng cm^{-2}. For more accurate

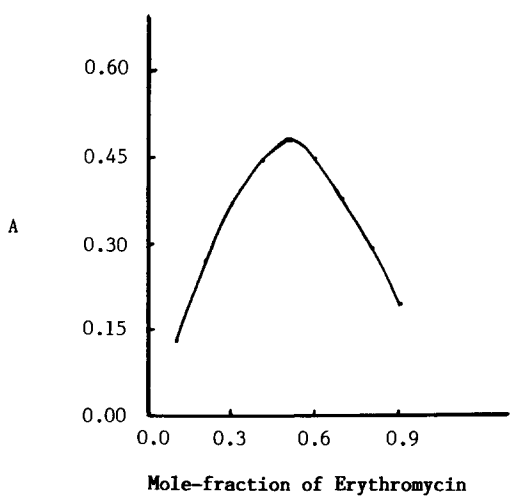

Fig. 1. Continuous variation method for the erythromycin–gentiana violet system.

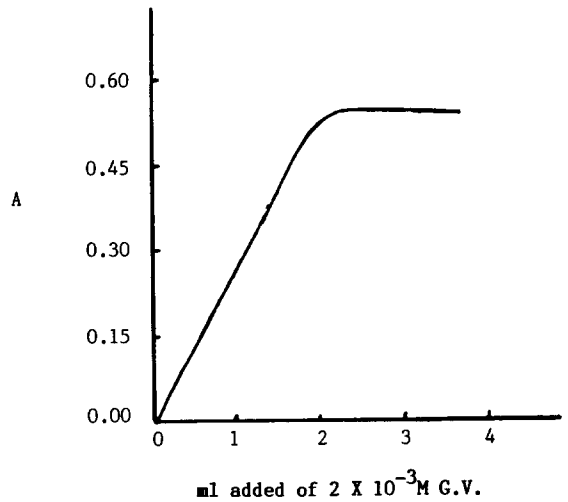

Fig. 2. Effect of gentiana violet concentration on the absorbance of the complex formed.

analysis, the Ringbom optimum concentration range was calculated and found to be 2.5–23 μg ml^{-1}.

The mean of ten replicate analyses of a solution of erythromycin at a concentration of 15 μg ml^{-1} assayed at 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. The recoveries of the different amounts tested determined from the calibration curve amounted to 99.8 ± 1.3%.

The performance of the suggested method was judged by calculation of *t*- and *F*-values and at the 95% confidence level, the corresponding values were 2.14 and 1.82 respectively, which are in good agreement with the theoretical values of 2.31 and 2.45 respectively.

3.7. Interferences

The influence of concomitant compounds was studied. Solutions of erythromycin and each com-

Table 1
Determination of erythromycin in pharmaceutical preparations

Sample	Nominal value (mg)	Found[a] (mg)			Recovery (%)
		Pharm. method [19]	Reference method [13]	Proposed method	
Erythrocin[b] tablet	250	245 ± 2.8	247 ± 2.4	251 ± 1.37	100.40
Erythrocin[b] tablet	500	505 ± 2.7	504 ± 2.8	497 ± 1.54	99.40
Erythrocin[b] granules	200	197 ± 2.6	198 ± 2.5	201 ± 1.19	100.50
Erythrocid[c] suspension	200/5 ml	197 ± 2.6	198 ± 2.5	202 ± 1.44	101.00
Erythrin[d] tablet	250	245 ± 2.8	247 ± 2.4	252 ± 1.13	100.80
Erythrin[d] suspension	200/5 ml	197 ± 2.6	198 ± 2.5	199 ± 0.89	99.50
Erythrin drops	200/5 ml	197 ± 2.6	198 ± 2.5	200 ± 0.27	100.00
Erythrocin[b] drops	200/5 ml	197 ± 2.6	198 ± 2.5	198 ± 2.33	99.00

[a] Average of six determination.
[b] Erythrocin (erythromycin stearate), manufactured by Kahira Pharm. and Chem. Ind. Co., under licence from Abbott Laboratories, North Chicago, IL.
[c] Erythrocid (erythromycin ethylsuccinate ester), produced by Chem. Ind. Development, S.A.A. Cairo, Egypt.
[d] Erythrin (erythromycin ethylsuccinate ester), produced by Misr Co. for Pharm. Ind. S.A.A. Mataria, Cairo, Egypt.

pound tested were mixed to obtain samples containing 15 μg ml^{-1} of 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 $\geq \pm 2.5\%$ 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, citric acid and acetylsalicylic acid were also tolerated.

3.8. Analysis of pharmaceutical preparations

In order to establish the validity of the proposed procedure, pharmaceutical preparations were analysed. Commercially available phamaceutical formulations were analysed using the gentiana violet procedure. Interference from tablet, oral suspension or drop matrices or the dyes present in the granules was not a problem. The data in Table 1 show that the erythromycin contents as measured by the proposed procedure were in excellent agreement with those obtained by the British Pharmacopoeia (1993) [19] and the method described by Tserng and Wagner [13].

4. Conclusions

The above results indicate that erythromycin, which has no characteristic spectrophotometric groups in its molecular form, can be determined by ion-pair complex formation with gentiana violet. The spectrophotometric procedure developed for erythromycin allows its determination in pharmaceutical preparations. Compared with the reference method, the recommended procedure offers considerable economy as regards reagent consumption and time required for the analysis without any loss of precision. The proposed procedure is useful for quality control of erythromycin in pharmaceutical dosage forms.

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