A New Colorimetric Method for the Assay of Erythromycin

M. Saeed Arayne (Department of Chemistry, Faculty of Pharmacy, University of Karachi.)
Najma Sultana, Shahnaz Begum (Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Karachi.)

Abstract
A chemical assay method which depends upon the development of intense absorption at 485 nm has been found satisfactory for the assay of erythromycin and its stearate from tablets, suspensions and other preparations. Its utility is brevokable for the determination of this antibiotic at all stages of manufacture, process and formulation, and it is more rapid and reproducible than the bioassay (JPMA 33:256, 1983).

Introduction
Erythromycin (McGuire et al., 1952; The Pharmaceutical Codex, 1979) C37H67N013, M.Wt 733.9 is a basic antibiotic that is used extensively for the treatment of various bacterial infections. An accurate, rapid and precise method for its determination at all stages of processing and formulation of this antibiotic was desired to supplement the Pharmacopoeial bioassay methods (Minimum requirements of antibiotic products, Japan, 1956; Pakistan Pharmacopoeia, 1974; British Pharmacopoeia, 1980; Pharmacopoeia of the United States, 1980; The National Formulary XW, 1975; Clarke, 1978). The intact antibiotic has been reported to be transparent in the visible portion of the spectrum and shows only a weak absorption at 280 - 285 mu (Tepe and John, 1955). During the course of this investigation many color reactions were studied for the development of a chemical method for analysis but those that gave positive reactions with pure erythromycin were either not specific for erythromycin or not sufficiently sensitive for dilute solutions, or degradation products of erythromycin caused variable results. Other reactions investigated and found useful for the analysis of pure samples were not applicable for process samples because of lack of sensitivity or specificity. The reactions studied include the treatment of erythromycin with transition metals like Fe and Mn, phosphotungstic acid and ascorbic acid.

During some preliminary experiments on degradation, it was observed that an intense yellow colour was produced when erythromycin was heated with 6N hydrochloric acid or with varying concentrations of sulfuric acid which absorbs at 485 nm. After considering the above procedures, it was found that the characteristic absorption properties of hydrolysed erythromycin offered most promising method for analysis. Erythromycin itself exhibits only a broad weak absorption band in the ultraviolet at 285 nm. After dilute alkaline hydrolysis, erythromycin exhibits strong absorption at 236 nm with an $E_{10^0\%} cm$ of 85 but in that case the acid untreated blank is not satisfactory because of the changes that may occur in impurities during the alkaline hydrolysis.

After strong acidic hydrolysis at elevated temperature it exhibits maxima at 226, 267 and 485 nm. The absorption at 226 nm has an $E_{10^0\%} cm$ of approximately 150 and obeys Beer Law Dut other degradation products in the process samples of erythromycin also have absorption in the 226-236 nm region and limit the usefulness of this absorption range. The absorption at 267 nm do not obey beer’s law. The only absorption left over is in the visible region of the spectrum at 485 nm which has been used in our present study to estimate the percentage of erythromycin in various formulations.

Material and Method
**Apparatus:**
A Beckman Spectrophotometer Model 25, Unicam SP 500 or Bausch and Lomb Spectronic 20 or 21 or any other convenient model.

**Reagents:**
i) 27N Sulphuric acid: Add 75 ml of the reagent grade acid (Sp. Gr. 1.84) to 35 ml of distified water slowly and cautiously with constant stirring and cooling.
ii) Methanolpure
iii) Erythromycin Reference Standard.

**Method**
In case of dry materials weigh and dissolve samples of erythromycin base or its salts, and make appropriate dilutions in case of aqueous solutions. Dilute this solution so that each ml of aliquot contains approximately 50 ug/ml of erythromycin activity.

Erythromycin base dissolves very slowly in water. The rate of solution is increased considerably by first dissolving the erythromycin in few ml of absolute methanol and diluting it with freshly prepared distilled water.

Pipette out 5 ml of this solution into a test tube, add 5 ml of 27 N sulfuric acid, heat on a water bath for 30 minutes at 50 °C, and measure the absorbance at 485 nm in a 1 cm cell using distilled water as blank.

Weigh accurately an amount of erythromycin primary standard equivalent to 25 mg of erythromycin base and transfer to a 25 ml volumetric flask. Add 1 ml of absolute methanol to dissolve and dilute to the mark with freshly prepared distilled water. Prepare this solution immediately before use. Dilute aliquots of this solution with fresh distilled water so that each ml of aliquots contain 25, 50, 75, 100, 125, 150, 175 and 200 ug of erythromycin activity respectively. Determine the absorbance of each aliquots as described above for sample solutions.

Determine the concentration of the sample solution by reference to the standard curve plotted by measuring the absorbance of the standard solutions at 485 nm versus concentration (as shown in the accompanying figure).

**Results and Discussion**
The described procedure has been employed for the determination of erythromycin from various formulations and standard erythromycin. Samples from one lot of the commercial production of each brand of erythromycin tablets were obtained directly from the market available in Pakistan at the time of study.
Table I lists results of various samples of erythromycin stearate tablets and powder from different sources. These were in good agreement with those obtained by the standard microbiological method (British Pharmacopoeia, 1980) and are compared in the sample table.

**Effect of Sulphuric Acid Concentration:**

The results obtained by varying the concentration of sulphuric acid are listed in table II.
As the colour intensity was found to be about the same for 12 N and 14N acids 27 N sulphuric acid was used as the reagent and added to an equal volume of water which contained the antibiotic. The resulting solution gave the same absorption maxima at about 485 nm and the intensity was found to be about twice than obtained with 6N sulphuric acid.

**Effect of heating time on colour development:**
When 5 ml portions of 27N sulphuric acid were pipetted into 5 ml samples of 150 Ug/mi of erythromycin solution and the test tubes were placed in a bath at 50°C for different time intervals to study the effect of heating time on the absorbance of erythromycin hydrolysate, the increase in absorbance was very slight after 30 minutes (Table III).
Although a 30 minute time period of heating at 50°C gave satisfactory result, it was not even necessary to use a 50°C bath, as the heat of dilution of the 27N sulphuric acid was sufficient to develop the colour.

**Reproducibility:**

<table>
<thead>
<tr>
<th>Heating Time in Minute</th>
<th>Absorbance at 485 ug/ml</th>
</tr>
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<tbody>
<tr>
<td>10</td>
<td>1.200</td>
</tr>
<tr>
<td>20</td>
<td>1.232</td>
</tr>
<tr>
<td>30</td>
<td>1.237</td>
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<tr>
<td>40</td>
<td>1.238</td>
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<td>50</td>
<td>1.239</td>
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<tr>
<td>60</td>
<td>1.239</td>
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<tr>
<td>70</td>
<td>1.239</td>
</tr>
<tr>
<td>80</td>
<td>1.238</td>
</tr>
</tbody>
</table>
The figure shows the absorbance of eight sets of duplicate determinations, and the evaluation of these results. These analysis were on different weights of erythromycin corrected to a unit weight in the table (50 Ug/mi).

**Interference**
The small amount of solvents present in the sample did not affect the test. Methanol which has no effect up to a concentration of 1% of final dilution, was used as a solvent whenever needed. One percent
of -acetone in the final dilution results in an assay that is 3 to 5% low.

References