*Int. J. Chem. Sci.: 14(3), 2016, 1569-1576*
*ISSN 0972-768X*
*www.sadgurupublications.com*

# VISIBLE SPECTROPHOTOMETRIC DETERMINATION OF AZITHROMYCIN IN PURE AND DOSAGE FORMS

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## ABSTRACT

Two simple, sensitive selective accurate and economical spectrophotometric methods A and B for the determination of azithromycin in bulk drug and pharmaceutical formulations (tablets) have been described in the present work. Method A is based on the formation of colored ion-association complex between azithromycin and tropaeolineo-oo (TPoo) to yield a color exhibiting absorption maximum at 485 nm and obeying Beer's law in the concentration range of 5-25 μg/mL. The method B is based on the formation of colored ion-association complex between azithromycin and alizarine Red S (ARS) to yield a colored chromogen exhibiting absorption maximum at 440 nm and obeying Beer's law in the concentration range of 5-25 μg/mL. The results are compared with those obtained using UV spectrophotometric method in chloroforms at 260 nm.

**Key words**: Azithromycin, Methods A and B, UV spectrophotometric method.

## INTRODUCTION

Azithromycin, chemically, (2R, 3S, 4R, 5R, 8R, 10R, 11R, 12S, 13S, 14R) -2-ethyl-3, 4, 10-trihydroxy-3, 5, 6, 8, 10, 12, 14-heptamethyl-15-oxo-11-{[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-]oxy}-1-oxa-6-azacyclo-pentadec-13-yl-2, 6-dideoxy-3-C methyl-3-O-methyl-α-Lribo-hexopyranoside is a semi-synthetic macrolide antibiotic widely used in the respiratory tract infections, like pharyngitis, pneumonia, chronic bronchitis, bronchopneumonia, skin and soft tissue infections and some sexually transmitted diseases, that acts on Gram positive bacteria and Gram negative bacteria[1-3]. The most commonly used techniques for the determination of azithromycin in pharmaceutical dosage forms are high performance liquid chromatography, liquid chromatography-mass spectrometry, microbiological, differential pulse voltammetric, amperometric diffuse reflectance near

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infrared spectroscopy and spectrophotometric methods. It is used in respiratory tract infections like pharyngitis, pneumonia, chronic bronchitis and bronchopneumonia[4-7]. The recommended dosage for azithromycin is 100-500 mg per day. Azithromycin is official in the United States Pharmacopoeia and it is assayed by the high performance liquid chromatographic method. Literature survey reveals that azithromycin is estimated in pharmaceuticals and biological fluids by spectrophotometric, HPLC and microbiological methods. Metal oxides play a very important role in many areas of chemistry, physics and materials science. The metal elements are able to form a large diversity of oxide compounds. In technological applications, oxides are used in the fabrication of microelectronic circuits, sensors, piezoelectric devices and fuel cells, coatings for the passivation of surfaces against corrosion and as catalysts[8-23]. Rao et al.[24-47] presented our results on different oxide materials in our earlier studies.

However, chromatographic techniques require long experimental procedures for sample clean up and demand expensive equipment. In differential pulse voltammetric method, the adsorption of the drug on the electrode surface has not been sufficiently strong and hence it has not been analytically useful. Most of the reported methods are highly sophisticated, costly and time-consuming. In the present investigation, an attempt has been made to develop a simple, accurate, and reproducible spectrophotometric method for estimation of azithromycin in pharmaceutical formulations. In Method A, the presence of secondary amine group in azithromycin enabled the uses of its ion association complex with TPoo to form orange red colored exhibiting absorption maximum $\lambda_{max}$ at 490 nm and obeying Beer's law in concentration range. 5-25 µg/mL. In method B Ion-association reaction with ARS The drug formed yellow colored due to the presence of secondary amine, which exhibited absorption $\lambda_{max}$ at 440 nm and obeying Beer's law in the concentration range 5-25 µg/mL. Spectrophotometric parameters were established for standardization for the two method A and B including statistical analysis of data. These methods have been successfully extended to the pharmaceutical preparations (tablets) containing azithromycin.

## EXPERIMENTAL

All spectral measurements were done An Elico, UV-Visible spectrophotometer (SL-159) with 1 cm matched quartz cells was used for the spectral and absorbance measurements. An Elico LI-120 digital pH meter was used for pH measurements.

## Reagents

### Method A

| | | |
|---|---|---|
| Tpoo solution (Fluka; 0.2%, 5.70 x $10^{-3}$M) | : | Prepared by dissolving 200 mg of tropaeoline oo in 100 mL of distilled water |
| HCl (E. Merck, 0.1 M) | : | Prepared by diluting 8.6 mL of concentrated hydrochloric acid to 1000 mL with distilled water and standardized. |
| Chloroform (Qualigens) | : | AR grade of chloroform was used. |
| Distilled water | | |

### Method B

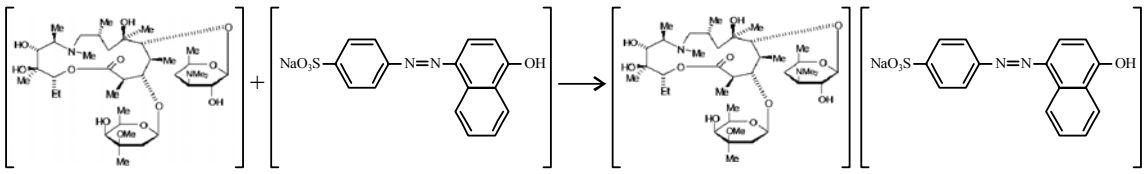
| | | |
|---|---|---|
| ARS reaction (0.2%, 5.84 x $10^{-3}$M) | : | 200 mg of ARS dissolved in 100 mL distilled water. |
| HCl solution (0.1M) | : | 8.6 mL of conc HCl in 100 mL with distilled water |
| Distilled water | | |

## Preparation of standard drug solutions

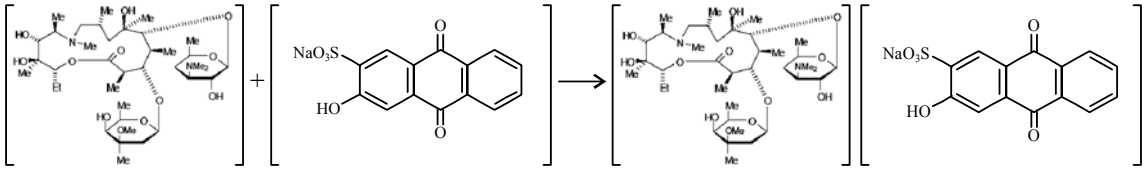
A 1 mg/mL solution was prepared by dissolving 100 mg of pure azithromycin in 100 mL of 0.1 N $CH_3COOH$ and this stock solution was diluted step wise with distilled water to get the working standard solutions of concentration of 250 µg/mL (Method A), 250 µg/mL (Method B).

## Method A and B

In to a series of 125 mL separating funnels contain aliquots of standard azithromycin solution (0.5-3 mL) 250 µg/mL 6.0 mL, 0.1 M HCl and 2.0 mL of 2% dye solution method A (TPoo), Method B (ARS) were added successively the total volume of aqueous phase in each separating funnel was made to 15.0 mL with distilled water. To each separating funnel 10 mL of chloroform was added as the contents were shaken for 2 min. The two phases were allowed to separate and the absorbance of the separated chloroform layer was mentioned at $\lambda_{max}$ 490 nm of TPoo, 440 nm of ARS against reagent blank (Scheme 1).



Azithromycin Tpoo Ion association complex



Azithromycin ARS Ion association complex

**Scheme 1**

## RESULTS AND DISCUSSION

The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity are presented in Table 1. The regression analyses using the method of least squares were made for the slope. Intercept and correlation obtained from different concentrations and the results are summarized in Table 1. The present relative standard deviation and percent range of error calculated from the six measurements ¾ of the upper Beer's law limits of azithromycin are given in Table 1.

**Table 1: Optical and regression characteristics, precision and accuracy of the proposed methods for AZA**

| Parameter | Method A | Method B |
|---|---|---|
| $\lambda_{max}$ (nm) | 490 | 440 |
| Beer's law limits (µg/mL) | 5 – 25 | 5 - 25 |
| Molar absorptivity (1 $mol^{-1}.cm^{-1}$) | $5.126 \times 10^3$ | $4.412 \times 10^3$ |
| Sandell's sensitivity (µg.$cm^{-2}$/0.001 absorbance unit) | 0.08554 | 0.2994 |
| Optimum photometric range (µg/mL) | 5-20 | 5-20 |
| Regression equation (Y = a + bc) | | |
| Slope (b) | 0.04053 | 0.01088 |
| Standard deviation on slope ($S_b$) | $2.070 \times 10^{-2}$ | $4.586 \times 10^{-3}$ |

Cont…

| Parameter | Method A | Method B |
|---|---|---|
| Intercept (a) | $2.499 \times 10^{-4}$ | $4.999 \times 10^{-3}$ |
| Standard deviation on intercept ($S_a$) | $1.373 \times 10^{-2}$ | $1.5211 \times 10^{-2}$ |
| Standard error on estimation ($S_e$) | $1.309 \times 10^{-2}$ | $1.450 \times 10^{-2}$ |
| Correlation coefficient (r) | 0.9998 | 0.9997 |
| Relative standard deviation (%)* | 1.226 | 1.6015 |
| % Range of error (confidence limits) | | |
| 0.05 Level | 1.410 | 1.841 |
| 0.01 Level | 2.210 | 2.887 |
| % Error in Bulk samples ** | 0.282 | -0.29 |

*Average of six determinations considered

**Average of three determinations

The results showed that these methods have reasonable precision. Comparison of the results obtained with the proposed and UV methods for dosage forms (Table 2) confirm the suitability of these methods for pharmaceutical dosage forms. In order to justify the reliability and suitability of the proposed methods, known quantities of pure azithromycin was added to its various reanalyzed formulations and the mixture was analyzed by the proposed methods. The results of recovery experiments were analyzed by the proposed methods and the results of recovery experiments are also summarized in Table 2. The other active in gradients and excipients usually present in pharmaceutical dosage forms did not interfere.

**Table 2: Estimation of Azithromycin in pharmaceutical formulations**

| Sample | Labelled amount (mg) | Amount obtained (mg) | | UV method | % Recovery of proposed methods** | |
|---|---|---|---|---|---|---|
| | | Proposed methods* | | | | |
| | | Method A | Method B | | Method A | Method B |
| Tablet - 1 | 250 | 248 | 247 | 248 | 99.59 | 99.19 |
| Tablet - 2 | 500 | 495 | 496 | 498 | 99.19 | 99.39 |

Average of six determinations

** Mean and standard deviation of six determinations

## CONCLUSION

The proposed methods are found to be simple, sensitive selective, accurate and economical when compared to quantitative methods by HPLC and LCMS. It can be used in the determination of azithromycin in bulk drug and its pharmaceutical dosage forms in a routine manner.

## ACKNOWLEDGEMENT

The authors are thankful to M/s Hetero Drugs Limit, Hyderabad for gift sample and Department of Chemistry, Andhra Loyola College, Vijayawada for providing laboratory facilities.

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*Accepted : 15.07.2016*