Spectrophotometric Method Development and Validation of Azithromycin in Tablet Formulation

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Abstract

A simple, accurate, precise and sensitive UV-visible spectrophotometric method was developed for the determination of Azithromycin (AZI). The solvent which was utilized in this method was 0.1 M Hydrochloric acid and there was a clear bathochromic shift in the λmax obtained when compared to the other methods available. The λmax was found to be 275 nm and the determination was found to be linear in the range of 1 – 4 mg/mL .The method was validated as per ICH guidelines, the mean correlation coefficient was found to be 0.9985. The method was found to be precise as %RSD was found to be less than 2%.The method was successfully validated and the results of the study revealed that the developed method is accurate, precise, sensitive and specific

Keywords – *Azithromycin, UV-Spectrophotometric Method, Validation, ICH Guidelines*

1. INTRODUCTION

Azithromycin (AZI) is chemically known as (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-exopyranosyl)-oxy]-2-ethyl-,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethyl-amino)-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-aza-cyclopentadecan-15-one dehydrate[1] (Fig. 1). AZI belongs to a branch of macrolide antibiotics, which are named as azalide and is categorized as semi-synthetic broad spectrum antibiotic.[2].AZI is used in various bacterial infections like ear infection, typhoid, strep throat and skin diseases.[3.4] [From the literature survey conducted, it was noted that many antimicrobial agents are available in the market and there is need to analyse these drugs for their quality and safety. Various methods which are available for AZI are HPLC, spectrophotometry and microbial assay methods.[5] These methods are either using costly reagents or the method is tedious. Therefore, a simple and cheap UV spectrophotometric method for AZI is developed and validated according to ICH guidelines.

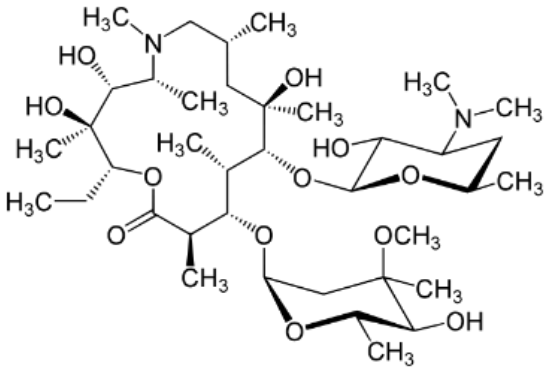

Fig. 1 Chemical structure of Azithromycin

2. MATERIALS AND METHODS

2.1 Instrument

A double beam UV-visible spectrophotometer Shimadzu, 1601) having two matched quartz cells with 1 cm light path and loaded with UV probe software was used for recording of spectra and measuring absorbance. Electronic weighing balance (Acculab, Bangalore) and sonicator (sonica) were used in this study.

2.2 Chemicals and Reagents

HPLC grade Methanol was obtained from (Rankem, Gujarat). HPLC grade Water was obtained from (Merck limited, Mumbai). LR grade Sodium hydroxide was obtained from Sigma Aldrich Chemical Pvt Ltd (Bangalore, India). Azithromycin standard drugs was procured from Yarrow Chem products (Bangalore, India). Marketed formulation with label claim of 250 mg in tablet dosage form was obtained from local pharmacy.

2.3 Preparation of Standard Stock Solution

500 mg of standard AZI was accurately weighed and taken in a 100 mL clean and dry volumetric flask containing 80 mL of 0.1 M hydrochloric acid and was made up to 100 mL. This is considered as the standard stock solution (5 mg/ml). Series of dilutions were prepared over a range of 1 - 4 mg/mL solution.

2.4 Preparation of Sample Solution

Twenty tablets with label claim of 250 mg of AZI were accurately weighed and finely powdered with the help of a motor and pestle. The powdered drug, equivalent to 500 mg of AZI, was dissolved in 60 mL of 0.1 M hydrochloric acid. The solution was kept for sonication for about 10 min, followed by filtration (whatman filter paper no.1). The filter was rinsed 3 times with 10 mL of 0.1 M hydrochloric acid and then the volume was made up to 100 ml.

3. VALIDATION OF THE ANALYTICAL METHOD [6]

3.1 Linearity

Standard solutions of AZI over a concentration range of 1 – 4 mg/mL were prepared from a 5 mg/mL stock solution and absorbance was measured. Five replicates were carried out. Calibration curve was constructed by plotting the concentration level of drug versus absorbance.

3.2 Precision

Three replicates each of intra and inter day studies in five cycles were carried out to determine the repeatability of the method.

3.3 Accuracy

Accuracy studies were carried out, by spiking the sample solution with standard of AZI 80, 100, & 120 %. Three replicates were carried out. This method was analysed, to estimate the recovery of the drug, at different levels.

3.4 Sandell's Sensitivity

Sensitivity of the proposed method is determined by calculating the Sandell's sensitivity, which is defined as the smallest weight of substance that can be detected in column of unit cross section.

Sandell's sensitivity is calculated by

Sandell's sensitivity = Concentration of the drug (µg/mL) x 0.001 /Absorbance

3.5 Limit of Detection and Limit of Quantification

Limit of detection and limit of quantification were calculated on the basis of the Standard Deviation of the Response and the Slope

The limit of detection (LOD) may be expressed as:

$LOD = 3.3 \times \sigma/S$

The limit of quantitation (LOQ) may be expressed as:

$LOQ = 10 \times \sigma/S$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The estimation of slope (S) can be done from the data obtained from calibration curve of the analyte. The estimate of σ may be carried out using standard deviation of the response.

Table 1. Optical characteristics of AZI

Parameters	Azithromycin
λ_{max}	275.2
Linearity (mg/mL)	1-4
Molar Absorptivity (L/ mol /cm)	0.0199 x 10^4
Sandell's Sensitivity (µg/ cm^2)	0.0376
Limit of Detection	0.6490 mg/mL
Limit of Quantification	1.9668 mg/mL

3.6 Analysis of the Marketed Formulation

2 mg/mL of AZI of the sample solution were applied in 5 replicates. The risk and possibility of interference of the excipient in the analysis was studied.

4. RESULTS AND DISCUSSIONS

4.1 Absorption Spectra for Azithromycin

Spectrum of AZI by spectrophotometric method is shown in (Fig. 2) given below. The λmax of AZI was found to be 275 nm in 0.1 M hydrochloric acid

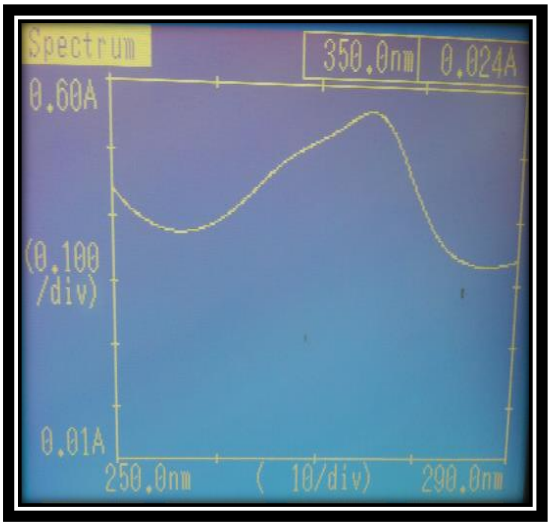

Fig. 2 UV-visible spectrum of AZI

4.2 Results of Validation Parameters

Table 2. Linear regression data of AZI

Parameters	Azithromycin
Correlation coefficient r^2	0.9985
Slope	0.2517
Intercept	0.0332

Table 3. Linearity data of AZI

Sl. no.	Concentration (mg/ml)	Mean Absorbance
1	1	0.2734
2	1.5	0.4221
3	2	0.5323
4	2.5	0.6647
5	3	0.8044
6	3.5	0.904
7	4	1.036

4.2.1 Optical Characteristics

Optical characteristics of AZI are tabulated in the (Table 1) given below. The data obtained for the optical characteristics, LOD and LOQ, clearly indicated that the proposed method for AZI is sensitive and even small quantities of compounds can be estimated accurately

4.2.2 Linearity

Good linear relationships were obtained over a concentration range of 1 - 4 mg/mL for AZI with respect to absorbance. The linear regression data for AZI is shown in (Table 2 and 3) and graphical representation is shown in (Fig. 3). From the graphical data of AZI, it is obvious that absorbance values of AZI are linear over the concentration range of 1 – 4 mg/mL.

Table 4. Recovery studies of AZI

Sl no	Conc. (mg/ml)		Mean Absorbance	Std.Dev	% RSD
1	1		0.278	0.0032	1.1511
2	1.5		0.430	0.0049	1.1395
3	2		0.550	0.0059	0.9636
4	2.5		0.663	0.0017	0.2564
5	3		0.806	0.0022	0.2730
6	3.5		0.920	0.0060	0.6522
7	4		1.025	0.0196	1.9122
		MEAN			**0.9069**

Table 5. Intra Day Studies of AZI

Sl. No	Conc, (mg/ml)	Mean Absorbance	Standard deviation	% RSD
1	1	0.273	0.0039	1.4286
2	1.5	0.422	0.0032	0.7583
3	2	0.532	0.0022	0.4135
4	2.5	0.665	0.0030	0.4511
5	3	0.804	0.0028	0.3082
6	3.5	0.904	0.0028	0.3097
7	4	1.036	0.0174	1.6795
MEAN				**0.7641**

Table 6. Inter Day Studies of AZI

Drug	Amount of drug present (mg/ml)	Amount of drug added (mg/ml)	Total amount of Azithromycin (mg/mL)	Mean % Recovery ± %RSD
Azithromycin	1	0.8	1.8	**99.9268 ± 0.2336**
	1	1	2	**100.0366 ± 0.1215**
	1	1.2	2.2	**101.1156 ± 0.6231**

Table 7. Assay values of AZI

Sl no	Concentration (mg/mL)	% Assay
1	2	99.12
2	2	99.12
3	2	99.12
MEAN		**99.12**

4.2.3 Accuracy

The proposed method was carried out by spiking the tablet formulation with 80, 100 and 120% of the standard. The results are tabulated in (Table 4). The excellent mean recoveries with % RSD obtained, suggests that the proposed method is accurate and no interference from formulations recipients.

4.2.4 Precision

Three replicates of each concentration in the range of 1-4 mg/mL were used for intra and inter day studies, five cycles were carried out. The results are tabulated in the (Table 5 and 6). Low values of the mean % RSD for inter and intra-day variation suggests, an excellent precision of the proposed method.

4.2.5 Analysis of Marketed Formulation

Sample solution of 2 mg/mL of AZI was prepared and measured for absorbance (Three trials were carried out). There was no interference from any of the formulation excipients and any other impurities in the sample. The mean amount of the drug present was found to be 99.12 % for AZI.

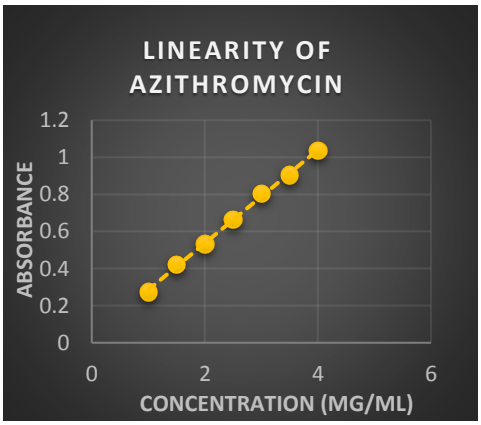

Fig. 3 Graphical representation of AZI for linearity studies

5. CONCLUSIONS

The proposed spectrophotometric method for azithromycin in bulk and formulation dosage showed a large bathochromic shift in the λ max from 240 nm to 275 nm when compared with other solvent systems when

performed in 0.1 M hydrochloric acid. The method was successfully validated and the results of the study revealed that the developed method is accurate, precise, sensitive and specific. Thus the developed method can be applied for the routine analysis of AZI in bulk and formulation dosage form

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