Development of the UV Spectrophotometric Method of Azithromycin in API and Stress Degradation Studies

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ABSTRACT: Azithromycin (AZI) is a semi-synthetic macrolide antibiotic drug, effective against a wide variety of bacteria. The present study describes a simple, accurate, reproducible and precise UV Spectrophotometric method for the estimation of AZI (pH 6.8 Phosphate buffer). The absorbance maximum (λ_{max}) for AZI was found to be 208nm. The method reveals high sensitivity, with linearity in the 10 $\mu\text{g/ml}$ to 50 $\mu\text{g/ml}$ range. The lower limit of detection was found to be 1.6 $\mu\text{g/ml}$ and the limit of quantification was found to be 5 $\mu\text{g/ml}$. All the calibration curves demonstrated a linear relationship between the absorbance and concentration, with the correlation coefficient higher than 0.99. The % recovery was found to be 99.72%. AZI was also subjected to stress degradation under different conditions recommended by the International Conference on Harmonization (ICH).

Introduction:

Azithromycin (AZI) is chemically known as (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-azacyclopentadec-13-yl-2,6-dideoxy-3-C-methyl-3-O-methyl- α -L-ribo-hexopyranoside (figure 1), is a broad spectrum semi-synthetic macrolide antibiotic, called azalides. According to Amantea and co-workers AZI protects mice against ischemic stroke injury by promoting macrophage transition towards M2 phenotype [1]. The chemistry, mechanism of action, antimicrobial spectrum, pharmacokinetics, clinical efficacy, adverse effects, drug interactions, and dosage and administration of AZI were described by elsewhere [2]. Foulds and co-workers studied the pharmacokinetics of AZI and found that approximately 37% of a single oral dose of 500 mg was bioavailable and produced a peak serum concentration of 04 mg/L while, multiple dose regimens produced only slight increases in peak serum concentrations [3]. Mendic and co-workers investigated the electrochemical oxidation of AZI in order to elucidate the mechanism for possible oxidative metabolic pathways in humans [4]. Various methods were proposed to estimate the AZI for both plasma and non-plasma medium like voltammetric assay [5], HPLC [6-8], HPTLC [9], LC-MS [10,11], LC-MS/MS [12]. Present study is aimed to develop a selective, precise, accurate and reliable UV method for determination of AZI.

Figure 1: Chemical structure of azithromycin

Materials and methods

Instruments and reagents

For spectral measurements, Shimadzu UV/VIS double beam spectrophotometer (model 1800) with 1 cm matched quartz cells was used. Shimadzu AX200 analytical balance was used for the weighing purpose. For robustness study Shimadzu (model 1700) was used. The API use was kindly gifted by Nectar Drugs Pvt. Ltd., Mumbai, India. Marketed formulation of Azithromycin used for the specificity study was purchased from local market (AZIPHAR 250 mg tablet). All other chemicals were of analytical grade.

Preparation of standard stock solution (SSS) and working standard solutions (WSS)

Accurately weighed 10 mg of AZI was transferred to a 10 mL volumetric flask and volume was made up with the 0.1 N HCl to get SSS of concentration 1000 µg/mL. From the SSS 1 mL was drawn out and diluted with pH 6.8 phosphate buffer up to 10 mL to get a WSS having concentration of 100 µg/mL and then further dilutions were made to obtain the concentration range of 10-50 µg/mL using pH 6.8 phosphate buffer as a solvent.

Phosphate buffer (pH 6.8) was prepared by taking 50.0 mL of 0.2 M potassium dihydrogen phosphate in a 200 mL volumetric flask, subsequently 22.4 mL of 0.2 M sodium hydroxide was added and then volume was make up with D/W water. Filter if necessary with whatman filter paper.

Determination of wavelength of maximum absorption

An UV spectroscopic scanning (200 - 400 nm) was carried out with the WSS solution to determine the λ_{max} for the detection of AZI using Phosphate buffer (pH 6.8) as blank.

Analytical method validation

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specified method will consistently produce a desired result, or a product meeting its predetermined quality. The method was validated for different parameters like linearity, specificity, selectivity, accuracy, precision, robustness, ruggedness, limit of quantification (LOQ) and limit of detection (LOD) as per the guideline prescribed by International conference on Harmonization (ICH) [13].

Linearity

The sample was analysed using UV-VIS spectrophotometer, using phosphate buffer pH 6.8 as a blank. The linearity of the AZI was studied using range of dilution of 10, 20, 30, 40, and 50 µg/mL. All experiments were performed in triplicate.

Accuracy

The accuracy of the method was determined by preparing solution of different concentrations, that is 80%, 100%, and 120%, in which the amount of marketed formulation was kept constant (30 mg) and the amount of pure drug was varied, that is, 24 mg, 30 mg, 36 mg for 80, 100, and 120%, respectively. All experiments were performed in triplicate.

Precision

The precision of method was performed by intra-day and inter-day variation studies. In the intra-day variation study, six different solution of same concentration (30 µg/mL) were prepared and analysed thrice a day (morning, afternoon, evening) and the absorbance were recorded. In inter-day variation study, the six different solution of same concentration (30 µg/mL) were prepared and analysed thrice, for three consecutive days, and the absorbance were recorded. All experiments were performed in triplicate.

Specificity

Specificity method was determined by preparing 10 mg of AZI solution in phosphate buffer (pH 6.8) along with 50% (5 mg), 100% (10 mg), and 150% (15 mg) of excipients. All the solutions were prepared and analysed for any change in absorbance and percentage drug recovery at respective wavelengths.

Ruggedness

The ruggedness of the method was determined by carrying out the analysis using two different analysts and the respective absorbance were noted.

Robustness

The ruggedness of the method was determined by using two different instruments and the linearity was taken.

Limit of detection

The limit of detection (LOD) was determines by preparing solutions of different concentrations ranging from 0.01 - 0.1 µg/mL. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected, but not necessarily quantitated as an exact value.

Limit of quantification

The limit of quantitation (LOQ) is the concentration that can be quantitated reliably with a specified level of accuracy and precision. The LOQ was calculated using the formula involving the standard deviation of response and the slope of the calibration curve.

Degradation studies

The ICH guidelines entitled stability testing of new drug substances and products that required stress testing to be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this study was to perform the stress degradation studies on Azithromycin using the method developed.

Stress degradation by acid hydrolysis

From the stock solution take 3 ml of solution in 10 mL volumetric flask and add 1 mL of 1 N HCl and make up the volume with methanol. Kept aside for 90 min in normal condition. After 30 min time interval 1 mL of sample was taken out and diluted to achieve a concentration of 30 µg/mL. For the blank solution 0.5 mL of 1 N HCl and 0.5 mL of 1 N NaOH were mixed in 10 mL volumetric flask and dilute up to the mark with phosphate buffer. Repeat the procedure for three times at an interval of 30 min.

Stress degradation by alkaline hydrolysis

From that SSS take 3 mL of solution in 10 mL volumetric flask and add 1 mL of 0.1 N NaOH and make up the volume with methanol. Kept aside for 90 min in normal condition. After 30 min time interval 1 mL of sample was taken out and diluted to achieve a concentration of 30 µg/mL. For the blank solution 0.5 mL of 0.1 N NaOH and 0.5 mL of 0.1 N HCl were mixed in 10 mL volumetric flask and dilute up to the mark with phosphate buffer. Repeat the procedure for three times at an interval of 30 minutes.

Degradation induced by dry heat

The AZI powdered drug is kept in petri plate at 70 °C for 48 hrs. After that weigh accurately 10 mg of drug from the sample and dilute with phosphate buffer to get the final concentration 30 µg/mL and analyse by previously validated UV method in triplicate.

Oxidative degradation

In a 10 mL of volumetric flask add mix 1.5 mL of SSS with 1 mL of 30% (v/v) of hydrogen peroxide and dilute up to the mark with methanol. Kept aside for overnight at room temperature. For blank solution, 1 mL of 30% w/v of hydrogen peroxide was kept under normal condition, overnight in a 10 mL volumetric flask. Both solutions were heated for 15 min to remove excess of hydrogen peroxide and analyse with UV.

Degradation by UV

A sample of azithromycin was kept near to UV in a stability chamber for 12 hrs, providing the illumination not less than 1.2 million lux hrs. From that sample 10 mg of drug is dissolve in a 10 ml of volumetric flask with HCl and then make up the volume with buffer solution. Make the dilution with methanol of 30 µg/ml and analyse in UV.

Result and discussion

SSS of AZI was prepared in HCl because AZI was not completely soluble in pH 6.8 phosphate buffer, while rest of the WSS was prepared using SSS and further dilution with phosphate buffer. UV spectrum of AZI shows λ_{max} at 208 nm (Figure 2), hence rest of the experiment were performed at 208 nm.

Figure 2: Azithromycin linearity absorbance spectrum

The calibration curve of azithromycin in phosphate buffer pH 6.8 was found to be linear in the concentration range 10-50 µg/ml (Figure 3) having R^2 value 0.997.

Figure 3: Calibration Curve of Azithromycin.

The developed method is precise as the % RSD values of intra-day (Table 1) and inter-day (Table 2) studies were found less than 2% which is acceptable by ICH requirement.

Table 1: Statistical analysis for INTRADAY ASSAY of the proposed method

Sr. No.	Concentration (µg/ml)	Absorbance (λ max=208nm)			Statistical Analysis
		Sample 1	Sample 2	Sample 3	
1	30	1.072	1.076	1.080	Mean: 29.97(µg/ml)
2	30	1.100	1.062	1.075	
3	30	1.050	1.068	1.085	
4	30	1.120	1.080	1.078	SD: 0.01465
5	30	1.068	1.075	1.080	
6	30	1.080	1.080	1.072	%RSD: 1.36%

Table 2: Statistical analysis for INTERDAY ASSAY of the proposed method

Sr. No.	Concentration (µg/ml)	Absorbance (λ max=203nm)			Statistical Analysis
		Day 1	Day 2	Day 3	
1	30	1.045	1.092	1.10	Mean: 29.88(µg/ml)
2	30	1.090	1.084	1.090	
3	30	1.068	1.060	1.072	
4	30	1.095	1.080	1.080	SD: 0.0150
5	30	1.078	1.075	1.098	
6	30	1.084	1.063	1.10	% RSD: 1.40%

The method is also specified as the % recovery was found to be 98.21%. The LOD (1.6 µg/mL) and LOQ (5 µg/mL) were performed at sub-microgram level indicating the sensitivity of the developed method. The method is also robust and rugged as the %RSD value were less than 3%. The formulation label claim meets with the claim as the performed accuracy shows the % recovery 99.91%. The results of the validation parameter are shown in the Table 3.

Table 3: Validation parameters

Sr. no	Parameter	Result
1.	Absorption maxima (nm)	208 nm
2.	Linearity range (µg/ml)	10-50 µg/ml
3.	Standard Regression Equation	$y = 0.0289x + 0.2477$
4.	Correlation coefficient (R^2)	0.9962
5.	Accuracy (%recovery $\pm$ SD)	99.72 $\pm$ 0.1081
6.	Precision (%RSD)	< 2%
7.	Specificity (%recovery $\pm$ SD)	98.21 $\pm$ 1.051
8.	LOD (µg/ml)	1.6 (µg/ml)
9.	LOQ (µg/ml)	5.0 (µg/ml)

The degradation studies were shown that Azithromycin undergoes degradation in acidic, alkaline and oxidation conditions; it is also less stable in UV, whereas, it was relatively stable under dry heat condition. The summary of the stress degradation studies is given in the Table 4.

Table 4: Stress degradation study

Condition	Time	Degradation (%)
1 N HCl (1 ml)	30 minutes	56%
	60 minutes	100%
	90 minutes	100%
0.1 N NaOH (1 ml)	30 minutes	56%
	60 minutes	83%
	90 minutes	100%
Dry heat (70°C)	48 hours	23.74%
30% Hydrogen peroxide (1 ml)	15 minutes	100%
Photolytic	12 hours	100%

Conclusion

All above studies lead to the conclusion that the method developed is accurate, precise, specific, cost effective, robust, rugged, simple and sensitive. This method can be applied in the estimation of AZI bulk and in pharmaceutical formulation. The method is also applicable for the stability testing of AZI in bulk as well as in pharmaceutical dosage form.

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