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Original Article

UV-Visible Spectrophotometric estimation of azithromycin and cefixime from tablet formulation by area under curve method

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ABSTRACT

Area under curve method was developed using UV-Vis spectrophotometer for simultaneous estimation of azithromycin and cefixime from tablet formulation by area under curve method. Two analytical wavelength ranges used were i.e. 219-224nm and 275-302 nm for simultaneous estimation of both the drugs from tablet formulation. The method was statistically validated for its linearity, accuracy, and precision as per ICH guidelines. The recovery study was performed at 80%, 100%, and 120% levels. Average recovery at these three levels was found to be 99.91%w/w and 100.11%w/w for azithromycin and cefixime, respectively. Observed linearity was found to be in the range of 2.5-15µg/ml for azithromycin and 2-12 µg/ml for cefixime. The precision of the method was ascertained by performing an assay of formulation in terms of intra-day (2 hr interval) and inter-day variation (3 different days). The result obtained in intra-day study was 99.94%w/v (azithromycin) and 99.96%w/v (cefixime), and in inter-day, the results were 99.84%w/v (azithromycin) and 99.80%w/v (cefixime). Both Intra and inter-day variations showed less %RSD values indicating the high grade of precision of this method. The recovery was found to be 99.91%w/w for azithromycin and 100.11%w/w for cefixime.

Keywords: Azithromycin; Cefixime; UV-Vis spectrophotometer; Area under curve

INTRODUCTION

Azithromycin (Fig.1) is macrolide azalide antibiotics [1]. It inhibits protein synthesis by binding with the 50S ribosomal subunit of the bacteria [2]. It is used for respiratory tract infection, cystic fibrosis, and also as an anti-inflammatory in COPD Patients [3]. Cefixime (Fig.2) is an orally active antibiotic with a similar antibacterial spectrum and resistance to β-lactamase as third-generation cephalosporins [4]. It inhibits an enzyme transpeptidase which is responsible for bacterial cell wall synthesis. It is used in lower respiratory tract infections, acute urinary tract infections, acute

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sinusitis, acute otitis media, helicobacter pylori infection [5]. Both the drugs are official in Indian Pharmacopoeia [6].

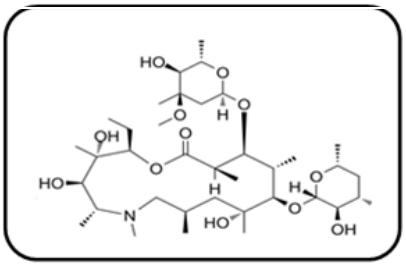

Fig. 1 Chemical structure of azithromycin

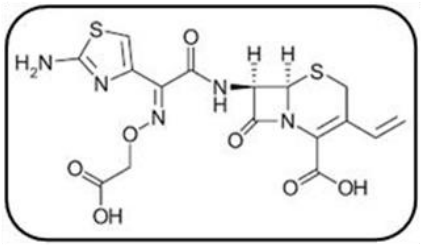

Fig. 2 Chemical structure of cefixime

There are several analytical methods for the analysis of various drugs from bulk and various formulations like tablets, capsules, injections, etc [7-51]. Literature survey revealed various analytical methods have been reported for estimation of azithromycin alone and in combination with other drugs [52-56]. Similarly, there are two analytical methods reported for Ambroxol HCl alone and in combination with other drugs [57-58]. However, nobody has covered the complete validation as per ICH guidelines [59].

MATERIALS AND METHOD

Instrument and reagents: Spectral scan was made on a Shimadzu UV-spectrophotometer, model 1800 (Shimadzu, Japan) with a spectral bandwidth of 2 nm with automatic wavelength corrections by using a pair of 10 mm quartz cells. All spectral measurements were done by using UV-Probe 2.42 software. Reference standard of azithromycin and cefixime were obtained from Ajanta pharmaceutical Ltd, Mumbai. The Tablet formulation used in the analysis was HYFEN-AZ (Hetro labs, Hyderabad, India) containing AZI 250 mg and CEFI 200 mg per tablet (1.25:1) was purchased from a local market.

Preparation of standard stock solutions: Individual standard stock solutions of azithromycin (50 µg/ml) and cefixime (40 µg/ml) were prepared in distilled water. The solutions were filtered through 0.45µ Whatman filter paper.

Preparation of mixed standard solution: Mixed standard solution containing azithromycin (5µg/ml) and cefixime (4µg/ml) was prepared in distilled water using the above-prepared solutions. The solutions were filtered through 0.45µ Whatman filter paper.

Preparation of calibration curves: Series of solutions containing 2.5-15 µg/ ml of azithromycin and 2 -10 µg/ ml of cefixime were used to determine linearity and range.

Selection of wavelength ranges: Mixed standard solution was scanned over the range 200 to 400 nm. Based on spectra obtained, the wavelength range selected for azithromycin was 219-224 nm and for cefixime, the range was 275-302 nm. Areas under curves for azithromycin and cefixime were recorded in wavelength ranges of 219-224 nm and 275-302 nm, respectively (**Fig. 3**).

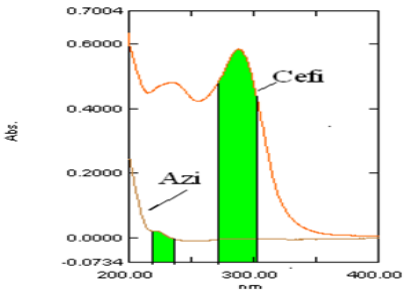

Fig. 3. UV-vis Spectra representing AUC of azithromycin (Azi) and cefixime (Cefi)

Area under curve method [60]: Area under curve method involves the calculation of integrated values of absorbance with respect to the wavelength between two selected wavelengths such as λ_1 and λ_2. The area under curve between λ_1 and λ_2 was calculated by UV probe 2.42 software. The concentration of each drug was calculated from the following AUC equation.

AUC Method Eq's

$$C_{AZI} = \frac{X^{CEF}_{219\text{-}224} \times AUC_{275\text{-}306} - X^{CEF}_{275\text{-}302} \times AUC_{219\text{-}224}}{X^{CEF}_{275\text{-}302} \times X^{AZI}_{219-224} - X^{CEF}_{219-224} \times X^{AZI}_{275\text{-}302}}$$

$$C_{CEF} = \frac{X^{AZI}_{275\text{-}302} \times AUC_{219\text{-}224} - X^{AZI}_{219\text{-}224} \times AUC_{215\text{-}302}}{X^{AZI}_{219\text{-}224} \times X^{CEF}_{275\text{-}302} - X^{AZI}_{275\text{-}302} \times X^{CEF}_{219\text{-}224}}$$

Here, C_{AZI} = Concentration of azithromycin; C_{CEF} = Concentration of cefixime; X^{AZN}= Area under curve of azithromycin at wavelength 275-302 nm; X^{CEF}= Area under curve of cefixime at wavelength 219-224nm; X^{CEF} = Area under curve of azithromycin at wavelength 219-224nm; X^{AZI} = Area under curve of cefixime at wavelength 275-302 nm; AUC^{M} = Area under curve of mixture

Assay of marketed tablet formulation: Based on marketed formation, a sample solution was prepared containing azithromycin (5 µg/ml) and cefixime (4 µg/ml) was prepared in distilled water using above prepared solutions. The solutions were filtered through 0.45µ whatman filter paper. The solution was scanned between 200-400 nm and AUC of each drug was recorded.

Method Validation

Studied validation parameters include accuracy and precision, linearity & range, LOD (limit of detection) & LOQ (limit of quantitation).

Accuracy & precision: To study the accuracy and precision, a recovery study was carried out by the addition of standard drug solutions to the pre-analyzed sample. Recovery study was undertaken at three levels i.e. 80%, 100%, and 120%.

Linearity & range: Linearity was studied by measuring the AUC of series of dilutions of mixed standard solution in the concentration range 2.5-15 µg/ml (azithromycin) and 2-12 µg/ml (cefixime). The calibration graph was plotted as concentration versus AUC.

Precision: The precision of the proposed method was determined by performing tablet assay at different time intervals (2-hour interval) on the same day (Intra-day precision) and on three different days (Inter-day precision).

RESULT AND DISCUSSION

Area under curve method involves the calculation of the integrated value of absorbance with respect to corresponding wavelengths between two selected wavelengths such as λ1 and λ2. Here, we have developed AUC method as both the drugs show discrete peaks at a suitable distance. It enables to analyse both drugs by simply measuring the peak area of each drug by selecting a suitable wavelength range for each. For the analysis of the formulation, AUC was recorded for each drug separately. Area under Curve spectra obtained shows the linear relationship between concentration and AUC.

Despite the nearly same concentration of both the drugs, azithromycin peak showed lesser AUC as compare to cefixime. This might be due to the lack of chromophoric group on azithromycin as cefixime has NH_2- attached to thiazole ring which imparts chromophoric activity to cefixime. Linearity of azithromycin and cefixime was observed in the range of 2.5-15 µg/ml and 2-12 µg/ml, respectively. Regression analysis was made for the slope, intercept, and correlation values. Result of the Analysis is given in **Table 1.**

The calibration curve yielded a correlation coefficient (r^2) of 0.999& 0.999 for azithromycin and cefixime, respectively. The coefficient of correlation of both drugs was found to be close to 1.00, indicating good linearity.

The assay results obtained by the proposed method were in good agreement. Recovery study was performed by standard addition method at 3 levels i.e. 80, 100, 120%.

The % RSD was found to be less than 1, which indicates the validity of the method. Average recovery of both drugs from tablet formulation was found to be 99.91%w/w & 99.97%w/w for azithromycin and cefixime, respectively.

Table 1. Validation Parameters

Parameters	Result	
	Azithromycin	Cefixime
Linearity (µg/ml)	2.5-15	2-12
Det. WL range	219-224	275-302
R^2	0.999	0.999
LOQ (µg/ml)	0.819	1.58
LOD (µg/ml)	2.46	4.60
% Recovery	99. 10	99. 97
Intra-day precision	99.94	99.96
Inter-day precision	99.84	99.80

The mean of intra- and inter-day precision was found to be 99.94 % and 99.96 %, respectively for azithromycin and 99.84 and 99.80, respectively for cefixime. The LOD and LOQ of azithromycin and cefixime were determined from standard deviation of the response and the slope. LOD value for AZI and CEFI was found to be 0.81µg/ml and 1.58 µg/ml, respectively. Whereas LOQ values for azithromycin and cefixime were found to be 2.46 µg/ml and 4.6 µg/ml, respectively (**see Table 1)**.

CONCLUSION

Calculations involved in the method are very simple. One can easily analyse the drug concentrations from area under curves of each drug. Thus proposed AUC method is simple, accurate, and precise. Hence, it can be directly used for the analysis of SAL and AMB from tablet formulation. This method can be adopted as an alternative to the existing methods. It can be easily and conveniently adapted for routine quality control analysis.

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