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Development and Validation of Spectrophotometric Method for Cefixime Trihydrate and Azithromycin Dihydrate by Absorbance Ratio Method

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ABSTRACT

A simple, accurate and precise spectroscopic method was developed for simultaneous estimation of Cefixime trihydrate and azithromycin dihydrate in marketed formulation using Q-Absorbance Ratio Method. In this spectroscopic method, 285nm (λmax of Cefixime trihydrate) and 219.40 nm (iso absorptive point for other drugs) were selected for measurement of absorptivity. Both the drugs show linearity in a concentration range of 10-50 µg/ml for Cefixime trihydrate and 2-10 µg/ml for azithromycin dihydrate at 285 nm and 219.40 nm respectively. Accuracy, precision and recovery studies were done by QC samples covering lower, medium and high concentrations of the linearity range. The relative standard deviation for accuracy, precision studies were found to be within the acceptance range (<2%). The recovery of Cefixime and azithromycin were found to be 99.84% and 100.76% respectively showing accuracy of the method. The method was validated statistically as per ICH guidelines.

INTRODUCTION

Cefixime (CFI) (6R,7R)-7-[[(Z)-2-(2-aminothiazol-4-yl)-2-[(carboxymethoxy) imino] acetyl] amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid trihydrate. The Molecular formula is $C_{16}H_{15}N_5O_7S_2.3H_2O$ and molecular weight: 507.50. Cefixime is an orally active antibiotic with similar antibacterial spectrum and resistance to β-lactamase as third generation cephalosporins. It inhibits an enzyme transpeptidase which is responsible for bacterial cell wall synthesis[1]. It is used in lower respiratory tract infections[2], acute urinary tract infections[3], acute sinusitis[4], acute otitis media[5], *Helicobacter pylori* infection[6].

Azithromycin (AZT) is macrolide antibiotics, it is an Azalide. It inhibits protein synthesis by binding 50S ribosomal subunit of the bacteria[7,8]. It is used for Otitis media[9], Respiratory tract infection[10], Cystic fibrosis [11], Anti-inflammatory in COPD Patient, in *P. Falciparum* Malaria with other Antimalarial drugs, Typhoid fever [14] and *Neisseria gonorrhoeae*.

The literature survey reveals that UV-Visible Spectrophotometry[23], HPLC[24, 25, 26], HPTLC [27],

Voltammetry[28], High-Performance Capillary Electrophoresis and LC-TMS methods were reported for the estimation of Cefixime alone or in combination with other drugs. As per literature survey, no analytical method has been reported for simultaneous estimation of Cefixime and Azithromycin in pharmaceutical dosage forms.

The aim of present research work was to develop and validate a simple method for estimate Azithromycin and Cefixime in their combined dosage form in routine analysis.

MATERIAL

Azithromycin and Cefixime were obtained as gift samples from MEHTA API Pvt. Ltd. and DALAS BIOTECH INDIA.

Instruments -

A Shimadzu model 1800 double beam UV/ Visible spectrophotometer with spectral width of 1 nm, wavelength accuracy of ± 0.1 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solution.

Digital balance (Mettler-toledo) was used for weighing the samples. Class 'A' volumetric glassware were used (Borosilicate).

EXPERIMENTAL

Preparation of stock solution of azithromycin dihydrate [8]:-

Accurately weighed quantity of azithromycin dihydrate 25 mg was transferred to 25 ml of methanol volumetric flask, dissolved and diluted up to mark with methanol to give a stock solution having strength of 1000 µg/ml. Then this solution further diluted to obtain concentration 100 µg/ml, further 10-15 ppm solution is prepared.

Preparation of stock solution of Cefixime trihydrate[8]-

Accurately weighed quantity of Cefixime trihydrate 10 mg was transferred to 10 ml volumetric flask, dissolved and diluted up to mark with methanol to give a stock solution having strength of 1000 µg/ml. Then this solution further diluted to obtain concentration 100 µg/ml. Further conc. 2-10 ppm is prepared with distilled water.

Preparation of standard mixture solution-

Stock solution of azithromycin and Cefixime were properly diluted to obtain the concentration 20µg/ml for azithromycin and 4 µg/ml for Cefixime.

Calibration curves for azithromycin and cefixime[9-12]:-

The solutions for Metformin were prepared by pipetting out 10, 20, 30, 40, and 50 ml of the working standard solution of azithromycin dihydrate (100 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with methanol. The absorbance of the solutions was measured at 219.40 nm against methanol as a reagent blank. The solutions for Cefixime were prepared by pipetting out 2, 4, 6, 8, 10 µg/ml of the working standard solution of Cefixime (10 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with methanol. The absorbance of the solutions was measured at 285 nm against methanol as a reagent blank. Plot the graph of absorbance versus respective concentration of azithromycin and Cefixime. Linearity range of azithromycin and Cefixime trihydrate was found with correlation coefficient. The overlain spectra for azithromycin and Cefixime trihydrate is shown in Figure.1

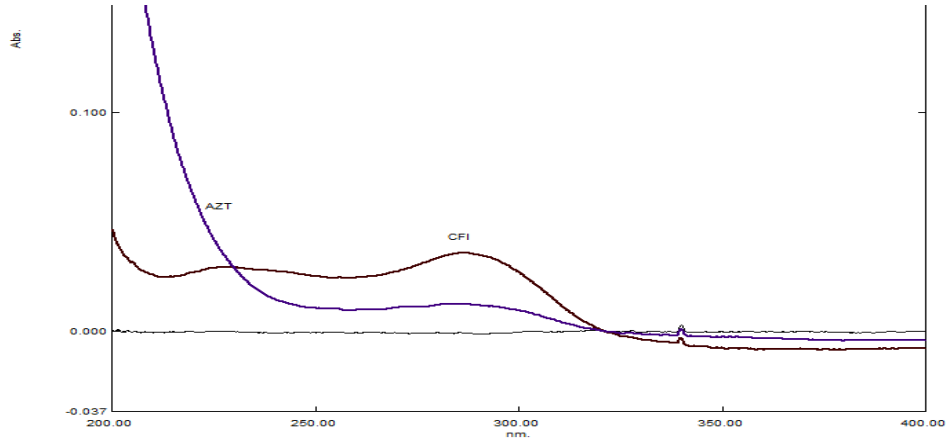


Fig. 1- Overlay spectra of azithromycin and cefixime trihydrate

METHODS

Absorbance ratio method (Q-analysis method)[13-16]:

The absorbance ratio method is a modification of the simultaneous equation procedure. It depends on the property that for a substance, which obeys Beer's law at all wavelength, the ratio of absorbance at any two wavelengths is constant value independent of concentration or path length e.g. two dilutions of the same substance give the same absorbance ratio A1/A2. In the USP, this ratio is referred to as Q value. In the quantitative assay of two components in mixture by the absorbance ratio method, absorbance is measured at two wavelengths, one being the λmax of one of the components (λ2) and the other being a wavelength of equal absorptivity of the two components (λ1), i.e., an isoabsorptive point. A series of standard solutions of Metformin and Sitagliptin in the concentration range of 5-25 µg/ml and 0.5-2.5 µg/ml respectively were prepared in methanol and the absorbance of these solutions was measured at 253.26 nm (iso-absorptive point) and 237.14 nm (λmax of Metformin). Calibration curves were plotted to verify the Beer's law and the absorptivity values calculated at the respective wavelengths for both the drugs.

The concentration of two drugs in mixture was calculated by using the following equations:

$$Cx = (Qm - Qy / Qx - Qy) \times (A1 / ax1)$$

$$Cy = (Qm - Qx / Qy - Qx) \times A1 / ay1)$$

Where,

ax1 = A (1%, 1cm) of Metformin at 237.14 nm

ay1 = A (1%, 1cm) of Sitagliptin at 237.14 nm

ax2 = A (1%, 1cm) of Metformin at 235.26 nm

ay2 = A (1%, 1cm) of Sitagliptin at 253.26 nm

A1 and A2 are the absorbances of mixture at 237.14 nm and 253.26 nm. Cx and Cy are the concentrations of Metformin and Sitagliptin in gm/100 ml respectively in sample solution.

$$Qm= A2 / A1, Qx = ax2 / ax1 \text{ and } Qy= ay2 /ay1$$

Assay of tablets by method:

Tablet Micef-Oax 200/250 contains 200 mg of Cefixime trihydrate and 250 mg of azithromycin dihydrate equivalent. It is marketed by Micro labs limited. Twenty tablets were weighed and triturated in a mortar pestle and the tablet powder equivalent to 100 mg of azithromycin and 10 mg of Cefixime was transferred to a 100 ml volumetric flask, dissolved and diluted up to mark with methanol. The solution was filtered through Whatman filter paper no. 42 and first few drops of filtrate were discarded. 1 ml of this solution was diluted to 10 ml with methanol and 0.4 ml of this solution was further diluted to 10 ml with methanol. Absorbance of the resulting solution was measured at 219.40 nm and 285 nm against methanol. The concentration of azithromycin and Cefixime can be obtained by using following equations,

$$Cx = (Qm-Qy / Qx-Qy) \times (A1/ ax1)$$

$$Cy = (Qm-Qx / Qy-Qx) \times A1 / ay1)$$

Method development and validation[17-18]

The method was validated for accuracy, precision, linearity, detection limit, quantitation limit and robustness.

1. Linearity[19]

Appropriate aliquots of AZT and CFI working standard solutions were taken in different 10 ml volumetric flasks and diluted up to the mark with 10,20,30,40,50 µg/ml of AZT and 2, 4, 6, 8, 10 µg/ml of CFI respectively. Calibration curves were constructed by plotting absorbance versus concentrations and regression equations were calculated for both the drugs.

Table no 1-Caliberation curve of Azithromycin

Conc.	Abs.
10	0.0112
20	0.0165
30	0.0213
40	0.0276
50	0.0321

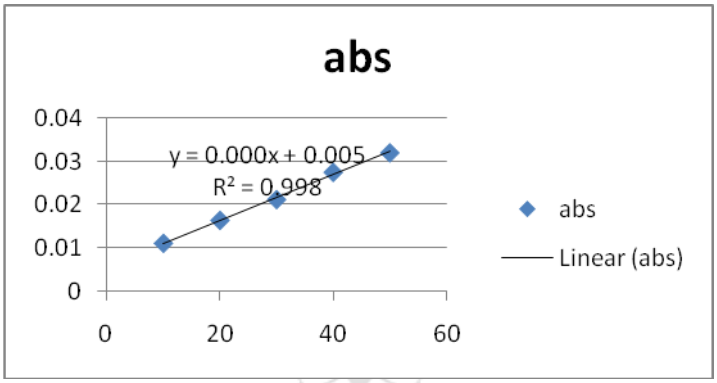
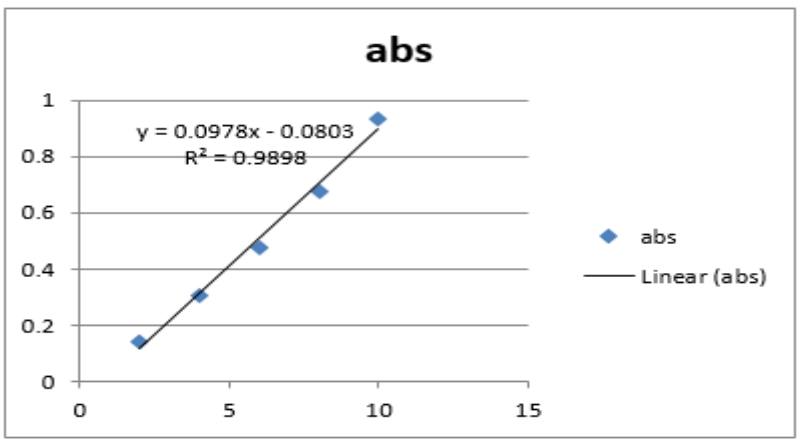


Table no 2-Calibration curve of Cefixime

Conc.	Abs.
2	0.143
4	0.3037
6	0.476
8	0.675
10	0.9357



2. Precision[20]

The repeatability studies were carried out by estimating response of AZT (10 µg/ml) and CFI (4 µg/ml) five times and results are reported in terms of relative standard deviation. The intra-day and inter-day precision studies (intermediate precision) were carried out by estimating the corresponding responses 3 times on the same day and on 3 different days for three different concentrations of AZT (20, 30, 40 µg/ml) and CFI (4, 6, 8 µg/ml), and the results are reported in terms of relative standard deviation.

3. Accuracy[21]

The accuracy of the method was determined by calculating recoveries of AZT and CFI by method of standard additions at three different levels 80, 100 and 120 %. Mean percentage recovery was determined.

Table 3-Recovery study of AZT

Sr.no	Recovery %	Amt. of drug in sample	Azithromycin dihydrate % recovery*	%RSD
1	80	10	100.28	0.08
2	100	10	100.45	0.10
3	120	10	100.33	0.18

Table 4- Recovery study of Cefixime

Sr.no	Recovery %	Amt. of drug in sample	Cefixime % recovery*	%RSD
1	80	6	100.13	0.24
2	100	6	100.39	0.40
3	120	6	99.89	0.41

Table 5-Data of the analysis of drug formulation

DRUG (mg/tab)	Label claim	Amount	% drug found
AZT	250	251.22	100.49
CFI	200	200.1	100.05

4. Detection limit[22]

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. The detection limit (LOD) may be expressed as

LOD = 3.3_/slope

Where □ □ = Relative standard deviation of the response.

5. Quantitation limit[22]

The Quantitation limit of an analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy.

Quantitation Limit (LOQ) may be expressed as = 10 _/slope

Where □ □ = Relative standard deviation of the response.

CONCLUSION

Proposed study describes method for the estimation of AZT and CFI combination. The method was validated and found to be simple, sensitive, accurate and precise as per ICH guidelines. The proposed method in routine quality control laboratories for determination of AZT and CFI in bulk and pharmaceutical formulation.

Table 6-Summary of validation parameter:

Validation parameters	*Azithromycin*	*Cefixime*
Linearity (µg/ml)	10-50	2-10
Correlation co-efficient	0.9996	0.998
Slope	0.0187	0.091
Intercept	0.0143	0.026
LOD (µg/ml)	1.67	1.51
LOQ (µg/ml)	5.06	4.57
Sandell's sensitivity (mg/cm^2/0.001 absorbance unit)	0.051	0.0094
% Recovery	100.28-100.33	99.68-100.29
Precision (%RSD)	0.82	0.72
Repeatability		
Intra day	0.35	0.42
Inter day	0.42	0.41
(n=5)		

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