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Simultaneous estimation of Azithromycin and Cefixime in Active Pharmaceutical Ingredients and Pharmaceutical dosage forms by Spectrophotometry

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Abstract

Plan: *An analytical method for estimation of Azithromycin and Cefixime in bulk and tablet formulation.*
Methodology: *A rapid, sensitive and specific uv-vis method was developed and validated for the estimation of Azithromycin and Cefixime in tablet dosage form. The method was validated in terms of linearity, accuracy, precision, specificity, limit of detection and limit of quantitation. The optimum conditions for the analysis of the drug were established. The maximum wavelength (λ_{max}) of Azithromycin and Cefixime were found to be 235 nm and 288nm respectively. The percentage recovery of Azithromycin and Cefixime were 100.28-100.33 and 99.68-100.29 respectively. Beer's law were obeyed in the concentration range of 10-50µg/ml for Azithromycin and 2-10µg/ml for Cefixime. The linear equation for Azithromycin and Cefixime were found to be y = 0.0187x - 0.0143, r^2 = 0.9996 and y = 0.0917x + 0.026, r^2 = 0.9985 respectively.Validation was performed as ICH guidelines for linearity, accuracy, precision, LOD and LOQ.*
Outcome: *The proposed method was successfully applied for the quantitative determination of Azithromycin and Cefixime in tablet dosage form.*
Key Words*: Azithromycin, Cefixime, Simultaneous estimation*

1. Introduction

Cefixime (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 $C_{16}H_{15}N_5O_7S2.3H_2O$ (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 Walls synthesis [1].

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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 is macrolide azalide antibiotics, [9-de-oxy-9a-aza-9a-methyl-9a-homoerythromycin A dihydrate]. 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 [12], in *P. falciparum* Malaria with other Antimalarial drugs [13], Typhoid fever [14] and *Neissaria gonorrhoeae* [15] .Both the drugs are official in Indian pharmacopoeia 2010 [16]. Literature survey reveals that HPLC [17,18] , LC-MS/MS [19], Micellar chromatography [20], UV-Visible Spectrophotometry [21] and UPLC [22], methods were reported for the estimation of Azithromycin alone or in combination.

The literature survey reveals that UV-Visible Spectrophotometry [23], HPLC [24, 25, 26], HPTLC [27], Voltametry [28], High Performance Capillary Electrophoresis [29] and LC-TMS [30] 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.

2. Experimental

2.1. Instrument

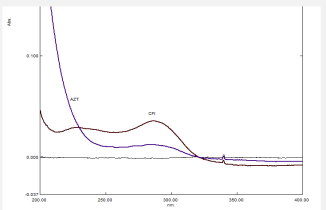
A shimadzu model 1800 double beam UV/ Visible spectrophotometer with spectral width of 1 nm, wavelength accuracy of ± 0.1 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software (ver.2.34).

2.2 Reagents and Materials

All chemicals and reagents were used of AR grade. Authentic of Azithromycin and Cefixime were obtained as gift samples from A to Z Pharmaceutical Chennai.

2.3 Selection of detection wavelength

Solutions of drug were scanned over the range of 200-400 nm. It was observed that both the drugs showed considerable absorbance at 235 nm for AZT and 288nm for CFI was selected as the wavelength for detection.



Spectra of AZT and CFI

2.4. Preparation of standard stock solutions

AZT and CFI were weighed (100 mg each) and transferred to two separate 100ml volumetric flasks and dissolved in 70 ml of methanol and make up the volume up to the mark with distilled water and the final concentration of solution containing 1000 µg/ml of AZT and CFI.

2.5. *Preparation of working solutions*

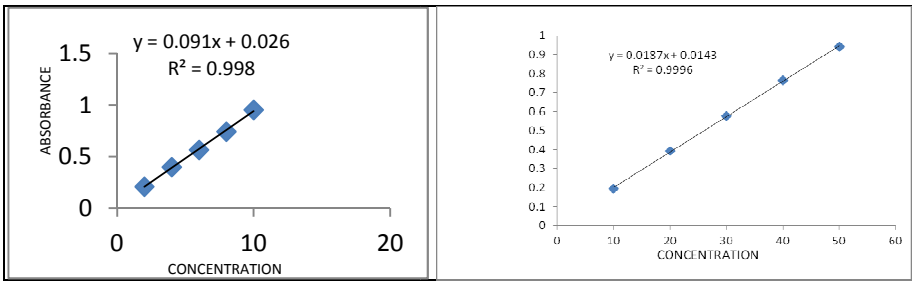
Aliquot from the stock solutions of AZT and CFI were appropriately diluted with distilled water to obtain working standard of AZT and CFI.

3. Method development and validation

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

3.1. Linearity

Appropriate aliquots of AZT and CFI working standard solutions were taken in different 10 ml volumetric flasks and diluted up to the mark with distilled water to obtain final concentrations of 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.



Calibration curve of Azithromycin *Calibration curve of Cefixime*

3.2 Precision

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.

3.3. Accuracy

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 1. Recovery study of AZT and CFI

S.N0	Recovery %	AZT			CFI		
		Amount of drug present in sample	*% recovery**	*%RSD*	*Amount of drug present in sample*	*% Recovery**	*%RSD*
1	80	10	100.28	0.07	6	100.13	0.24
2	100	10	100.45	0.10	6	100.29	0.40
3	120	10	100.33	0.18	6	99.68	0.41

*average of three determinations.

3.4. Assay procedure for tablets

Twenty tablets were weighed accurately and finely powdered. Tablet powder equivalent to 250 mg AZT and 200 mg of CFI was taken in 100 ml volumetric flask. Methanol (70 ml) was added to the above flask and the flask was sonicated for 30 minutes. The solution was filtered using whatman filter paper No.41 and volume was made up to the mark with distilled water. From this solution prepare working solutions they have concentration 10µg/ml of AZT and 6µg/ml of CFI.

Table 2. Data of the analysis of drug formulation

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

3.5. Detection limit

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 = 3.3σ/slope

Where σ = Relative standard deviation of the response.

3.6. Quantitation limit

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 σ/slope

Where σ = Relative standard deviation of the response.

4. 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 3. Summary of validation parameters.

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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