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# Analytical Method Development and Validation for The Simultaneous Estimation of Azithromycin and Cefixime by Rp-Hplc Method in Bulk and Pharmaceutical Formulations

**K.Nagaraju[1]*, Y.A. Chowdary [2]**

[1]Research scholar, Krishna university, Machilipatnam , Krishna, Andhra Pradesh, India

[1]Sir C. R Reddy college of Pharmaceutical Sciences, Eluru, W.G

[2]NRI College of Pharmacy, Pothavarappadu (V), Via Nunna,Agiripalli (M), Vijayawada Rural, Krishna, Andhra Pradesh, India

## ABSTRACT

To develop and validate a simple, rapid, accurate and precise RP-HPLC method for the simultaneous determination of azithromycin and cefixime in bulk and pharmaceutical formulation. Chromatographic separation was performed on a supleco C18 (25cm×4.6 mm, 5 µm) column from thermo isocratic mode with mobile phase 80:20 Na2HPO4: Methanol with pH adjusted to 8 with methanol at flow rate 1 ml/min. Peak intensity of both the drugs was monitored at 273 nm with PDA detection.The retention time (RT) of Azithomycin and Cefixime was found to be 2.77 and 4.93 min, respectively. The linearity of Azithromycin and Cefixime were found in the range of 50-150 µg/ml . The limit of detection and limit of quantitation was3mg/ml and 10 mg/ml for Azithromycin and 3 mg/ml and 10 mg/ml for CHL.The proposed method was validated in terms of accuracy, precision, linearity, limit of detection and limit of quantification. Furthermore, no interference was observed with extra pharmacopoeial excipients in tablet suggesting its utility for routine quality control analysis of Azithromycin and Cefixime in pharmaceutical formulations.

**Keywords:** Azithomycin and Cefixime,RP-HPLC,

## I. 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 $C_{16}H_{15}N_5O_7S2.3H_2O$ 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 walls 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 [9-de-oxy-9a-aza-9a-methyl-9a-homoerythromycin A dihydrate] 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 [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 litrature 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.

## II. EXPERIMENTAL

### Apparatus

The liquid chromatographic system consists of WATERS 2695 with Photo diode array detector(PDA), with an automated sample injector. The output signal was monitored and integrated using Empower 2 software. SUPLECO C18,25cm x 4.6mm,5µm was used for seperation

### REAGENTS AND MATERIALS

All chemicals and reagents were used of AR grade. Authentic of CFI and AZT were obtained as gift samples Ato Z Pharmaceutical Chennai., India.

### Selection of detector wavelength

Solutions of drug were scanned over the range of 200-400 nm. It was observed that the isobestic point of drugs showed considerable absorbance at 273 nm was selected as the wavelength for detection

### Chromatographic Conditions

The SUPLECO $C_{18}$ column (25 x 4.6mm, 5µm) equilibrated with mobile phase 0.01M Disodium hydrogen phosphate ($Na_2HPO_4$): Methanol in the ratio of 80:20 (v/v) at pH 8 was used. The flow rate was maintained at 1 ml/min. Detection wavelength with PDA detector at 273 nm, and the injection volume was 10 µl and run time was kept 10min.

### Preparation of Standard and Stock solutions

CFI and AZT were weighed (100 mg each) and transferred to two separate 100ml volumetric flasks and dissolved in 50 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 CFI and AZT.

### Preparation of Working Solution

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

### Method Development

Lots of mobile phase and there different proportions were tried and finally was selected as 0.01M Disodium hydrogen phosphate ($Na_2HPO_4$): Methanol in the ratio of 80:20 (v/v) at pH 8 appropriate mobile phase which gave good resolution and acceptable system suitability parameters. The chromatogram of working standard solution is shown in fig 1.

### Calibration Curve

Accurately measured volumes of working standard solution of CFI and AZT were transferred into a series of 10ml volumetric flasks and diluted appropriately with mobile phase. 10µl of each solution was injected at same chromatographic conditions. Calibration curves were obtained by plotting the peak area versus concentration of drug. Regression equations were calculated. The method was found linear over a concentration range 50-150µg/mL of CFI and 50-150 µg/mL of AZT. (Fig. 2, 3)

**Precision**

The repeatability studies were carried out by estimating response of CFI (100 µg/mL) and AZT (100µg/mL) five times and results are reported in terms of % CV .

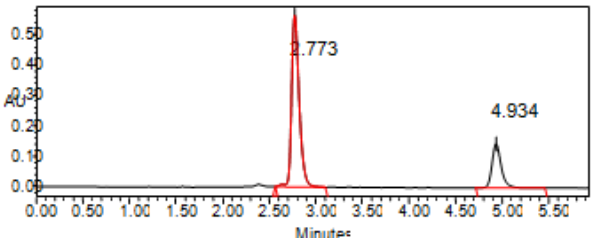


**Figure 1.** A typical chromatogram show sharp peaks of AZT and CFI

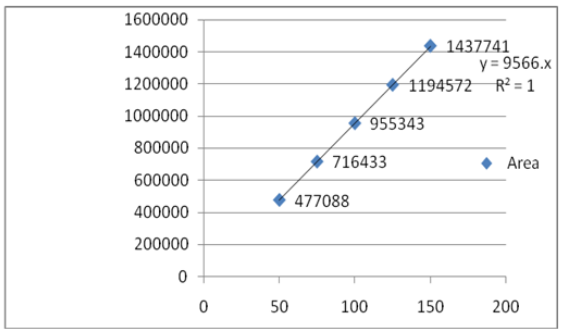


**Figure 2.** Calibration curve of cefixime

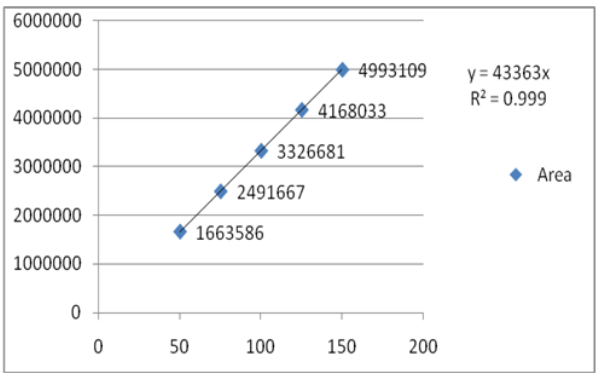


**Figure 3.** Calibration curve of Azithromycin

**Accuracy**

Recovery assessment was obtained by using standard addition technique which was by adding known quantities of pure standards at three different levels in 50%, 100% and 150% to the pre analysed sample formulation. From the amount of drug found, amount of drug recovered and percentage recovery were calculated which sense to conformation that the proposed method was accurate.

**Analysis of marketed formulation**

Ten tablets were weighed accurately and finely powdered. Tablet powder equivalent to 25mg AZT and 20mg of CFI was taken in 100 ml volumetric flask. Methanol (50 ml) was added to the above flask and the flask was sonicated for 30 minutes. The solution was filtered using whatman filter paper No.1 and volume was made up to the mark with distilled water.

**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

**Quantification limit**

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

**Specificity**

The method was determined as specific by comparing test results obtained from analyses of sample solution containing placebo ingredients with that of test results those obtained from analyses of standard solution.

## III. RESULTS AND DISCUSSION

The present work done on this combination comprises a simple, precise and accurate method by reverse phase high performance liquid chromatography. The present combination of CFI and AZT was marketed as one formulation. An attempt has been made to estimate CFI and AZT by RP-HPLC. Calibration curve depicting the linearity

and range for CFI and AZT were determined from mixed standards and were found to be of the order 50-150 µg /ml of CFI and 50-150 µg /ml of AZT. The formulation was diluted in the linearity range and peak areas were determined, the concentrations of both like CFI and AZT were then determined by comparing the peak areas of sample with that of standard peak areas of CFI and AZT in mixture can be identified by their retention times being 2.77 Minutes for AZT and 4.93 minutes for CFI. The results obtained from HPLC method were reproducible and encouraging. The values percentage deviation was within limit

## IV. CONCLUSION

Proposed study describes method for the estimation of AZT and CFI combination in mixture. The method was validated and found to be simple, sensitive, accurate and precise as per ICH guidelines .The method was successfully used for determination of drugs in their pharmaceutical formulation.

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**Table 1.** Analytical parameters

| S.NO. | PARAMETERS | Cefixime | Azithromycin |
|---|---|---|---|
| 1 | Limit of linearity (µg/ml) | 50-150 | 50-150 |
| 2 | Regression equation | Y=9566.x | Y=43363x |
| 3 | Correlation coefficient(r | 0.99 | 0.99 |
| 4 | Retention time(min) | 2.77 | 4.93 |
| 5 | Detection limit( µg/ml) | 3.48 | 3.89 |
| 6 | Quantification limit( µg/ml) | 10.05 | 10.59 |
| 7 | Accuracy (%) | 101 | 101 |
| 8 | Precision(%CV) | 0.14 | 0.23 |
| 9 | Assay | 99.2 | 99.1 |