SIMULTANEOUS ESTIMATION OF AZITHROMYCIN AND CEFPODOXIME PROXETIL IN BULK AND COMBINED TABLET DOSAGE FORM BY UV-SPECTROPHOTOMETRIC METHODS


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KEYWORDS:
Azithromycin, Cefpodoxime, Q-absorbence ratio method, Area under curve method.

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ABSTRACT

Two simple, accurate, rapid and precise UV Spectrophotometric methods have been developed for simultaneous estimation of Azithromycin and Cefpodoxime in combined tablet dosage form. The methods employed were (A) Q-absorbence ratio and (B) Area under curve method. Method-A involves measurement of absorbence at 270nm (Iso-absorptive point) and 281nm ($\lambda_{\text{max}}$ of Azithromycin). Method-B involves measurement of peak area in the wavelength range 271-291nm and 224-244nm for Azithromycin and Cefpodoxime respectively. In both the methods linearity was found in the concentration range of 5-25 $\mu$g/ml and 4-20 $\mu$g/ml respectively. Both the methods were found to be rapid, specific, precise and accurate. Hence these methods can be applied for routine analysis of Azithromycin and Cefpodoxime in combined dosage form without any interference by the excipients. The above methods are validated according to ICH guidelines.
1. INTRODUCTION:
Azithromycin (AZI) is chemically, (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-hexopyranosyl)oxy]-1-oxa-6-azacyclopentadec-13-yl 2,6dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranoside is a semi-synthetic macrolide antibiotic widely used in the respiratory tract infections, like pharyngitis, pneumonia, chronic bronchitis, bronchopneumonia, skin and soft tissue infections and some sexually transmitted diseases, that acts as a gram positive bacteria and gram negative bacteria. Azithromycin prevents bacteria from growing by interfering with their protein synthesis. It binds to the 50S subunit of the bacterial ribosome, and thus inhibits translation of mRNA. Nucleic acid synthesis is not affected. It is an official drug in IP, BP

![Fig. 1 Chemical structure of Azithromycin](image1)

![Fig. 2 Chemical structure of Cefpodoxime](image2)

Cefpodoxime Proxetil (CEF) is chemically, 1-(isopropoxy carbonyloxy) ethyl (6R,7R)-7-[2-(2-amino-4-thiazolyl)-(z)-2-(methoxyimino)acetamido]-3-Methoxymethyl-3-cephem-4-arboxylate. Cefpodoxime Proxetil is orally active ester prodrug of 3rd generation cephalosporin-Cefpodoxime. It is active against most Gram positive and Gram negative organisms. The antibacterial action of Cefpodoxime is through inhibition of bacterial cell wall synthesis probably by acylation of membrane bound transpeptidase enzymes; this prevents cross linkage of peptidoglycan chains, which is necessary for bacterial cell wall strength and rigidity. It is commonly used to treat respiratory, urinary, skin and soft tissue infection.

Survey of literature revealed that few analytical methods have been developed for the determination of AZI and CEF individually and in combination with other drugs. Hence an attempt has been made to develop a simple, accurate, precise and reproducible UV-Visible spectroscopic method for simultaneous estimation of AZI and CEF in combined dosage form with validation as per recommendation of ICH guidelines.
2. EXPERIMENTAL:

2.1 Instrument and materials:- For both the methods Shimadzu 1800 UV-Vis Spectrophotometer was used with 1 cm match quartz cell of 10 mm optical path length, spectral band width of 1 ± 0.2nm, wavelength accuracy of ± 0.3 nm. The tablet formulation of AZi and CEF (Label claim: Azithromycin 250 mg and Cefpodoxime 200 mg), GUDCEF-AZ (Theon Pharmaceuticals Ltd, H.P, India.) was purchased from the local market. Standard Azithromycin and Cefpodoxime were obtained as gift samples from Microlabs Pvt. Ltd, Bangalore and FDC Pvt. Ltd, Goa. AR grade methanol was used as solvent throughout the experiment.

2.2 Preparation of standard stock solution:- 100 mg each of Azithromycin and Cefpodoxime were weighed separately and transferred in two different 100 ml volumetric flasks. Both the drugs were dissolved in 50 ml of solvent by ultrasonication and then volume was made up to the mark with Solvent to obtain concentration of 1000 µg/ml of each component (stock A and A’ solution). From the above stock A and A’ solution 10 ml of aliquot was pipetted out into a 100 ml volumetric flask and the volume was made up to the mark with solvent to obtain the final concentration of 100µg/ml of each component (stock B and B’ solution).

2.3 Preparation of sample stock solution using formulation:- Twenty tablets of Azithromycin and Cefpodoxime (GUDCEF-AZ) in combination were weighed and their average weight was determined. The tablets were crushed to fine powder and from the triturate, tablet powder equivalent to 250 mg of Azithromycin was weighed which also contains 200 mg of Cefpodoxime and transferred to 100 ml volumetric flask and dissolved in 50 ml solvent and the content was kept in ultrasonicator for 15 min. The solution was filtered through Whatmann filter paper No.41, finally the volume was made up to the mark with solvent, which gave a concentration of 2500µg/ml of Azithromycin and 2000µg/ml of Cefpodoxime and this solution was used as stock ‘A’ solution.

From the above stock ‘A’ solution, 5 ml of the aliquot was pipetted out and was transferred to a 50 ml volumetric flask. The volume was made up to 50 ml with solvent to obtain a solution with final concentration of 250µg/ml Azithromycin and 200µg/ml of Cefpodoxime (stock B).

2.4 Methods:

a) Method A (Q –Absorbance ratio):- It uses the ratio of absorbance at two selected wavelengths, one which is an isoabsorptive point and other being the λ-max of one of the two
components. From the overlay spectra of two drugs, it is evident that AZI and CEF show an isoabsorptive point at 270.0 nm. The second wavelength used was 281 nm, which is the λ-max of AZI. Five working standard solutions having concentration 4, 8, 12, 16, 20μg/ml for CEF and 5, 10, 15, 20, 25μg/ml for AZI were prepared in methanol: water in the ratio of 70: 30 and the absorbances were measured at 270.0 nm (isoabsorptive point) and 281.0 nm (λ-max of AZI), absorptivity coefficients were calculated using calibration curve.

Absorptivity = Absorbance/ Concentration of that component in gm/100 ml.

The concentration of two drugs in the mixture can be calculated using following equations.

\[ CA = \frac{[(QM - QI) / (QA - QI)] \times A1/aX1}{(A1/aX1) - CA} \]  

(1)

Where, \( A1 \) and \( A2 \) are absorbances of mixture at 270.0 nm and 281.0 nm;
\( aX1 \) and \( aY1 \) are absorptivities of AZI and CEF at 270.0 nm;
\( aX2 \) and \( aY2 \) are absorptivities of AZI and CEF respectively at 281.0 nm;
\( QM = A2 / A1, \)
\( QA = aX2 / aX1 \) and
\( QI = aY2 / aY1. \)

b) **Method B (Area under curve):**

It involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths \( \lambda_1 \) and \( \lambda_2 \). Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated.

This wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration. For the selection of analytical wavelength suitable dilutions of Azithromycin (5-25μg/ml) and Cefpodoxime (4-20μg/ml) of the standard stock solutions (100μg/ml) of both were prepared separately and scanned in the range of 400-200 nm. Maximum absorbence was observed at 281nm and 234nm for Azithromycin and Cefpodoxime respectively.

The wavelength ranges selected for the estimation of Azithromycin and Cefpodoxime are 271-291 nm (\( \lambda_1 \) and \( \lambda_2 \)) and 224-244 nm (\( \lambda_3 \) and \( \lambda_4 \)) respectively. Aliquots were prepared for the sample solution in the concentration range of 5-25μg/ml and 4-20μg/ml for AZI and CEF and their area under curve was measured at above selected wavelengths.

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2.4 Validation of the methods:- All the methods were validated according to ICH guidelines by carrying out analysis of six replicate samples of tablet. Recovery studies were carried out at three different levels i.e., 80%, 100% and 120% by adding the pure drug to previously analyzed tablet powder sample. From the amount of the drug found, percentage recovery was calculated.

3. RESULT AND DISCUSSION:-

The estimation of Azithromycin and Cefpodoxime in bulk and tablet formulation was found to be accurate and reproducible with a linearity range of 5-25µg/ml and 4-20µg/ml respectively for both the methods and the correlation coefficient was found to be 0.9999 and 0.9998 for the methods A and B respectively. The optical characteristics such as linearity range, molar absorptivity, percentage relative standard deviation of recovery studies and precision in each method were calculated and the results were reported in Table 1 and Table 2 for method A and B respectively.

Also the regression characteristics like slope (m), intercept (c) and correlation coefficient (r) were calculated and are presented in Table 1 and Table 2 for method A and B respectively. The accuracy was found by recovery studies at different levels i.e. 80%, 100% and 120%. The values of standard deviation were satisfactory and the recovery studies were close to 100%. The % RSD value was less than 2, an indicative of the accuracy of the methods. The results for formulation were reported in Table 3.

The spectra of Azithromycin and Cefpodoxime are reported by Q-Absorbence ratio method (Fig. 3,) and calibration curve was plotted (Fig. 4, 5, 6 and 7).
Fig: 3: Isoabsorptive point of Azithromycin and Cefpodoxime.

Fig: 4: Calibration curve of AZI at 270.0 nm by Q - Absorption ratio Method.
Fig: 5: Calibration curve of CEF at 270.0 nm by Q – Absorption ratio Method

Fig: 6: Calibration curve of Azithromycin at 281.0 nm by Q - Absorption ratio Method.
The absorption spectra of Azithromycin, Cefpodoxime and formulation by Area Under Curve method are reported (Fig. 8, 9 and 10) and calibration curve was plotted (Fig. 11, 12, 13 and 14)

Fig. 7: Calibration curve of Cefpodoxime at 281.0 by Q - Absorption ratio Method.

Fig. 8: Spectra showing AUC for AZI in the wavelength range, 271 ($\lambda_1$) to 391 ($\lambda_2$) nm and 224($\lambda_3$) to 244 ($\lambda_4$) nm
Fig. 9: Spectra showing AUC for CEF in between the wavelength range of 271 (\(\lambda_1\)) to 291 (\(\lambda_2\)) nm and 224 (\(\lambda_3\)) to 244 (\(\lambda_4\)) nm.

Fig. 10: Spectra showing AUC for Formulation in Between the wavelength range, 271 (\(\lambda_1\)) to 391 (\(\lambda_2\)) nm and 224 (\(\lambda_3\)) to 244 (\(\lambda_4\)) nm in the Tablet dosage form.
Fig: 11: Calibration curve of Azithromycin at 271 - 291nm by Area Under Curve Method.

Fig: 12: Calibration curve of Cefpodoxime at 224-244nm by Area Under Curve Method.

Fig: 13: Calibration curve of Formulation at 271 - 291 nm by Area Under Curve Method.
Fig: 14: Calibration curve of Formulation at 224 - 244 nm by Area Under Curve Method.

Table 1: Optical characteristics and other parameters for Method A

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AZI</th>
<th>CEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear range (µg/ml)</td>
<td>05-25</td>
<td>04-20</td>
</tr>
<tr>
<td>λ_max / wavelength range (nm)</td>
<td>281</td>
<td>270</td>
</tr>
<tr>
<td>Coefficient of correlation</td>
<td>0.9999</td>
<td>0.9998</td>
</tr>
<tr>
<td>Slope*(m)</td>
<td>0.012</td>
<td>0.016</td>
</tr>
<tr>
<td>Intercept*(c)</td>
<td>0.002</td>
<td>0.005</td>
</tr>
<tr>
<td>Accuracy (%RSD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80%</td>
<td>0.3814</td>
<td>0.1175</td>
</tr>
<tr>
<td>100%</td>
<td>0.2683</td>
<td>0.5705</td>
</tr>
<tr>
<td>120%</td>
<td>0.2898</td>
<td>0.3110</td>
</tr>
<tr>
<td>Precision (%RSD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intra-day</td>
<td>0.3184</td>
<td>0.2683</td>
</tr>
<tr>
<td>Inter-day</td>
<td>0.7735</td>
<td>0.1671</td>
</tr>
<tr>
<td>Limit of Detection (µg/ml)</td>
<td>0.4125</td>
<td>0.2949</td>
</tr>
<tr>
<td>Limit of Quantification (µg/ml)</td>
<td>1.2508</td>
<td>0.8975</td>
</tr>
</tbody>
</table>

*y = mx + c; when x is the concentration in µg/ml and y is absorbance unit.
### Table 2: Optical characteristics and other parameters for Method B

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AZI</th>
<th>CEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear range (µg/ml)</td>
<td>05-25</td>
<td>04-20</td>
</tr>
<tr>
<td>( \lambda_{\text{max}} / ) wavelength range (nm)</td>
<td>271-291</td>
<td>224-244</td>
</tr>
<tr>
<td>Coefficient of correlation</td>
<td>0.9999</td>
<td>0.9998</td>
</tr>
<tr>
<td>Slope*(m)</td>
<td>0.032 x</td>
<td>0.043x</td>
</tr>
<tr>
<td>Intercept*(c)</td>
<td>0.004</td>
<td>0.005</td>
</tr>
<tr>
<td>Accuracy (% RSD)</td>
<td>80%</td>
<td>0.4766</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>0.2857</td>
</tr>
<tr>
<td></td>
<td>120%</td>
<td>0.3011</td>
</tr>
<tr>
<td>Precision (% RSD)</td>
<td>Intra-day</td>
<td>0.27516</td>
</tr>
<tr>
<td></td>
<td>Inter-day</td>
<td>0.6289</td>
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<tr>
<td>Limit of Detection (µg/ml)</td>
<td>0.1907</td>
<td>0.1619</td>
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<tr>
<td>Limit of Quantification (µg/ml)</td>
<td>0.5781</td>
<td>0.4906</td>
</tr>
</tbody>
</table>

\*y = mx + c; when x is the concentration in µg/ml and y is absorbance unit.

### Table 3: Results of Formulation

<table>
<thead>
<tr>
<th>Method</th>
<th>Brand name</th>
<th>Label claim of AZI (mg)</th>
<th>Label claim of CEF (mg)</th>
<th>Amount found for AZI (mg)</th>
<th>Amount found for CEF (mg)</th>
<th>% Recovery ±SD** for AZI</th>
<th>% Recovery ±SD** for CEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>GUDCEF-AZ</td>
<td>250</td>
<td>200</td>
<td>249.42</td>
<td>199.19</td>
<td>99.76±0.20952</td>
<td>99.64±0.26372</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>250</td>
<td>200</td>
<td>248.35</td>
<td>196.86</td>
<td>99.38±0.51291</td>
<td>99.57 ±0.3881</td>
</tr>
</tbody>
</table>

** Average of six determinations

4. **CONCLUSION:-**
The proposed UV- spectrophotometric methods were found to be simple, accurate, precise and inexpensive and can be used for routine analysis of Azithromycin and Cefpodoxime in bulk and its formulation.

5. **ACKNOWLEDGEMENT:-**
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6. REFERENCES:


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