SIMULTANEOUS ESTIMATION OF AZITHROMYCIN AND CEFPODOXIME PROXETIL FROM ITS TABLET DOSAGE FORM BY UV VISIBLE SPECTROSCOPIC METHODS.

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ABSTRACT

New simple, precise, accurate and cost effective UV spectrophotometric methods are developed for the estimation of azithromycin and cefpodoxime proxetil from its combined tablet dosage form by simultaneous equation method and derivative spectroscopic method. Both the method utilized methanol as solvent. Method – I is simultaneous equation method in which wavelength selected for azithromycin is 251 nm (λmax) and cefpodoxime shows maximum absorption at 234 nm. Method – II is derivative spectroscopic method in which zero order (D0) and first order (D1) derivative spectroscopic methods were explicated. Zero order derivatives utilize λmax of both the drugs as 251 nm and 234nm for analysis. While first order derivative spectroscopic method uses 233 nm (zero crossing point of cefpodoxime) for azithromycin and 245 nm (zero crossing point of azithromycin) for cefpodoxime analysis. Both the drugs follow beer-lamberts law in the concentration range of 5-30 µg/ml. The correlation coefficient of azithromycin and cefpodoxime was found to be as 0.999. Percent recovery for both the drugs is found to be around 99.95 %. Both the developed UV spectrophotometric methods were validated according to ICH guidelines with respect to accuracy, precision, ruggedness, specificity, LOD and LOQ. Both the proposed methods can be implemented for routine analysis of azithromycin and cefpodoxime in its combined tablet dosage forms.
KEYWORDS: Azithromycin, Cefpodoxime, UV Spectroscopy, Simultaneous equation method, First order derivative.

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)-ß-D-xylo-hexo pyranosyl} oxy}-1-oxa-6-azacyclopentadec-13-yl 2,6-dideoxy-3-C-methyl-3-O-methyl-ß-L-ribo-hexopyranoside (Fig. 1.). AZI is an antibiotic useful for the treatment of bacterial infections. It is an azalide category of drug, which is subclass of the macrolide antibiotic. AZI is mainly used in upper respiratory tract infections.[1]

Cefpodoxime proxetil (CEF) is chemically (6R,7R)-7-{{(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-methoxyimino-acetyl}amino}-3-(methoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (Fig. 2.). CEF is a third-generation cephalosporin antibiotic. It is active against most Gram-Positive and Gram-Negative organism. CEF is used in treatment of urinary tract and soft tissues infections. AZI and CEF are official in Indian Pharmacopoeia, 2010 and United States Pharmacopoeia, 2007.[2]

Literature survey reveals the Spectrophotometric methods like absorption ratio method, dual wavelength spectroscopy and First Order derivative Spectrophotometric method for estimation of CEF and AZI as single drug and in combination with other drugs. An RP-HPLC method for determination of AZI and CEF with other drugs is also reported in literature. Literature survey does not reveal any simultaneous equation method and derivative spectroscopic method for simultaneous estimation of AZI and CEF in combined dosage forms.[4–12]

Fig. 1. Chemical Structure of Azithromycin
MATERIAL AND METHODS

Chemicals and reagents

Active Pharmaceutical ingredients (API) of azithromycin and cefpodoxime proxetil were obtained as a gift sample from Wockhardt Pharmaceutical Ltd., Aurangabad and Adora pharmaceutical Ltd. Aurangabad, India respectively. The formulations Macpod AZ tablets were procured from local market with labeled amount of 250 mg of azithromycin and 200 mg of cefpodoxime proxetil of Macleods Pharmaceuticals Pvt. Ltd. Methanol AR grade, was purchased from Lab Trading Ltd. Aurangabad.

Instruments used

UV-Visible double beam spectrophotometer Shimadzu UV 1800, wavelength range 190-1100 nm band width 2nm, 1 cm quartz cells, slit width of 2 nm, instrument scan speed of 600 nm min^{-1} was used for analytical method development. The spectral data is processed by Shimadzu software UV Probe Ver.2.33.

Preparation of stock solution

A stock solution of AZI and CEF was prepared, by accurately weighing 10 mg of each drug and dissolving in separate 100 ml volumetric flasks and then diluted with methanol up to the mark to get final concentration of 100 µg/ml each. Further appropriate aliquots were pipette out from the standard stock solution into a series of 10 ml volumetric flasks, to get a set of dilutions for each drug.

Fig. 2. Chemical Structure of Cefpodoxime.
Method development\textsuperscript{[13 - 25]}

Method I: Simultaneous equation method

Appropriate dilution of standard stock solutions of both the drugs to 20 µg/ml dilution each is scanned in the spectrum mode from 400 nm to 200 nm. The absorption spectra of both the drugs are obtained, from the overlain spectra (Fig. 3.), wavelength selected for quantitation are 251 nm and 234 nm for AZI and CEF respectively, which are the $\lambda_{\text{max}}$ of both the drugs. The calibration curves for AZI and CEF were plotted in the concentration range of 5-30 µg/ml exhibiting the Beer’s and Lamberts law. The concentration of individual drug present in the mixture was determined by using the simultaneous equation calculations.

![Fig. 3. Overlay Spectra of azithromycin and cefpodoxime proxetil](image)

Method II: Derivative Spectroscopic methods

Zero order derivative ($D^0$) spectroscopic determination of AZI and CEF was done at a wavelength 251 nm and 234 nm respectively. While, first order derivative ($D^1$) spectroscopic determination for AZI was done at 233 nm, where CEF is showing zero crossing point, and wavelength selected for CEF was 245 nm, where AZI is showing zero crossing point (Fig. 4.). The concentration of individual drug present in the mixture was determined by using the calibration curve equations of both the drugs in zero and first order derivative spectroscopic method.
Stability of Sample and Standard Solutions

The results of analysis of solution stability of the drug substance and drug product (20 µg/ml of each drug) ensure that they are stable up to 48 h at 2°C – 8°C and ambient temperature. This indicates that there is no degradation occurring due to hydrolysis, photolysis, or adhesion to glassware over the course of the run period and can be utilize for UV Visible Spectroscopic analysis for the specified time period Table 1.

Table 1: Evaluation data of solution stability study of AZI and CEF for UV Visible Spectroscopy.

<table>
<thead>
<tr>
<th>Intervals (hours)</th>
<th>% Assay for Drug sample solution stored at 2°C – 8°C</th>
<th>% Assay for Drug sample solution stored at ambient temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AZI</td>
<td>CEF</td>
</tr>
<tr>
<td>Initial (0)</td>
<td>-----</td>
<td>------</td>
</tr>
<tr>
<td>12</td>
<td>99.81</td>
<td>100.02</td>
</tr>
<tr>
<td>24</td>
<td>99.78</td>
<td>99.88</td>
</tr>
<tr>
<td>48</td>
<td>99.75</td>
<td>99.85</td>
</tr>
<tr>
<td>Average Mean</td>
<td>99.78</td>
<td>99.92</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.1890</td>
<td>0.2457</td>
</tr>
</tbody>
</table>

Tablet Analysis

Twenty tablets were weighed accurately and triturated in mortar and pestle to powder form. Powder equivalent to 250 mg of AZI and 200 mg of CEF was weighed and transferred to 100 ml volumetric flask. The drugs are extracted with methanol by sonicating the tablet mixture for 20 min. Then it is filter through Whatman filter paper no. 41. Further, the sample
solutions were diluted with methanol to get final working dilution of 25 µg/ml and 20 µg/ml of AZI and CEF respectively. The absorbance of this final dilution was measured and results are calculated by both simultaneous equation method and derivative spectroscopic methods. The results are tabulated in Table 2.

Table 2: Results of tablet analysis of AZI and CEF.

<table>
<thead>
<tr>
<th>UV Method</th>
<th>Sample</th>
<th>Contents</th>
<th>Amount present (mg/tab)</th>
<th>Amount found (mg/tab)</th>
<th>%Percent Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simultaneous equation</td>
<td>Tablet</td>
<td>AZI</td>
<td>250</td>
<td>249.68</td>
<td>99.67 ± 1.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CEF</td>
<td>200</td>
<td>199.85</td>
<td>99.85 ± 0.91</td>
</tr>
<tr>
<td>Zero Order Derivative Method</td>
<td>Tablet</td>
<td>AZI</td>
<td>250</td>
<td>249.70</td>
<td>99.70 ± 0.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CEF</td>
<td>200</td>
<td>199.65</td>
<td>99.65 ± 1.16</td>
</tr>
<tr>
<td>First Order Derivative Method</td>
<td>Tablet</td>
<td>AZI</td>
<td>250</td>
<td>249.46</td>
<td>99.46 ± 1.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CEF</td>
<td>200</td>
<td>199.93</td>
<td>99.93 ± 0.46</td>
</tr>
</tbody>
</table>

*n = 5

Validation as per ICH guidelines.[26–29]

Linearity

Linearity of concentration range of 5-30 µg/ml for both drugs follows beers law, for which six dilutions (5, 10, 15, 20, 25, 30 µg/ml) are selected. The absorbances of solutions were then measured at 251 nm and 234 nm. The calibration curves were constructed by plotting absorbance versus concentration and the regression equations were calculated.

Precision

The method precision was determined by repeatability as intra-day (within a day) precision and inter-day precisions (within days). Concentration of 20 µg/ml of each drug was analyzed in triplicate; results are observed and calculated from corresponding absorbance for AZI and CEF Table 3.

Table 3: Results of precision studies of AZI and CEF.

<table>
<thead>
<tr>
<th>UV method</th>
<th>Intraday Precision</th>
<th>Interday Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AZI (20 µg/ml)</td>
<td>CEF (20 µg/ml)</td>
</tr>
<tr>
<td></td>
<td>% Recovery</td>
<td>% RSD</td>
</tr>
<tr>
<td>Simultaneous Equation Method</td>
<td>99.98</td>
<td>0.25</td>
</tr>
<tr>
<td>Zero Order Derivative Method</td>
<td>99.78</td>
<td>0.17</td>
</tr>
<tr>
<td>First Order Derivative Method</td>
<td>99.66</td>
<td>0.35</td>
</tr>
</tbody>
</table>

*n = 3
Accuracy
Accuracy of the method was obtained by performing recovery studies by the standard addition method at different levels of pure standard drug i.e., 80%, 100% and 120% of AZI and CEF to previously analyzed tablet powder sample and mixtures were reanalyzed by the proposed methods. From the amount of drug found percentage recovery was calculated and tabulated as in Table 4.

Table 4. Results of accuracy studies of AZI and CEF

| UV method                  | Level | Label Claim (mg/tab) | Amount of standard added (mg) | Total Amount recovered (mg) | % Recovery  
|----------------------------|-------|----------------------|-----------------------------|---------------------------|---------------
| Simultaneous Equation Method. | 80    | 250 200              | 200 160                     | 199.85 160.05           | 99.85 100.05
|                            | 100   | 250 200              | 250 200                     | 249.99 199.65           | 99.99 99.65
|                            | 120   | 250 200              | 300 240                     | 299.23 239.54           | 99.23 99.54
| Zero Order Derivative Method. | 80    | 250 200              | 200 160                     | 199.21 159.26           | 99.21 99.26
|                            | 100   | 250 200              | 250 200                     | 249.22 199.12           | 99.22 99.12
|                            | 120   | 250 200              | 300 240                     | 300.03 239.33           | 100.03 99.33
| First Order Derivative Method. | 80    | 250 200              | 200 160                     | 199.75 159.84           | 99.75 99.84
|                            | 100   | 250 200              | 250 200                     | 250.09 200.15           | 100.09 100.15
|                            | 120   | 250 200              | 300 240                     | 299.92 239.55           | 99.92 99.55

Specificity
The specificity of method was determined by comparing the results obtained from bulk drug analysis with that of marketed formulation analysis and it is observed that no excipients are interfering with drug analysis and thus all developed methods are said to be specific.

LOD & LOQ
The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with acceptable accuracy. The LOD and LOQ were calculated as LOD = 3.3 standard deviation of Y intercept/ slope of the calibration curve. LOQ = 10 standard deviation of Y intercept/slope of the calibration curve. LOD for AZI and CEF was found to be 0.25 μg/ml and 0.32 μg/ml respectively. While LOQ for AZI and CEF is found to be 0.5 μg/ml and 0.7 μg/ml respectively.
Ruggedness

The ruggedness was established through analysis of tablet by different analysts on the same UV instrument. The working dilution of 25 µg/ml and 20 µg/ml of AZI and CEF respectively, was used by analysts – I and analysts – II and analyzed by developed UV Spectroscopic methods (Table 5).

Table 5: Results of ruggedness studies of AZI and CEF.

<table>
<thead>
<tr>
<th>UV method</th>
<th>Analysts – I</th>
<th>Analysts – II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AZI</td>
<td>CEF</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>RSD</td>
</tr>
<tr>
<td>Simultaneous Equation Method.</td>
<td>100.05</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>99.65</td>
<td>0.24</td>
</tr>
<tr>
<td>Zero Order Derivative Method.</td>
<td>99.54</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>100.05</td>
<td>0.33</td>
</tr>
<tr>
<td>First Order Derivative Method.</td>
<td>99.77</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>99.45</td>
<td>0.21</td>
</tr>
</tbody>
</table>

n = 3

RESULT AND DISCUSSION

The developed methods are simple, precise, accurate and cost effective for the estimation of azithromycin and cefpodoxime from its combined tablet dosage form. This method utilized methanol as common solvent. The wavelength selected for simultaneous equation method for AZI shows maximum absorption at 251 nm and CEF shows maximum absorption at 234 nm. The drugs follow the Beer-Lamberts law in the concentration range of (5 – 30 µg/ml for both drugs). The method was validated by following analytical parameters as suggested by the ICH guideline which included accuracy, precision, specificity, ruggedness, LOD and LOQ. All validation parameters were within acceptable range. The correlation coefficient of AZI and CEF was found to be 0.999. Percent recovery was found to be 99.66 % for AZI and CEF. The proposed method is recommended for routine analysis of both the drugs its combined dosage forms.

CONCLUSION

The validated spectrophotometric methods developed are simple, rapid, accurate, precise, and cost effective and can be used for routine quality control analysis of azithromycin and cefpodoxime from combined tablet dosage form.
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