Validated Simultaneous Derivative Spectrophotometric Estimation of Azithromycin, Fluconazole and Secnidazole in Bulk and Pharmaceutical Formulation

M.A. Raskar*1, P.A. Kate1, S.S. Mungse1, G. R. Godge2

1 Department of Pharmaceutical Chemistry, Dr. Vithalrao Vikhe Patil Foundation’s College of Pharmacy, Vilad Ghat, Ahmednagar
2 Department of Pharmacology, Dr. Vithalrao Vikhe Patil Foundation’s College of Pharmacy, Vilad Ghat, Ahmednagar

INTRODUCTION

Azithromycin (AZI) chemically is (2R,3S,4R,5R,8R,10R,11R,12S,13R,14R)-13-[2,6-dideoxy-3-C-methyl-3-O-methyl-D-ribohexopyranosyloxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,9,10,12,14-heptamethyl-11-[[3,4,6-trIDEOxy-3-(dimethylamino)-4,6-D-xylOhexopyranosyloxy]-1-oxa-6-azacyclopendecan-15-one monohydrate or dehydrate. It is a semisynthetic macrolide antibiotic used to treat certain bacterial infections, such as bronchitis and pneumonia, and infections of the ears, lungs, skin, and throat. It is official in Indian Pharmacopeia1. The most commonly used techniques for determining AZI in pharmaceutical dosage forms are UV-Visible spectrophotometric2-5, RP-HPLC6-8, HPTLC9-10, LC-MS11-13, microbiological14, differential pulse voltametric15-17, amperometric18, IR spectroscopy19, diffuse reflectance near-infrared spectroscopy20 and UPLC21.

Fluconazole (FLU) chemically is 2-(2,4-Difluorophenyl)-1,3-bis (1H-1,2,4-triazole-1-y1)propan-2-ol. It belongs to the antifungal triazole class. It is widely used to treat fungal infections caused by cryptococci, candida and coccidia, and it is official in Indian Pharmacopoeia1. The most commonly used techniques for determining FLU in pharmaceutical dosage forms and biological fluid are the UV-Spectrophotometric22-23, HPLC24-27, UPLC28 and LC-MS29-30.

Secnidazole (SEC) chemically is (RS) - 1-(2-methyl-5-nitroimidazole-1-yl) propan-2-ol. It is used to treat protozoal infections and anaerobic bacterial infections. It is official in Indian Pharmacopoeia1. The most commonly used techniques for determining SEC in pharmaceutical dosage forms are UV spectrophotometric31-32, HPLC33-35, supercritical fluid chromatography36 and electrochemical method37. Other method has been performed to determine SEC in biological fluids, such as polarography38.

As per our knowledge, no derivative UV spectrophotometric method has been reported for simultaneous estimation of Azithromycin, Fluconazole and Secnidazole in tablet formulation. Hence we have developed two derivative spectrophotometric methods for simultaneous estimation of these three drugs from bulk and pharmaceutical formulation.

MATERIALS AND METHODS

Chemicals and Reagents

AZI, FLU and SEC were purchased from Balaji Drugs, Surat(Gujarat). The tablet dosage form of FLU, AZI and SEC...
Combokit, Hetero Healthcare Limited, Assam, India (Label Claim: 150mg FLU, 1gm AZI and 1gm SEC) was procured from the local market. AR grade methanol was used throughout the analysis.

**Instrument**

A double-beam UV-Visible Spectrophotometer (Jasco, Model V-630) was employed with a pair of 1cm quartz cells for all analytical work.

**Selection of Common Solvent**

For all three drugs, methanol was used as a common solvent for developing spectral characteristics by assessing the solubility in various solvents.

**Preparation of Standard Stock Solution**

The standard stock solutions of AZI, FLU and SEC were prepared separately by dissolving 10mg of each drug in 40ml of methanol. The final volume was adjusted with methanol to get a solution containing 100 µg/ml of each drug. For the selection of analytical wavelength, a standard solution of 20 µg/ml of each AZI, FLU and SEC was prepared separately by appropriate dilution of standard stock solution with methanol and scanned in the entire UV range of 200-400nm. The spectral data were processed to obtain each drug’s first-order derivative spectrum, and the above process was repeated for the second-order derivative method.39-41

**Derivative Spectrophotometric Method**

**Method 1: First-Order Derivative Method**

Each pure drug’s first-order derivative (D1) overlain spectra showed zero crossing points (ZCP). They assisted in their simultaneous estimation, as shown in Fig.1. The first-order derivative wavelength considered for AZI was 215nm, at which FLU and SEC show zero absorbance. Similarly, the estimation of FLU and SEC was carried out at 275 and 333nm, at which the other two drugs show zero absorbance. Calibration Curves were plotted between absorbance observed at D1 for three drugs at selected wavelengths against the concentration in the ranges of 5-30, 5-60 and 5-40 µg/ml for AZI, FLU and SEC respectively.42-45

**Method 2 – Second Order Derivative Method**

The second order derivative (D2) overlain spectra of each pure drug was found to show Zero Crossing Point (ZCP) and assisted in their simultaneous estimation, as shown in Fig.2. The second derivative wavelength considered for AZI was 220nm at which FLU and SEC show zero absorbance. Similarly, the estimation of FLU and SEC was carried out at 225 and 211nm, at which the other two drugs show zero absorbance. Calibration Curves were plotted between absorbance observed at D2 for three drugs at selected wavelengths against the concentration in the ranges of 5-35, 5-40 and 5-40 µg/ml for AZI, FLU and SEC respectively.46
Figure 2: Second-order derivative overlain spectra of AZI, FLU and SEC

Analysis of Tablet Formulation

Twenty tablets of each AZI, FLU and SEC (FAS-Kit) were weighed, and the average weight of each tablet was determined individually. The tablets of each drug were crushed into a fine powder, accurately weighed tablet powder equivalent to 1000mg (14.4 mg) of AZI, 150 mg (24.2 mg) of FLU and 1000 mg (14 mg) of SEC respectively and dissolved in methanol, sonicated for 10 min and diluted to 100ml with methanol and transferred into three individual 100ml volumetric flasks. The tablet solution of each drug was filtered through Whatman filter paper (no.41). After appropriate dilution, the absorbance of sample solutions was recorded at corresponding wavelengths and the results were recorded as shown in Table no.1.

Table 1: Result of Tablet Analysis

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Method 1</th>
<th>Method 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AZI</td>
<td>FLU</td>
</tr>
<tr>
<td>%Drug Content</td>
<td>99.23</td>
<td>97.65</td>
</tr>
<tr>
<td>SD*</td>
<td>0.0072</td>
<td>0.0023</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.099</td>
<td>0.12</td>
</tr>
</tbody>
</table>

*Mean of three determinations

Validation

The methods were validated according to International Conference on Harmonization (ICH) Q2B guidelines for validation of analytical procedures to determine linearity, precision and accuracy of each analyte. Both precision and accuracy were determined with standard samples prepared in triplicates at different concentration levels covering the entire linearity range39.

RESULTS AND DISCUSSION39-47

Linearity

The linearity for the first-order derivative method was determined from 5-30, 5-60 and 5-40 µg/ml for AZI, FLU and SEC respectively. For the second-order derivative method, linearity ranges from 5-35, 5-40 and 5-40 µg/ml for AZI, FLU and SEC respectively.

Precision

Precision was determined by studying repeatability and intermediate precision. The experiment was repeated three times a day for intra-day and on three different days for inter-day precision. The results of the precision study are presented in Table No. 2. In both methods, SD in the intra- and inter-day precision study was not more than 2.0%, indicating excellent repeatability and intermediate precision.
<table>
<thead>
<tr>
<th>Parameters</th>
<th>AZI</th>
<th>FLU</th>
<th>SEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working wavelength (nm)</td>
<td>Method 1</td>
<td>Method 2</td>
<td>Method 1</td>
</tr>
<tr>
<td></td>
<td>215 nm</td>
<td>220 nm</td>
<td>275 nm</td>
</tr>
<tr>
<td>Beer-Lambert’s Law range (µg/ml)</td>
<td>5-30</td>
<td>5-35</td>
<td>5-60</td>
</tr>
<tr>
<td>Precision* Interday precision (SD)</td>
<td>0.22</td>
<td>0.29</td>
<td>0.47</td>
</tr>
<tr>
<td>Intraday precision (SD)</td>
<td>0.03</td>
<td>0.25</td>
<td>0.20</td>
</tr>
<tr>
<td>LOD (µg/ml) *</td>
<td>0.16</td>
<td>0.21</td>
<td>0.39</td>
</tr>
<tr>
<td>LOQ (µg/ml) *</td>
<td>0.49</td>
<td>0.65</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Regression Values

<table>
<thead>
<tr>
<th></th>
<th>Method 1</th>
<th>Method 2</th>
<th>Method 1</th>
<th>Method 2</th>
<th>Method 1</th>
<th>Method 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope*</td>
<td>0.031</td>
<td>0.023</td>
<td>0.005</td>
<td>0.005</td>
<td>0.0448</td>
<td>0.031</td>
</tr>
<tr>
<td>Intercept*</td>
<td>0.035</td>
<td>0.037</td>
<td>0.009</td>
<td>0.002</td>
<td>0.0091</td>
<td>0.028</td>
</tr>
<tr>
<td>Regression Coefficient (R²)</td>
<td>0.995</td>
<td>0.995</td>
<td>0.998</td>
<td>0.999</td>
<td>0.998</td>
<td>0.998</td>
</tr>
</tbody>
</table>

*Mean of three determinations

**Accuracy**

Recovery studies by standard addition method assessed the validity and reliability of the proposed methods. The results are shown in Table No.3. The SD for mean of recovery (%) values was found to be < 2.0 for both methods.

Table 3: Results of Recovery Studies

<table>
<thead>
<tr>
<th>Drug</th>
<th>Recovery Level</th>
<th>% Recovery ± SD*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Method 1</td>
</tr>
<tr>
<td>AZI</td>
<td>50%</td>
<td>99.8 ± 0.10</td>
</tr>
<tr>
<td>FLU</td>
<td></td>
<td>99.2 ± 0.18</td>
</tr>
<tr>
<td>SEC</td>
<td></td>
<td>99 ± 0.23</td>
</tr>
<tr>
<td>AZI</td>
<td>100%</td>
<td>99 ± 0.099</td>
</tr>
<tr>
<td>FLU</td>
<td></td>
<td>97.65 ± 0.12</td>
</tr>
<tr>
<td>SEC</td>
<td></td>
<td>98.15 ± 0.11</td>
</tr>
<tr>
<td>AZI</td>
<td>150%</td>
<td>98.32 ± 0.074</td>
</tr>
<tr>
<td>FLU</td>
<td></td>
<td>100 ± 0.06</td>
</tr>
<tr>
<td>SEC</td>
<td></td>
<td>98.96 ± 0.27</td>
</tr>
</tbody>
</table>

*Mean of three determinations

**CONCLUSION**

The proposed UV spectrophotometric derivative methods for estimation of AZI, FLU and SEC were found to be simple, accurate and precise. The results obtained were found to be within the acceptable limit. The developed methods are applicable for estimating AZI, FLU and SEC in pure and tablet dosage forms. The good validation criteria of the proposed methods allow their use in quality control laboratories.

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CONFLICT OF INTEREST
There is no conflict of interest involved by the authors.

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