Development and Validation of UV-Visible Spectrophotometric Method for Estimation of Moxifloxacin in Human Plasma

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Abstract

A new reversed phase simple, economic and specific validated high performance liquid chromatography method for estimation of moxifloxacin in human plasma. Mobile phase used is acetonitrile: trisodium phosphate (5:95% v/v). UV detection at 294nm. The bioanalytical procedure involves de proteination of plasma with liquid–liquid extraction. The percentage of relative recovery and coefficient of variation of accuracy and precision were within acceptable limits. The method proved in simple, cost effective and sensitive for estimation of moxifloxacin in human plasma.

Keywords: Moxifloxacin, liquid–liquid extraction, human plasma, method development, validation, bioanalytical procedure.

INTRODUCTION

Moxifloxacin (MFX) one of the newer fluoroquinolones, has a broad spectrum of antimicrobial activity and issued for treating bacterial infections of the respiratory tract and soft tissues. Fluoroquinolones (FQs) are among the most important antibacterial agents used in human medicine [1-5]. They are active against both Gram-positive and Gram-negative bacteria through inhibition of their DNA gyrase and also possess some activity against mycobacteria, mycoplasmas and rickettsia [6-10]. Moxifloxacin is an 8-methoxy-fluoroquinolone that has proved an important fluoroquinolone for the treatment of a wide range of infections, including community-acquired pneumonia, and has a good safety record [11-13]. In vitro and in vivo experiments showed bactericidal activity of MFX against Mycobacterium tuberculosis (M. tuberculosis), which was high in comparison with other fluoroquinolones and is equal to or greater than isoniazid. Moxifloxacin is more frequently introduced as a second-line agent in the treatment of tuberculosis (TB) in case there is resistance or intolerance to first-line agents like rifampicin, isoniazid, pyrazinamide, and ethambutol [14-16]. The most commonly used method of analysis to determine MFX in human plasma, uses high-performance liquid chromatography (HPLC) followed by fluorescent or UV detection. However, these methods require liquid–liquid extraction and are therefore time-consuming [17-19].

IUPAC name: Moxifloxacin (1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-[(4aS,7aS)-octahydro-6H-pyrrolo-[3,4-b]pyridin-6-yl]-4-oxo-3-quinolinecarboxylicacid hydrochloride).

Molecular weight: 401.431g/mol.
Molecular formula: C$_2$H$_3$FN$_3$O$_4$.

![Fig-1: Structure of moxifloxacin](image_url)
Moxifloxacin is a slightly yellow to yellow crystalline substance. It was first patented in US in 1991 by Bayer A.G., and again in 1997 with the trade name of “Avelox”. Avelox was subsequently approved by the USFDA for use in the United States in 1999 to treat bacterial infections. Moxifloxacin is also manufactured by Alcon as “Vigamox” [20-22].

Table 1: Pharmacokinetic data of moxifloxacin

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioavailability</td>
<td>86%</td>
</tr>
<tr>
<td>Protein binding</td>
<td>47%</td>
</tr>
<tr>
<td>Metabolism</td>
<td>Glucuronide and Sulfate conjugation; cytochrome P450 system not involved.</td>
</tr>
<tr>
<td>Shelf-life</td>
<td>12.1 hours</td>
</tr>
</tbody>
</table>

Routes of administration: By mouth, IV, local (eye drops)

**Introduction to UV Spectroscopy**

UV spectroscopy is a type of absorption spectroscopy in which light of ultra-violet region (200-400 nm) is absorbed by the molecule. Any molecule has either n, π or σ or combination of these electrons. These bonding (σ and π) and non-bonding (n) electrons absorb the characteristic radiation and undergo transition from ground state to excited state. By the characteristic absorption peaks and the nature of the electron present the molecular structure can be elucidated UV spectroscopy obeys the Beer-Lambert law.

**Beer law:** This law can be stated as follows: “When a beam of monochromatic radiation is passed through a solution of absorbing substances, the intensity of a beam of monochromatic light decreases exponentially with the increase in concentration of the absorbing substances exponentially”.

\[
I = I_0 e^{-k_1 c} \tag{1}
\]

Where, 
- \(I_0\) = intensity of light incident upon sample cell
- \(I\) = intensity of light leaving sample cell
- \(C\) = molar concentration of solute
- \(K_1\) = constant

**Lambert’s law:** This law can be stated as follows “When a beam of light is allowed to pass through a transparent medium, the rate of decrease of intensity with the thickness of medium is directly proportional to the intensity of the light”.

\[
I = I_0 e^{-k_2 l} \tag{2}
\]

Where, 
- \(I_0\) = intensity of light incident upon sample cell
- \(I\) = intensity of light leaving sample cell
- \(L\) = length of sample cell (cm.)
- \(K_2\) = constant

After combining equation 1 and 2 and deriving we get the following equation 3 of Beer-Lambert law as:

\[
A = \log \left( \frac{I_0}{I} \right) = εcl \tag{3}
\]

Where, 
- \(A\) = absorbance
- \(I_0\) = intensity of light incident upon sample cell
- \(I\) = intensity of light leaving sample cell
- \(C\) = molar concentration of solute
- \(L\) = length of sample cell (cm.)
- \(ε\) = molar absorptivity

A literature search has shown that there are only few quantitative analytical methods for estimation of moxifloxacin further, very few methods were available that shows the quantification of moxifloxacin in biological fluids, these methods include LC-MS, GC-MS, which needs high end instrumentation which are costly and not available in conventional bioanalytical laboratory. Thus, the conclusion was to develop a rapid, simple and economical method which was based on liquid–liquid extraction (LLE) for sample preparation and UV detection for quantification of moxifloxacin from spiked human plasma.

**MATERIALS AND METHODS**

**Chemicals and reagents:** Moxifloxacin were gifted by pharma company, Hyderabad, Telangana, India. Hplc grade acetonitrile procured from rankem chemicals limited, New Delhi, India. Human samples are procured as a.

**Instrumentation**

Double beam UV spectrophotometer; Model: SL 210; Make: ELICO. The data was obtained using Spectra Treats 3.11.01Rel 2b.

Vortex mixer; Model:CM 101; Make: REMI

The analysis was performed using UV SL120 using UV detector used for method development and validation. The output signal was checked and the acquisition and integration of data was performed using spectral threats. Software on a computer. The diluents are filtered through 0.25µm detection was monitored at 294nm.

**Procedure**

**Selection of Wavelength**

10mg of moxifloxacin drug was accurately weighed and transferred into 10 ml of volumetric flask and the volume was made up to the mark with acetonitrile as diluent. Then from this 0.1 ml was
pipetted out and transferred into another 10 ml volumetric flask and the volume was made up to the mark with acetonitrile to give 10ppm solution and this was scanned between 200 to 400nm and its absorbance was measured at 294nm (Figure-2).

Blood was collected into an EDTA containing tube and then it was centrifuged for 10 min at 3000rpm blood was separated into two layers after centrifugation. The supernatant which contains stray yellow colour (plasma) was collected and used for sample preparation.

Preparation of Plasma Solution
2.5ml plasma was deproteinised to this 2.5ml of moxifloxacin was taken in a centrifuge tube and the contents are vortex for 30 seconds. To that add 1ml of acetonitrile and vortex it for 3 mins in cyclomixer. Then the solution was centrifuged for 10 min at 13000rpm. Then the organic layer was transferred to another centrifuge tube and evaporated at 40°C. Under stream of air. Then the dried extract was reconstituted with 200µl of acetonitrile. The aliquot was collected and absorbance was measured at 294nm.

Method Validation Parameters
Method validation: ICH guidance for industry was followed for validation of the method. linearity, accuracy, robustness, LOD, LOQ were assessed during method validation.

Linearity
Calibration standard solutions were prepared in plasma from the working solutions. Five calibration curves ranging from the 2 to 10 ppm were run to establish the linearity by using linear regression analysis. From the stock solution 0.2ml, 0.4 ml, 0.6 ml, 0.8 ml and 1.0 ml was pipetted out and transferred into 10ml volumetric flask and the volume was made up to 10ml with acetonitrile to give 2ppm, 4ppm, 6ppm. 8ppm and 10ppm concentration. respectively and absorbance was measured at 294nm using acetonitrile as blank and the calibration curve is plotted.

Precision
10ppm standard solution of moxifloxacin pure drug is selected for Precision study. From the standard stock solution 0.1ml was pipetted out and transferred into 10ml volumetric flask and the volume was made up to 10ml using acetonitrile to give 10ppm solution. This procedure is repeated 6 time and observances of all were measured at 294nm using acetonitrile as blank and its %RSD was calculated by using the formula:

\[ \% \text{RSD} = \left( \frac{\text{standard deviation of the measurement}}{\text{mean value of measurement}} \right) \times 100 \]

Accuracy

Quality control of samples was prepared at four different levels. The concentration of moxifloxacin was calculated from a standard calibration curve that was concurrently obtained. Accuracy was analysed at each level by comparing the observed concentration as a mean relative percentage recovery. Standard quantity equal into 50%, 100% and 150 % is to be added in sample. 2ml of standard solution was spiked with 4ml of sample solution, 2ml of standard solution was spiked with 6ml of sample solution, 2ml of standard solution was spiked with 8ml of sample solution. Absorbance was measured for three times at 294nm.

Repeated three times and their absorbance is measured at 294nm and the %recovery is calculated by using the formula:

\[ \% \text{Recovery} = \left( \frac{\text{amount found}}{\text{amount added}} \right) \times 100 \]

Limit of Detection

The detection limit (DL) may be expressed as:

\[ \text{DL} = 3.3 \times \frac{\sigma}{S} \]

Where,
\[ \sigma = \text{the standard deviation of the response} \]
\[ S = \text{the slope of the calibration curve} \]

The slope S may be estimated from the calibration curve of the analyte.

Limit of quantification

The quantitation limit (QL) may be expressed as:

\[ \text{QL} = 10 \times \frac{\sigma}{S} \]

Where,
\[ \sigma = \text{the standard deviation of the response} \]
\[ S = \text{the slope of the calibration curve} \]

The slope S may be estimated from the calibration curve of the analyte.

Robustness

Robustness: 6 aliquots of 6ppm of standard solution was prepared and it was scanned at wavelength at (±)1nm of λmax. The absorbance was noted down.

RESULTS AND DISCUSSION

Method development and optimization of chromatographic condition:
The % assay was found to be 99%.

<table>
<thead>
<tr>
<th>Table-2: Conc. Vs Abs. table for Linearity Study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Concentration(ppm)</strong></td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>10</td>
</tr>
</tbody>
</table>

Fig-3: linearity curve of Moxifloxacin
Table-3: Evaluation data of precision study

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.3534</td>
</tr>
<tr>
<td>2</td>
<td>0.3529</td>
</tr>
<tr>
<td>3</td>
<td>0.3620</td>
</tr>
<tr>
<td>4</td>
<td>0.3511</td>
</tr>
<tr>
<td>5</td>
<td>0.3622</td>
</tr>
<tr>
<td>6</td>
<td>0.3506</td>
</tr>
<tr>
<td>Mean</td>
<td>0.3554</td>
</tr>
<tr>
<td>SD</td>
<td>0.005321</td>
</tr>
<tr>
<td>%RSD</td>
<td>1.497</td>
</tr>
</tbody>
</table>

Table-4: Evaluation data of accuracy study

<table>
<thead>
<tr>
<th>% Recovery level</th>
<th>% Recovery</th>
<th>Mean % recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>99.76</td>
<td>99.65</td>
</tr>
<tr>
<td></td>
<td>99.65</td>
<td></td>
</tr>
<tr>
<td></td>
<td>99.55</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>99.85</td>
<td>99.77</td>
</tr>
<tr>
<td></td>
<td>99.79</td>
<td></td>
</tr>
<tr>
<td></td>
<td>99.67</td>
<td></td>
</tr>
<tr>
<td>150%</td>
<td>99.88</td>
<td>99.74</td>
</tr>
<tr>
<td></td>
<td>99.79</td>
<td></td>
</tr>
<tr>
<td></td>
<td>99.56</td>
<td></td>
</tr>
</tbody>
</table>

The limit of detection was found to be 0.51 ppm and limit of quantification found to be 1.57ppm.

Table-5: Evaluation data of robustness study

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>293nm</th>
<th>294nm</th>
<th>295nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.6411</td>
<td>0.6431</td>
<td>0.6435</td>
</tr>
<tr>
<td>2</td>
<td>0.6399</td>
<td>0.6428</td>
<td>0.6430</td>
</tr>
<tr>
<td>3</td>
<td>0.6381</td>
<td>0.6421</td>
<td>0.6429</td>
</tr>
<tr>
<td>4</td>
<td>0.6376</td>
<td>0.6416</td>
<td>0.6420</td>
</tr>
<tr>
<td>5</td>
<td>0.6355</td>
<td>0.6410</td>
<td>0.6416</td>
</tr>
<tr>
<td>6</td>
<td>0.6345</td>
<td>0.6406</td>
<td>0.6410</td>
</tr>
<tr>
<td>Mean</td>
<td>0.6378</td>
<td>0.6419</td>
<td>0.6423</td>
</tr>
<tr>
<td>SD</td>
<td>0.002514</td>
<td>0.0009873</td>
<td>0.0009543</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.3942</td>
<td>0.1538</td>
<td>0.1486</td>
</tr>
</tbody>
</table>

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REFERENCES


