DEVELOPMENT AND VALIDATION OF ZERO AND FIRST ORDER DERIVATIVE AREA UNDER CURVE SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF AXITINIB IN BULK MATERIAL AND in-house TABLETS

Ravsaheb H. Rathod, Amod S. Patil, Atul A. Shirkhedkar*
Department of Pharmaceutical Chemistry, R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, Dist. Dhule (MS), India 425 405

Date of Submission: 03 Oct, 2017
Date of Revision: 05 Nov, 2017
Date of Acceptance: 15 Dec, 2017

*Corresponding Author:
Email ID: shirkhedkar@gmail.com

KEYWORDS
Axitinib; UV- Spectrophotometry; First Order Derivative; Spectrophotometry; Area under Curve

ARTICLE INFO

ABSTRACT
The proposed study demonstrates simple, precise and accurate UV-Spectrophotometry methods for the estimation of Axitinib in bulk and in-house tablets. Axitinib is tyrosine kinase inhibitor; four simple UV-Spectrophotometric methods were established for estimation of Axitinib, using double beam UV-Spectrophotometer (UV-2450, Shimadzu). Methanol was used as a solvent. Axitinib showed maximum absorbance ($\lambda_{\text{max}}$) at 330 nm. The calibration curve were plotted in the concentration range of 2 -12 $\mu$g/ml. The % recovery was found to be in the range of 98 - 100 %. Furthermore the precision of methods were calculated in terms of % RSD less than 2 showed, methods are precise. The developed methods have been validated according to ICH guidelines. The depicted methods can routinely be used for determination of Axitinib in bulk and in-house tablets.

1. INTRODUCTION
Axitinib, chemically is N-methyl-2-[(3-[((1E)-2-(2-pyridinyl) ethenyl]-1H-indazol-6-yl) thio]- benzamide (Figure 1). Axitinib is a small molecule tyrosine kinase inhibitor (TKI). It inhibits the VEGFR-1, VEGFR-2 and VEGFR-3 by platelets, and thus interferes with platelet aggregation and hence is used in reducing risks of breast cancer in xenograft models. It gives short respond in clinical trials with renal cell carcinoma (RCC) and several other tumor types. The Oncologic Drugs Advisory Committee (ODAC) selected unanimously to suggest the approval of Axitinib for the second-line treatment of patients with advanced renal cell carcinoma (RCC).

Literature review revealed that Axitinib was resolved by the liquid chromatography-mass spectrophotometry (LC-MS), and high performance liquid-chromatography (RP-HPLC) methods, moreover one UV- Visible Spectrophotometry estimation of anticancer drug has been reported. Our objective is to establish zero and first order derivative UV-Spectrophotometry methods applying amplitude and also AUC techniques. The current investigate emphasize a simple, sensitive and effective UV-Spectrophotometry method for estimation of Axitinib in bulk material and in-house tablets. Further, methods were validated as per ICH guidelines.

2. MATERIALS AND METHOD

Chemicals:
Pure Axitinib were obtained as a gift sample from Glenmark Pharmaceutical Ltd., Mumbai. Methanol purchased from Merck Ltd., Worli, and Mumbai, India.

Instrumentation: A double beam UV-VIS spectrophotometer (UV-2450, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe 2.21 with 1 cm quartz cells was used. An electronic balance (Model Shimadzu AUX 120) was used for weighing purpose.

Figure 1: Structure of Axitinib
2. MATERIALS AND METHODS

Selection of common solvent:
Methanol of analytical reagent grade was selected as common solvent for developing spectral characteristics of drug. The choice of the solvent was made after evaluating the solubility in different solvents.

Preparation of Stock Standard Solution and determination of $\lambda_{\text{max}}$

The stock standard solution of Axitinib was prepared by weighing accurately, 10 mg of Axitinib transferred in to 100 ml volumetric flask and volume was made up to the mark with methanol, obtaining a concentration of 100 $\mu$g/ml.

From the stock standard solution, 1 ml of solution was transferred into 10 ml of volumetric flask and volume was make up with the same to get concentration of 10 $\mu$g/ml. The resulting solution was scanned in UV region 400 - 200 nm, the spectrum showed maximum absorption at ($\lambda_{\text{max}}$).

Methods A (Zero Order Spectrophotometry) and Method B (Zero order Spectrophotometry–AUC)

For Method A, absorbance was recorded at 330 nm shown in Figure 2. While in Method B, AUC was selected in the wavelength range of 319 – 338.40 nm, shown in Figure 2. The calibration curves were constructed by plotting concentration versus absorbance and AUC of zero order spectrum in Method A and B, respectively shown in Figure 3.

Methods C (First order derivative-UV Spectrophotometry) and D (First order derivative-UV Spectrophotometry–AUC)

For Method C and D, spectra of above solutions were derivatized into first order using software UV-Probe 2.21 with delta lambda 4 and scaling factor 10. In Method C, the amplitude was recorded at 350.40 nm shown in Figure 2. While in Method D, AUC of the derivative spectrum was selected wavelength range 344.40 – 359.40 nm shown in Figure 2. The calibration curves were constructed by plotting concentration versus amplitude and AUC of first order spectrum in Method C and D, respectively, is shown in Figure 3.

Preparation of Sample Solution:
Since the pharmaceutical formulation of Axitinib is not available in the Indian market; therefore, in-house tablets were prepared with 5 mg of Axitinib using common excipients. The sample solution was prepared from in-house formulated Axitinib tablets.
Twenty *in-house* tablets were weighed and ground into fine powder. A quantity of the powdered drug equivalent to about one tablet was transferred into a volumetric flask containing 50 ml of methanol sonicated for about 15 min and then volume made up with same solvent. The resulting solution was filtered through Whatmann filter paper. An appropriate 0.6 ml was transferred into 10 ml volumetric flask and volume was made to the mark using methanol. The resulting solutions were scanned using UV-Spectrophotometer in the range of 400 - 200 nm.

**Validation of Method:**

The method was validated with respect to various parameters including linearity, limit of detection and quantification, precision and accuracy according to ICH guidelines [10].

**Study of linearity curves:**

Aliquots 0.2, 0.4, 0.6, 0.8, 1 and 1.2 ml of standard stock solution of Axitinib was transferred to series of 10 ml volumetric flask and volume was made up to the mark using methanol. The calibration curve was obeyed in the concentration of range 2 - 12 μg/ml and the graph was plotted between concentrations versus absorbance, amplitude and AUC.

**Accuracy/ Recovery studies:**

To study the accuracy of the anticipated methods and to confirm the obstruction from excipients used in the dosage forms, recovery experiments were performed by the standard addition method. It was carried out by adding known amount of standard drug to the developed *in-house* tablet formulation at 80, 100 and 120 % level. It was then re-analyzed by the proposed methods. The % recovery of noted.

**Precision**

The precision of proposed method was determined in terms of intra-day and inter-day precision. Intra-day precision was resolute by examine the 4, 6 and 8 μg/ml of Axitinib for three times in the similar day. Inter-day precision were determine the concentration of 4, 6 and 8 μg/ml of Axitinib for three days.

**Sensitivity**

The limit of detection (LOD) and limit of quantification (LOQ) of the drug were calculated by using the equations designated by International Conference on Harmonization (ICH) guidelines.

\[
\text{LOD} = 3.3 \times \sigma / S
\]

\[
\text{LOQ} = 10 \times \sigma / S,
\]

Where \(\sigma\) is the standard deviation of intercept, \(S\) is the slope.

**Ruggedness**

Ruggedness of the introduced method was determined for 6 μg/ml concentration of Axitinib by analysis of aliquots from a homogenous slot by two analysts using same operational and environmental conditions. The results are in acceptable range that is % RSD values < 2 for all the methods.
3. RESULTS AND DISCUSSION

Axitinib showed a good correlation coefficient for all methods are shown in Table 1, the given concentration range 02-12 μg/ml.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Method A</th>
<th>Method B</th>
<th>Method C</th>
<th>Method D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer-Lambert's</td>
<td>02 - 12</td>
<td>02 - 12</td>
<td>02 - 12</td>
<td>02 - 12</td>
</tr>
<tr>
<td>range (μg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>λ max (nm)</td>
<td>330.00</td>
<td>319.40</td>
<td>350.40</td>
<td>344.40</td>
</tr>
<tr>
<td>Slope</td>
<td>0.090</td>
<td>0.10</td>
<td>0.037</td>
<td>0.100</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.028</td>
<td>0.079</td>
<td>0.026</td>
<td>0.048</td>
</tr>
<tr>
<td>Correlation</td>
<td>0.999</td>
<td>0.999</td>
<td>0.998</td>
<td>0.998</td>
</tr>
</tbody>
</table>

The % amounts predicted from in-house tablet formulation show that there is no interference from excipients present in it. The percentage amounts of Axitinib estimated from in-house tablets using all methods are shown in Table 2.

<table>
<thead>
<tr>
<th>Methods</th>
<th>% Amount found</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>98.43</td>
<td>0.96</td>
</tr>
<tr>
<td>B</td>
<td>100.00</td>
<td>0.98</td>
</tr>
<tr>
<td>C</td>
<td>98.28</td>
<td>1.06</td>
</tr>
<tr>
<td>D</td>
<td>99.17</td>
<td>0.69</td>
</tr>
</tbody>
</table>

The LOD and LOQ of proposed methods were shown in Table 3. Intra-day and inter-day precision was carried out by performing three replicates of three of three different concentration 4, 6 and 8 μg/ml of Axitinib showed % RSD less than 2 was shown in Table 3. The percentage recovery of Axitinib at three concentration levels 80, 100, and 120 % was calculated and results are shown in Table 3. The results of validation parameters are summarized in Table 3.

3.3 CONCLUSION

Overall four methods were established for quantitative analysis of Axitinib in bulk and in-house tablets using zero order, first order and AUC technique of UV-Spectrophotometry. The results and statistical boundary shows that the developed UV-Spectrophotometric methods are simple, accurate and precise and specific. Therefore, these methods can routinely be used for estimation of Axitinib in bulk and pharmaceutical formulations.

4. ACKNOWLEDGMENT

Authors are thankful to Principal of R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, Dist: Dhule (MS) for providing necessary laboratory facility.

5. DISCLOSURE OF INTEREST

The authors declare that they have no competing interest.

6. REFERENCES:


