Analytical Method Development and Validation of Vandetanib by Using RP-HPLC of Bulk Drug
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Abstract

RP-HPLC is fast, simple, sensitive, precise, and reproducible (liquid chromatography) method was developed and validated for the analysis of vandetanib bulk dosage form. Using C-18 HPLC column separation was carried out. Which was maintained at ambient temperature. During separation mobile phase consist of methanol (100 v/v) was delivered at a rate of 1mL/min. Analysis was carried out by using UV detector at the wavelength 328 nm. In RP-HPLC method was validated by using various parameters like, precision, limit of quantitation (LOQ), linearity and robustness. The RP-HPLC method was found to be linear over the concentration ranges from 50-100 μg/mL (r² =0.9996). Retention time for bulk vandetanib was found to be 5.496±25 min. LOQ of method was 6.8339 μg/mL and LOD 2.7036μg/mL. Thus, the RP-HPLC developed method was found to be robust and rugged which can be applied for the regular analysis of vandetanib in the bulk as well as pharmaceutical dosage form.

Key words: C18, RP-HPLC, Methanol, Vandetanib.

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INTRODUCTION

Vandetanib(N(4-bromo-2-fluorophenyl))6-methoxy-7[(1-methyl-4-piperidinyl)methoxy]4-quinazolinamine) is a recently identified small molecule inhibitor, which shows antitumor efficacy by inhibiting tumor cell proliferation and survival via epidermal growth factor receptor (EGFR) and RET inhibition, as well as inhibiting tumor angiogenesis via vascular EGFR-2 (VEGFR-2) inhibition. Its preclinical and clinical activity against several tumor types including advanced and metastatic papillary thyroid cancer, non-small cell lung cancer (NSCLC), and advanced colorectal cancer (CRC) either in monotherapy or in combination with other anticancer agent as first or secondline therapy has been demonstrated.

In literature survey reveals that a few spectrophotometric, RP-HPLC methods are reported for the estimation of Vandetanib in combination with other drugs. Main purpose of this work to develop a RP-HPLC method for the determination of vandetanib in bulk form for the provide more scope in further research study on the drug and pharma industry [1-4].

MATERIALS AND METHOD

Chemicals

Vandetanib was received as a gift sample from Mylan Laboratories Limited, Hyderabad, India. HPLC grade methanol and double distilled water as solvent was used for the other purpose.

Instrumentation

The chromatographic technique was performed on a Shimadzu LC-2010CIR Liquid chromatography with UV-visible detector and LC-Solution software, reversed phase C18 column (Inerstil ODS-3V 5 um 250x4.6 mm), Shimadzu analytical balance AY-220, Vacuum micro filtration unit with 0.45μ membrane filter was used in the study. Double beam UV-visible spectrophotometer (UV-probe 2.32 software) [5].

Determination of Working Wavelength (λmax)

10 mg of vandetanib was weighed and transferred in to 100 mL volumetric flask and dissolved in methanol and then make a dilution of that stock solution. Prepare 10 μg/mL solution by diluting 1 mL to
10 mL with methanol. Wavelength of maximum absorption for 10 µg/mL solution of the bulk drug in methanol was scanned using UV-Visible spectrophotometer within the range of 200-400 nm wavelength with methanol as reference. The absorption curve shows at 328 nm for vandetanib bulk [6-20].

**Chromatographic Condition**

Mobile phase for this developed method methanol 100% and it was filtered through a 0.45µm membrane filter degassed with a helium spurge for 30min and pumped from the respective solvent reservoir to the column inerstil C18 column (250x4.6 mm) at flow rate 1.0 mL/min. This HPLC developed method run time was set at 10 min and column temperature was maintained at RT. Then prior to inject the bulk drug solution in to the column, column was equilibrated for at least 30 min with the same mobile phase flowing through the system. The eluent was monitored at 328 nm. Using “LC-Solution” software data was stored and analysed [2].

**Selection of Mobile Phase**

At the beginning solution of bulk drug vandetanib was injected into the HPLC system and run in various solvent system. Various mobile phase methanol, water, acetonitrile, and phosphate buffer in different ratio were tried and finally methanol (100%) was selected as an appropriate mobile phase which gave good resolution and acceptable peak parameters for bulk vandetanib [20].

**Evaluation of Analytical Methods**

**Linearity**

For the linearity study were prepared suitable dilution (ranging from 50-100 µg /mL) of standard stock solution using mobile phase. Though linear response was obtained at lower concentrations for vandetanib and higher concentration range was used to improve signal to noise ratio. Linearity was determined by analysing five working standards over the concentration range of 50-100 µg /mL for vandetanib [20].

**Limit of Detection (LOD)**

The limit of detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value. LOD is calculated from the formula:

\[
\text{LOD} = \frac{3.3\sigma}{S}
\]

Where,

- \(\sigma\) = standard deviation of the response
- \(S\) = slope of the calibration curve.

**Limit of Quantification (LOQ)**

The limit quantification (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined with precision and accuracy. LOQ is calculated from formula:

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

Where,

- \(\sigma\) = standard deviation of the response
- \(S\) = slope of calibration curve

**Accuracy**

Accuracy of this method was carried out using one set of different standard addition methods at different concentration levels 80%, 100% and 120%, and then comparing the difference between the spiked value (theoretical value) and actual found value [10, 11].

**Precision**

Five sets of aliquots with same concentration (90 µg /mL) were prepared and these solutions were analysed to record any intra and inter day variations in the results. The results obtained for Intra and inter day variations.

**Robustness**

Robustness of the proposed method for vandetanib was carried out by the slight variation in flow rate, temperature and mobile phase ratio. The percentage recovery and RSD were noted for vandetanib.

**RESULT AND DISCUSSION**

**Checking Resolution of Drug and Materials**

The column was saturated with the mobile phase (indicated by constant back pressure at desired flow rate). Standard solution of vandetanib was injected to get the chromatogram. The retention time for vandetanib was found to be 5.496 min. It is shown in the Table-1.

**Table-1: Resolution of bulk drug**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Ret. Time</th>
<th>Area</th>
<th>Height</th>
<th>Theoretical plate</th>
<th>Tailing factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vandetanib</td>
<td>5.496</td>
<td>35957814</td>
<td>340511</td>
<td>3206.216</td>
<td>1.387</td>
</tr>
</tbody>
</table>

**Linearity**

The data of the peak area vs drug concentration were evaluated by linear regression analysis as shown in the Table-2 and calibration curve obtained after plotting drug concentration vs area shown in the Fig-1. Linear regression analysis demonstrated that chromatograph response for the drug was highly linear (\(r^2=0.9996\)) in the studied concentration range of 50-100 µg/mL. A typical
chromatogram of vandetanib (50 μg/mL) shown in Fig-1.

Table-2: Calibration of Vandetanib

<table>
<thead>
<tr>
<th>Sr No</th>
<th>Concentration (μg/mL)</th>
<th>Peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>35957814</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>46217278</td>
</tr>
<tr>
<td>3</td>
<td>70</td>
<td>56914020</td>
</tr>
<tr>
<td>4</td>
<td>80</td>
<td>66967139</td>
</tr>
<tr>
<td>5</td>
<td>90</td>
<td>76629861</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>86282828</td>
</tr>
</tbody>
</table>

Precision

The result depicted in the Table 3a & 3b indicated that the given method has sufficient precision as indicated by the corresponding values of %RSD ranging 0.13 for inter day studies respectively. The values of %RSD for both the studies are well below 1.0% constructing adequate precision.

Table-3a: Intra-day Precision for Vandetanib

<table>
<thead>
<tr>
<th>Concentration (μg/mL)</th>
<th>Peak area</th>
<th>Mean (n=5)</th>
<th>S.D.</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>76629865</td>
<td>76618861</td>
<td>12622.96</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Table-3b: Inter-day Precision for Vandetanib

<table>
<thead>
<tr>
<th>Concentration (μg/mL)</th>
<th>Peak area</th>
<th>Mean (n=5)</th>
<th>S.D.</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>75629865</td>
<td>75100844</td>
<td>38217.53</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Limit of Detection and Quantification

Standard error and slope of linear data is used to predict LOD and LOQ of rivastigmine and precision was established at the predict concentration. The result was shown in the Table-4.

Table-4: Limit of detection and Limit of quantification

<table>
<thead>
<tr>
<th>Limit of Detection</th>
<th>Limit of Quantification</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.7036μg/mL</td>
<td>6.8339μg/mL</td>
</tr>
</tbody>
</table>

CONCLUSION

From the results and discussion, RP-HPLC methods were developed and validated as per ICH guidelines Q2 (R1). In this paper described for the determination of vandetanib in bulk is simple, sensitive and reproducible. The proposed methods can be successfully applied for vandetanib without any interference in quality control.

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REFERENCE


