Method development and validation of cabozantinib by LC-MS/MS

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Abstract

The objective of this method is to be simple, precise, and economical performed by LC-MS/MS instrument. The mass spectrometric determination was performed using electrospray ionization in the positive mode with multiple reaction monitoring (MRM) mode and precursor to product ion transition to product ion of m/z 502.2 > 323 for cabozantinib. The effective separation of cabozantinib was achieved X-Bridge (2.1 mm × 100 mm, 3.5 µ) column and the mobile phase composition is 0.2% formic acid: acetonitrile (40:60 v/v), pumped at 0.12 ml/min flow rate. The Rt of cabozantinib was found to be 1.34 minutes. The LOD and LOQ were found at 1.5 ng/ml and 5 ng/ml concentrations and linearity concentrations were in a range of 5 ng/ml to 75 ng/ml with a regression correlation coefficient of 0.999. The % RSD value of accuracy was observed at 1.2–2.0. The marketed formulation assay was found to be 99.82%. The developed method and validation parameters were accepted as per USFDA guidelines.

Keywords

Cabozantinib, LC-MS/MS, Validation, Limit of detection, % RSD

Introduction

Cabozantinib is an anticancer drug and its molecular structure is shown in Fig. 1. The mechanism action of cabozantinib is tyrosine kinase inhibitor (TKI) and affects on vascular permeability factor (VPF) (Yakes FM et al. 2011). A phase-3 randomized controlled study of cabozantinib has higher progression-free-survival (PFS) overall survival (OS) or overall response rate (ORR) as opposed to drug affinitior in patients who progressed following before vascular endothelial factor (VEGF) growing molecularly targeted drugs resulting to its accredited by USFDA (Choueiri TK et al. 2015). Cabozantinib is additionally accepted for utilization in the front line position for patients with midway / low-risk patients (Choueiri TK et al. 2018). Tyrosine kinase (TKs) are considered possible attack for the latest drug progress mostly for cancer and rheumatoid arthritis drugs inhibitors the past various tyrosine kinase inhibitors (TKIs) have been grown and accepted for medicaments of different classifications of cancer with each one targeting certain sign pathways (Nguyen L et al. 2015). Moreover modern further have conducted findings of the janus kinases (JAKs) (Tolaney SM et al. 2016) which by their inhibition established a novel curative path for cancer and immunity disorders (Lacy S et al. 2015).

The heart rhythm problems including long QT intervals observed in inpatient history i.e. the drug should be used with caution (Qaseem A et al. 2012; Takeda H et al. 2017; Osmani L et al. 2018; Van Schil PE et al. 2018; Wienand K et al. 2019; Poole and Jeanne E 2020). Cabo-
Cabozantinib and nivolumab drugs were marketed under the brand name of opdivo and used for various classifications of cancer treatment include melanoma, lung cancer renal cell cancer, Hodgkin lymphoma head, and neck cancer and colon cancer, and liver cancer. The usual side effects contain tiredness rash, liver problems, muscle pain, and cough (Comi G et al. 2001; Qaseem A et al. 2012; Takeda H et al. 2017; Ashok G and Mondal S 2018; Osmani L et al. 2018; Wienand K et al. 2019).

Materials and methods

Cabozantinib standard powder and API (purity > 98%) were procured from the API industry and marketed tablets procured from the pharmacy store. All HPLC grade solvents were procured from merc india Ltd, india. All chemical reagents and aqueous solvents are purified by using millipore (0.45 µm) filters.

Instrumentation and optimized chromatography conditions

The chromatography analysis was performed by using UPLC instrument waters with an acquity model with an auto sampling system. MS detector is waters Quattro premier XF model triple quadrupole MS was used. The software of the LC-MS/MS system is open lab software. Mass spectroscopy specifications are electro spray ionization (ESI), positive ionization mode, the capillary voltage was set at 3KV, and nitrogen was used as a desolvation gas at a flow rate of 850 L/Hr. The cone voltage is 35 and the cone gas flow is 102 L/Hr.

Separation of the cabozantinib was achieved by the X-Bridge (2.1 × 100 mm, 3.5 µ) column and mobile phase composition of 0.2% formic acid: acetonitrile (40:60 v/v), pumped with 0.12 ml/min flow rate and injection volume is10 µL.

Preparation of standard solution

10 mg of cabozantinib standard pure powder was transferred into 10 ml of volumetric flask and diluted with 10 ml of methanol. This solution concentration is 1000 µg/ml.

Preparation of standard stock solution

0.1 ml of the above standard sample solution was transferred into 10 ml of volumetric flask and diluted with methanol and the resulting solution concentration is 10 µg/ml. This solution was considered a standard stock solution.

Preparation of sample solution

10mg of cabozantinib active pharmaceutical ingredient powder was transferred into 10 ml of volumetric flask and diluted with 10 ml of methanol. This solution concentration is 1000 µg/ml.

Preparation of sample stock solution

0.1 ml of the above sample solution was transferred into 10 ml of volumetric flask and diluted with methanol and the resulting solution concentration is 10 µg/ml. This solution was considered a sample stock solution.

Method validation

System suitability

The 100% level of cabozantinib standard solution (50ng/ml) was injected 6 times into LC-MS/MS system.

Linearity

The linearity method was determined in the range of LOQ levels (5 ng/ml) to 150% level (75 ng/ml) of cabozantinib samples were injected in the LC-MS/MS system. The regression coefficient value was found from the linearity calibration graph.

Sensitivity (LOD and LOQ)

Limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the following formulas.

\[
\text{LOD} = 3.3\sigma/s
\]
\[
\text{LOQ} = 10\sigma/s
\]

Whereas, as \(\sigma\) is the SD of the response (y-intercept) and \(S\) is the slope of the linearity plot.

Accuracy

The accuracy method was determined by calculating recovery values at different intervals of LOQ level, 50%, 100%, and 150% level. The % recovery and % RSD values were calculated.

Method precision (repeatability)

The method precision was determined at 100% level (50 ng/ml) of cabozantinib sample 6 replicates were injected and calculated the % RSD.
Intermediate precision

This method was performed by cabozantinib at 100% level (50 ng/ml) of 6 samples injected for different days and calculated the % RSD.

Assay of marketed formulation

Preparation of standard drug solution

10 mg of cabozantinib powder was transferred into 10 ml of a volumetric flask and diluted with methanol. The resulting solution concentration is 1000 µg/ml. Pipette out 0.1 ml of the above solution taken into 10 ml volumetric flask and diluted with methanol. The resulting solution concentration is 10 µg/ml. Transferred 0.5 ml of the above solution and dilute with methanol. The resulting concentration is 50 ng/ml and the percentage purity of cabozantinib was calculated.

Preparation of sample drug solution

Weighed 10 tablets and calculated the average weight of the tablet (10.02 mg). Weight equivalent to one tablet of powder was transferred into 10 ml of a volumetric flask and diluted with methanol. The resulting solution concentration is 1000 µg/ml. Pipette out 0.1 ml of the above solution taken into 10 ml volumetric flask and diluted with methanol. The resulting solution concentration is 10 µg/ml. Transferred 0.5 ml of the above solution and dilute with methanol. The resulting concentration is 50 ng/ml and the percentage purity of cabozantinib was calculated.

Solution stability

The analyte stability ST% indicates the part of the analyte in a sample that does not degrade before the authentic LC-MS analysis. Prepare 50 ng/ml sample from the stock solution and injected in LC-MS/MS system. The sample solution checks the stability.

Bracketing standard

Bracketing standards are used to analyze the samples, one run before and one after the samples. Prepare 50 ng/ml sample and inject LC-MS/MS system.

Results

MS detection

The predominant protonated precursor [M+H] + ions at m/z 502.27 were obtained from mass spectra of cabozantinib. The detection of ions was determined in MRM mode by transition pairs (precursor to product ion) of m/z 502.13–323.07 for cabozantinib. The molecular ion and product ion is shown in Fig. 2.

Figure 2. Mass spectra of cabozantinib molecular ion and production.
**Optimized method**

The cabozantinib method was optimized by using an X-Bridge (2.1 × 100 mm, 3.5μ) column and mobile phase composition of 0.2% formic acid: acetonitrile (40:60 v/v), using 0.12 ml/min flow rate and 10 µL of injection volume, with methanol used as diluents. The retention time was observed at 1.35 min. The optimized chromatogram was given in Fig. 3.

**Method validation**

**System suitability**

The system suitability parameters were evaluated and analyzed to check system performance by using 100% level (50 ng/ml) of the standard solution of cabozantinib. The system suitability % RSD was found to be 1.88. The results data are shown in Table 1.

**Sensitivity (LOD and LOQ)**

The LOD and LOQ of the cabozantinib 1.5 ng/ml and 5ng/ml of sample concentrations were determined. The LOD and LOQ values are shown in Table 3.

**Accuracy**

The accuracy % recovery values were found to be 86.66 – 114.57% and % RSD values were found to be 0.8–2.0%. The accuracy results data was shown in Table 4.

**Method precision (Repeatability)**

The %RSD values for method precision of the cabozantinib were found to be 1.70% for the 100% level concentration (50 ng/ml).

**Intermediate precision**

% RSD values of cabozantinib intermediate precision were found to be 1.82%. The method precision and intermediate precision results data were shown in Table 5.
The % purity of cabozantinib was found to be 99.82%. The assay results data was shown in Table 6.

### Solution stability

The sample solution stability was found to be 99.49%. The solution stability results data was given in Table 7.

### Bracketing standard

Bracketing standard % R.S.D was found to be 1.84%. The bracketing standard results data was given in Table 8.

### Assay of marketed formulation

The % purity of cabozantinib was found to be 99.82%. The assay results data was shown in Table 6.

### Discussion

The cabozantinib is mass detection was performed by positive ionization mode due to the drug's basic nature. The optimization of the chromatogram is achieved by X–bridge column which gives good results. Cabozantinib eluted before 2 min, RT in the existing technique was 1.34 min, and run time which proves it is economical due to the less consumption of mobile phase solvents. Linearity concentration was taken LOQ level and the correlation coefficient of the developed method was very nearest value to 1.0, which supports the sensitivity of the method. This accuracy method % RSD values within limits so that is this method is accurate. Method and intermediate precision were performed which proves that the developed method was precise. The

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**Table 4.** Accuracy data of cabozantinib.

<table>
<thead>
<tr>
<th>Accuracy levels</th>
<th>Concentration (ng/mL)</th>
<th>Peak area</th>
<th>Amount Recovery (ng/mL)</th>
<th>Mean % Recovery ± SD</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOQ</td>
<td>5</td>
<td>504.892</td>
<td>4.3</td>
<td>86.66 ± 1.15</td>
<td>1.3</td>
</tr>
<tr>
<td>50%</td>
<td>25</td>
<td>504.997</td>
<td>4.3</td>
<td>101.60 ± 2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>100%</td>
<td>50</td>
<td>514.299</td>
<td>4.4</td>
<td>106.40 ± 0.872</td>
<td>0.8</td>
</tr>
<tr>
<td>150%</td>
<td>75</td>
<td>1807.594</td>
<td>25.1</td>
<td>114.57 ± 1.46</td>
<td>1.2</td>
</tr>
</tbody>
</table>

**Table 5.** Method precision and intermediate precision of cabozantinib.

<table>
<thead>
<tr>
<th>Injection</th>
<th>Concentration (ng/mL)</th>
<th>Method Precision Peak area</th>
<th>Intermediate Precision Peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>3627.573</td>
<td>3334.442</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>3452.188</td>
<td>3486.185</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>3486.185</td>
<td>3478.166</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>3540.686</td>
<td>3364.151</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>3550.935</td>
<td>3427.706</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>3536.402</td>
<td>3381.354</td>
</tr>
<tr>
<td>Mean</td>
<td>3532.3281</td>
<td>3412.001</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>60.093</td>
<td>62.245</td>
<td></td>
</tr>
<tr>
<td>% RSD</td>
<td>1.70</td>
<td>1.82</td>
<td></td>
</tr>
</tbody>
</table>

**Table 6.** Assay data of cabozantinib.

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>Standard Peak area</th>
<th>Sample Peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3486.185</td>
<td>99.82</td>
</tr>
<tr>
<td>2</td>
<td>3540.686</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>3513.436</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>358.538</td>
<td></td>
</tr>
<tr>
<td>% RSD</td>
<td>1.09</td>
<td></td>
</tr>
</tbody>
</table>

**Table 7.** Solution stability data of cabozantinib.

<table>
<thead>
<tr>
<th>Concentration (ng/mL)</th>
<th>Peak area</th>
<th>% Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution sample</td>
<td>50</td>
<td>3364.151</td>
</tr>
<tr>
<td>System suitability 1st sample</td>
<td>50</td>
<td>3381.354</td>
</tr>
</tbody>
</table>

**Table 8.** Bracketing standard data of cabozantinib.

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>Concentration (ng/mL)</th>
<th>Peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>3427.706</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>3381.354</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>3364.151</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>3257.524</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>3334.442</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>3353.035</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>63.185</td>
</tr>
<tr>
<td>% RSD</td>
<td></td>
<td>1.88</td>
</tr>
</tbody>
</table>
marketed formulation assay value was found at 99.82%. All the method validation parameters were validated as per USFDA guidelines.

**Conclusion**

The present research work LC-MS method was successfully developed and validated for the estimation of cabozantinib. This method was economical and precise. The developed method could be practical and reliable to the quality control department of the pharmaceutical industry.

**Acknowledgement**

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**References**


