INTRODUCTION

Tabrecta, also known as capmatinib, is a medicine that is used to treat individuals who have metastatic non-small cell lung cancer (NSCLC) and whose tumors contain mutations that produces the mesenchymal-epithelial transition (MET) exon 14 skipping. This mutation may be identified using a US Food and Drug Administration (FDA) approved test. NSCLC is one of the numerous types of cancer that has been linked to abnormal stimulation of the c-Met gene. These mutant proteins have a diminished capacity to regulate negatively, leading to a pathological increase in their downstream activity of downstream.

Mutations produced in the skipping of MET exon 14 lead to the development of a mutant c-Met lacking the regulatory domain.

Capmatinib inhibits c-Met-mediated phosphorylation of proteins downstream signaling and the propagation and survival of tumor c-Met-dependent cells. It does this by inhibiting the phosphorylation of both wild-type and mutant variants of c-Met, which is triggered by the binding of its endogenous ligand, hepatocyte growth factor.

It is chemically named as 2-fluoro- N-methyl-4- {7-[quinolin-6-yl] methyl} imidazo [1,2-b] [1,2,4] triazin-2-yl} benzamide, with chemical formula and weight of C$_{23}$H$_{17}$FN$_{6}$O and 412.428 g·mol$^{-1}$ (Figure 1).

According to the published research on capmatinib, only two analytical approaches may be used to determine the amount of capmatinib using liquid chromatographic tandem mass spectrometric (LC-MS/MS) technique for the quantitation of capmatinib in plasma was developed and subjected for validation. Canagliflozin was employed as an internal standard (IS).

 Extraction of plasma was executed utilizing 5 mL of ethyl acetate solvent. Analysis was performed Zorbax ODS (50 mm × 2.1 mm × 3 µ) stationary phase at room conditions and a movable solvent composition of 0.1% HCOOH, acetonitrile and methyl alcohol (15:50:35). The flowing rate of the movable phase was 0.4 mL/min. Drug and IS were identified in positive mode of ionization with electrospray mode to get the mass transitions of (m/z): Capmatinib, 413.15/386.14 and canagliflozin (IS), 445.15/267.12. The correlation between capmatinib concentrations and their respective peak proportions to canagliflozin was a straight line over 0.2 to 3200 ng/mL concentration levels. Intra day and inter-day precisions were ≤5.18% for capmatinib. Inter and intra day bias were within the range of −4.17 to 4.53%. The mean measured extraction recovery of capmatinib was 99.23%. Recovery of IS was 98.31%. Capmatinib was subjected for long-term, freeze thaw, bench top, short-term stability, auto-sampler, dry extract, and stock solution stability at low QC and high QC levels and it was stable at all these conditions. The established technique can be utilized to regularly quantify of capmatinib in plasma samples in industries, forensic labs, and clinical research organizations.

Keywords: Capmatinib, Liquid chromatographic tandem mass spectrometric, Lung cancer, Stability, Validation.


How to cite this article: Hema, Panigrahi N. Stability Indicating an LC-MS Method Development and Validation for the Quantification of Capmatinib in Human Plasma. International Journal of Pharmaceutical Quality Assurance. 2023;14(4):953-958.

Source of support: Nil.

Conflict of interest: None

MATERIALS AND METHODS

Reagent Chemicals

Capmatinib and canagliflozin were obtained from Novartis and Dr Reddys, Hyderabad, India, respectively. HCOOH of analytical quality and methanol and acetonitrile of HPLC grade was purchased from JT Bakers in Hyderabad, India. The Milli-Q®RO system’s built-in water supply was used for
the production of the moveable system and washing solutions.

**LC-MS/MS Instrument**

Analytes were extracted from plasma samples with standard C-18 Sep-Pak tubes made by Waters Corporation, MA, USA. Chromatographic elution was done on an LC-MS/MS instrument with a Waters 2695 Alliances separating model (Waters corporation, USA) for sample introduction and delivery of solvent. Micro mass Quattromicro API triple quadruple mass spectrometric system connected to a ESI (Z-spray) source as detection system (Micromass, Manchester, UK). Estimation of drug and IS were executed on RP-Zorbax ODS (50 mm × 2.1 mm x 3 µ) column, which was combined with the symmetry C_{18} (3.9 mm × 20 mm, 5 µm) guard column. Mass Lynx software (Version 4.0) running on Microsoft Windows XP professionals was employed to handle the device, get the data, measure the signals-to-noise fraction, and integrate and smoothen of peaks.

**LC and Mass System Conditions**

A Analysis was performed using Zorbax ODS (50 mm × 2.1 mm x 3 µ) column at room temperature and a movable solvent composition of 0.1% HCOOH, methyl alcohol and acetonitrile (15:35:50). The flowing rate of movable phase was 0.4 mL/min. The electrospray ionization (ESI) source interface operated in our study’s positive ionization modes. Throughout the course of our research, the ESI interface was managed using the +ve ionisation method. The following characteristics were considered in this study: 2.5 kV for the capillary voltage, 200°C for the source temperature, and 450°C for the desolvation temperature. At a flow rate of 700 L/h, nitrogen was used as the desolvation gas, and at a flow rate of 50 L/h, nitrogen was utilized as the cone gas. In the collision cell, an argon flow rate of 0.17 mL/min was employed as the collision gas. Collision energies and cone voltages of 24V/18V and 30V/25V, respectively, were shown to be optimal for capmatinib and canagliflogin. Drug and IS were identified in positive mode of ionization with electrospray mode to get the mass transitions of (m/z): Capmatinib, 413.15/386.14 and canagliflogin(IS), 445.15/267.12.

**Quality Control and Standard Solutions**

Separate stock solutions of capmatinib and IS at a 1.0 mg/mL concentration were produced in the mobile phase. They underwent further diluting with plasma in order to get workable solutions that were 10 µg per mL. Eight calibration standards with concentrations ranging from 0.2 to 3200 ng/mL in human plasma and four quality control (QC) samples with concentrations of 0.2, 0.6, 1600, and 2400 ng/mL were produced. After vortexing for one minute and transferring 1.0 mL aliquots into borosilicate glass (13 x 100 mm) culture tubes, the calibration standards and quality control samples were kept at a temperature of -20°C until they were utilized.

**Sample Preparation**

It was decided to let aliquots of one milliliter of blank plasma, calibration curve, or quality control samples acclimate to room temperature. After adding 5 mL of ethyl acetate to each tube, the final concentration was adjusted to 750 ng/mL by adding 100 µL of the IS solution and then vortexing the mixture for 20 seconds. After the process was complete, the solution that was obtained was centrifuged at 3500 rpm for 15 minutes. After that, the clear solution that had formed at the top was separated out, and it was evaporated to dryness under a moderate stream of nitrogen in a heating block at 45°C. The residues were then reconstituted in 100 µL of mobile phase, placed into an auto-sampler vial, and 5 µL were injected into the LC-MS/MS system.

**Stability Studies**

Keeping the processed quality control samples in an auto sampler that was kept at a temperature of 5 ± 3°C for a time of 02 days, 20 hours, and 27 minutes allowed it to assess their...
LC-MS/MS Method for the Quantification of Capmatinib in Human Plasma

Table 2: Capmatinib precision and accuracy for inter-batch and intra-batch

<table>
<thead>
<tr>
<th>Concentration level (ng/ml)</th>
<th>Nominal concentration</th>
<th>Intra-batch</th>
<th>Inter-batch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>% CV</td>
<td>Bias</td>
</tr>
<tr>
<td>LLoQ 0.2</td>
<td>0.193 (0.01)</td>
<td>5.18</td>
<td>-3.50</td>
</tr>
<tr>
<td>LQC 0.6</td>
<td>0.617 (0.022)</td>
<td>3.56</td>
<td>2.87</td>
</tr>
<tr>
<td>MQC 1600</td>
<td>1672.56 (54.23)</td>
<td>3.24</td>
<td>4.53</td>
</tr>
<tr>
<td>HQC 2400</td>
<td>2317.84 (94.41)</td>
<td>4.07</td>
<td>-3.42</td>
</tr>
</tbody>
</table>

Table 3: Recovery of analytes after the extraction

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>C</th>
<th>D</th>
<th>%Recovery</th>
<th>%Mean recovery</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>LQC</td>
<td>1023</td>
<td>1006</td>
<td>98.34</td>
<td>99.23</td>
<td>1.46</td>
</tr>
<tr>
<td>MQC</td>
<td>2728234</td>
<td>2763155</td>
<td>101.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HQC</td>
<td>4092857</td>
<td>4013865</td>
<td>98.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IS</td>
<td>2545698</td>
<td>2528133</td>
<td>99.31</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

C, unextracted sample recoveries; D, extracted sample recoveries.

autosampler stability. By screening at -20°C for two months, long-term stabilities for the analyte and internal standard was processed at LQC and HQC. By holding at 2 to 8°C for 10 days, stock solution stability for analyte was processed at LQC and HQC levels. Three cycles of freeze and thaw stability processing were performed at -20°C and room temperature (Darshan et al., 2017). The drug and IS were treated for stability of short-term by placing at room conditions for 8 hours. A 17 hours, 28 minutes storage duration at room temperature was used to test the bench top condition of the quality control spiked sample solutions. The quality control spiked samples for dry extract stability was assessed for the duration of two days by placing those samples at -28 ± 5°C.14

Validation of the Analytical Method

The technique was validated in accordance with the accepted practices outlined in the US FDA guidelines (US FDA, 2001) on the validation of bioanalytical methods. Specificity, accuracy, linearity, precision, stability, and recovery were among the validation criteria.12-19

RESULTS AND DISCUSSION

Optimization of LC-MS/MS Parameters

The precursor and product ions of capmatinib and canagliflogin (IS) were measured by pumping the standard solution (1.0 µg/mL in acetonitrile) into an MS instrument utilizing a pump for syringes at a rate of flow of 20 µL. The standard mixture was used to determine both drugs' product and precursor ions. The collection of the mass spectrometry data was carried out using the MRM technique, with the + ve ion transitions mechanism chosen at the following m/z values: capmatinib, 413.15/386.14 and IS, 445.15/267.12, respectively. The source temperature was set at 200°C, capillary voltage at 2.5 kV, and desolvation temperature at 450°C. At a flow rate of 700 L/h, N2 was applied as the desolvation gas, and using flowing rate of 50 L/h, N2 was utilized as the cone gas. In the collision cell, an argon circulation rate of 0.17 mL/min was employed as the collision gas. Collision energies and cone voltages of 24V/18V and 30V/25V, respectively, were shown to be optimal for capmatinib and canagliflogin, respectively. Electrospray ionization was used to detect eluent in the positive ion mode. The transitions of mass to charge (m/z) used were capmatinib, 413.15/386.14 and canagliflogin (IS), 445.15/267.12.

Specificity

We tested six different types of blank plasma as well as ones with capmatinib or IS. No other pharmacological or endogenous component was shown to be co-eluting with capmatinib or IS. The chromatogram shown in Figure 2(A) is an example of a typical chromatogram of drug-free human plasma (blank), which was used in the creation of standards and QC samples. Figure 2B shows blank plasma that has been spiked with the IS at a concentration of 750 ng/mL.15-18

Linearity

The linear nature of the study was validated by conducting an analysis on a series of standard blends that included capmatinib.
and IS in human plasma at eight different concentrations ranging from 0.2 to 3200 ng/mL. A regression analysis was carried out on the peak proportions that corresponded to the IS as well as the concentrations.\textsuperscript{17,20} For capmatinib, the mean equations that were found were $y = 0.0007x - 0.0019$, and the $r^2$ value was 0.9998 ($n = 6$). By doing a back calculation to determine the concentration of capmatinib in human plasma based on the calibration curves, it was determined that the curves used for calibration were appropriate for use (Table 1). Every computed concentration was found to be substantially below the maximum permissible level. Figure 3 represents mean calibration curve of capmatinib. The LLOQ of capmatinib was 0.2 ng/mL (signaling-to-noise proportion>10) and was sufficient for accurate quantification of capmatinib in the analysis of plasma samples.

\textit{Precision and accuracy (bias)}

We assessed the precision and bias for four different QC concentrations (0.2, 0.6, 1600, and 2400 ng/mL). For capmatinib, the intraday precision ($n$ equaled 10) was 5.18\%, while the interday precision ($n$ equaled 20 and spanned three consecutive days) was 4.39\%. The intraday bias varied from a low of 3.50\% to a high of 4.53\%,\textsuperscript{19} while the interday bias ranged from 4.17 to 3.95\%. Table 2 provides a summary of the outcomes obtained. The LC-MS/MS chromatograms of low QC (0.6 ng/mL), MQC (1600 ng/mL), and high QC (2400 ng/mL) both spiked with IS (750 ng/mL) are shown in Figure 4.

\textit{Recovery}

Comparison of the peak heights of spiked-before-extraction samples and spiked-after-extraction samples (6 sets) was used to calculate the extraction recovery of capmatinib at three different concentrations (0.6, 1600, and 2400 ng/mL) and the IS at one concentration (750 ng/mL).\textsuperscript{18} This was done in order to determine the IS at one concentration. The average measured recovery of capmatinib after extraction was 99.23\% (Figure 4 and Table 3). Recovery of IS was 98.31\%.

\textit{Matrix effect}

Matrix effect was quantitatively evaluated by comparing peak heights of capmatinib in presence and absence of matrix components. It was measured at LQC and HQC levels by infusing the six sample solutions. The $\%CV$ findings at LQC and HQC level were 3.64 and 3.87, respectively (Table 4).

\textit{Carryover effect}

The auto sampler’s carryover impact was investigated by injecting mobile phase, aqueous ULoQ, LLoQ, and STD Blank samples. This experiment showed no carryover. The results are summarized in Table 5.

\textit{Dilution integrity}

The method’s dilution integrity was tested by spiking the plasma with a DIQC concentration of 9600 ng/mL from a DI spiking solution that was 3 times the ULoQ. DIQC sample is diluted 1/5 (1920 ng/mL) and 1/10 (960 ng/mL). Analyzing samples against calibration curve standards established the

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
\textbf{Analyte} & \textbf{Capmatinib} & \textbf{IS} \\
\hline
\textbf{Sample ID} & \textbf{Peak Area} & \textbf{Drug} \\
\hline
\textbf{Unextracted samples} & & \\
Mobile phase & 0 & 0 \\
Aqueous ULoQ & 5456254 & 2545657 \\
Mobile phase & 0 & 0 \\
Aqueous LLoQ & 363 & 2544952 \\
\hline
\textbf{Extracted samples} & & \\
STD Blank & 0 & 0 \\
ULoQ & 5457015 & 2545335 \\
STD Blank & 0 & 0 \\
LLoQ & 357 & 2545954 \\
\hline
\end{tabular}
\caption{Carry-over effect}
\end{table}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
\textbf{S.No} & \textbf{1/10\textsuperscript{th} Dilution (960 ng/mL)} & \textbf{1/5\textsuperscript{th} Dilution (1920 ng/mL)} \\
\hline
1 & 954.3279 & 1911.12 \\
2 & 956.0041 & 1931.762 \\
3 & 949.9021 & 1900.848 \\
4 & 956.1513 & 1924.479 \\
5 & 966.3853 & 1935.218 \\
6 & 968.6088 & 1927.05 \\
\hline
\textbf{n} & 6 & 6 \\
\textbf{Mean} & 958.56 & 1921.75 \\
\textbf{SD} & 6.68 & 12.02 \\
\textbf{\%CV} & 0.69 & 0.63 \\
\textbf{\%Mean Accuracy} & 99.85 & 100.09 \\
\hline
\end{tabular}
\caption{Dilution integrity}
\end{table}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4}
\caption{Capmatinib chromatograms at A) Low-QC B) Medium-QC and C) HQC level.}
\end{figure}
LC-MS/MS Method for the Quantification of Capmatinib in Human Plasma

IJPQA, Volume 14 Issue 4, October - December 2023

Page 957

precision and accuracy of 1/5 and 1/10 dilution integrity standards. The results are summarized in the Table 6.

**Stability**
The stability of capmatinib and the IS in unprocessed and processed plasma sample at LQC an HQC levels were examined. Table 7 summarizes the findings of the stability investigations.

**CONCLUSION**
A precise and linear liquid chromatographic tandem mass spectrometric (LC-MS/MS) technique for the determination of capmatinib in plasma was developed and subjected for the validation. Canagliflozin was employed as an internal standard (IS). Extraction of plasma was executed utilizing 5 mL of ethyl acetate solvent. Analysis was performed Zorbax ODS (50 mm × 2.1 mm x 3 µ) stationary phase at room conditions and a movable solvent composition of 0.1% HCOOH, acetonitrile and methyl alcohol (15: 50:35). The flowing rate of movable phase was 0.4 mL/min. Drug and IS were identified in positive mode of ionization with electrospray mode to get the mass transitions of (m/z): Capmatinib, 413.15/386.14 and canagliflozin (IS), 445.15/267.12. The correlation between capmatinib concentrations and their respective peak proportions to canagliflozin was a straight line over 0.2 to 3200 ng/mL concentration levels. Intra day and inter-day precisions were ≤5.18% for capmatinib. Inter and intra day bias were within the range of −4.17 to 4.53%. Mean measured extraction recovery of capmatinib was 99.23%. Recovery of IS was 98.31%. Capmatinib was tested for stability in a variety of circumstances, including long-term, bench top, freeze-thaw, short-term, auto-sampler, dry extract, and stock solution stability at low QC and high QC levels, and it was shown to be stable in each of these settings. The developed method may be used to routinely measure capmatinib in plasma samples in companies, forensic laboratories, and clinical research organizations. This is possible since the method has been validated.

**REFERENCES**

5. Archana Upadhya, Khushwant S. Yadav and Ambikanandan

---

**Table 7:** Capmatinib stability findings

<table>
<thead>
<tr>
<th>Stability study</th>
<th>Concentration level</th>
<th>Comparison samples area mean</th>
<th>Stability samples area mean</th>
<th>%Mean stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long term stability</td>
<td>HQC</td>
<td>4084862</td>
<td>4063080</td>
<td>99.47</td>
</tr>
<tr>
<td></td>
<td>LQC</td>
<td>351</td>
<td>335</td>
<td>95.41</td>
</tr>
<tr>
<td>Short term stability</td>
<td>LQC</td>
<td>4086959</td>
<td>4074689</td>
<td>99.69</td>
</tr>
<tr>
<td></td>
<td>HQC</td>
<td>4085191</td>
<td>4116893</td>
<td>100.78</td>
</tr>
<tr>
<td>Bench top stability</td>
<td>LQC</td>
<td>355</td>
<td>337</td>
<td>94.93</td>
</tr>
<tr>
<td></td>
<td>HQC</td>
<td>4085191</td>
<td>4097829</td>
<td>100.31</td>
</tr>
<tr>
<td>Freeze thaw stability</td>
<td>LQC</td>
<td>356</td>
<td>335</td>
<td>93.93</td>
</tr>
<tr>
<td></td>
<td>HQC</td>
<td>4085191</td>
<td>4030468</td>
<td>98.66</td>
</tr>
<tr>
<td>Dry extract stability</td>
<td>LQC</td>
<td>349</td>
<td>333</td>
<td>95.37</td>
</tr>
<tr>
<td></td>
<td>HQC</td>
<td>4085191</td>
<td>4146175</td>
<td>101.49</td>
</tr>
<tr>
<td>Stock solution stability</td>
<td>LQC</td>
<td>357</td>
<td>338</td>
<td>94.78</td>
</tr>
<tr>
<td></td>
<td>HQC</td>
<td>4085191</td>
<td>4039762</td>
<td>98.88</td>
</tr>
<tr>
<td>Auto sampler stability</td>
<td>LQC</td>
<td>355</td>
<td>337</td>
<td>94.93</td>
</tr>
</tbody>
</table>


8. Ulrike Glaenzel, Yi Jin, Regine Hansen, Kirsten Schroer, Gholamreza Rahmanzadeh, Ulrike Pfaar, Jan Jaap van Lier, Hubert Borell, Axel Meissner, Gian Camenisandha Sylvia Zhao. Absorption, Distribution, Metabolism, and Excretion of Capmatinib (INC280) in Healthy Male Volunteers and In Vitro Aldehyde Oxidase Phenotyping of the Major Metabolite. Drug Metabolism and Disposition. 2020;48(10):873-885. DOI: https://doi.org/10.1124/dmd.119.090324


11. Xiaoqiang Fan, Guanghu Yang, Wenjuan Cui, Qin Liu, Zhaolong Zhang, Zhikun Zhang. Development and full validation of an LC–MS/MS methodology to quantify capmatinib (INC280) following intragastric administration to rats, 2020; 34(3):e4768. DOI: 10.1002/bmc.4768


