RESEARCH ARTICLE

Stability Indicating an LC-MS/MS Method Development and Validation for the Quantification of Capmatinib in Human Plasma

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ABSTRACT

A linear and accurate liquid chromatographic tandem mass spectrometric (LC-MS/MS) technique for the quantitation of capmatinib in plasma was developed and subjected for validation. Canagliflogin 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 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 canagliflogin (IS), 445.15/267.12. The correlation between capmatinib concentrations and their respective peak proportions to canagliflogin 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 quantitfy of capmatinib in plasma samples in industries, forensic labs, and clinical research organizations.

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

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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 an food and drug administration (FDA) approved test.[1-3] 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.[4] 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.[5] 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.[6-8]

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 $C_{23}H_{17}FN_6O$ and 412.428 $g \cdot mol^{-1}$ (Figure 1).[9,10]

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).[6,11] In light of this, the purpose of the current work was to establish an LC-MS/MS approach for the quantitative analysis of capmatinib in plasma samples that was both more accurate and precise.

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

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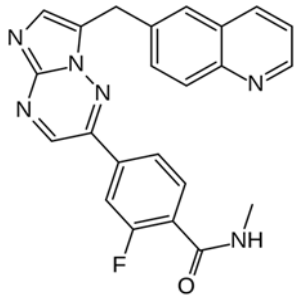

Figure 1: Structure of Capmatinib

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

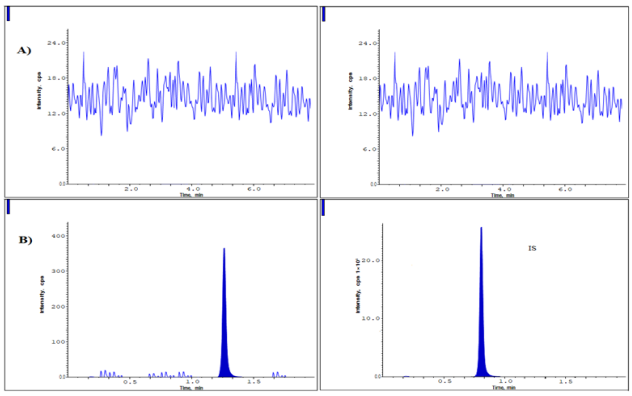

Figure 2: Capmatinib chromatograms at A) Blank and B) LLoQ levels.

Table 1: Calibration standards for capmatinib

L S-ID	*Concentrations (ng/mL)*	*Average response*	*IS response*	*Analyte/IS response*
LS -1	0.2	394	2545954	0.000155
LS -2	40	70200	2545003	0.027583
LS -3	210	375050	2546021	0.147308
LS -4	450	695641	2545657	0.273266
LS -5	900	1565418	2544952	0.615107
LS -6	1600	2724235	2545259	1.070317
LS -7	2400	4096541	2544058	1.610239
LS -8	3200	5458456	2545335	2.144494

LS: Calibration standard.

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.[12] 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.[13] 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

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

Concentration level	Nominal concentration (ng/ml)	Intra-batch			Inter-batch		
		Mean (SD)	% CV	% Bias	Mean (SD)	% CV	% Bias
LLoQ	0.2	0.193 (0.01	5.18	-3.50	0.207 (0.009)	4.33	3.95
LQC	0.6	0.617 (0.022)	3.56	2.87	0.619 (0.024	3.88	3.20
MQC	1600	1672.56 (54.23)	3.24	4.53	1533.25 (46.87)	3.06	-4.17
HQC	2400	2317.84 (94.41)	4.07	-3.42	2324.88 (102.08)	4.39	-3.13

Table 3: Recovery of analytes after the extraction

Concentrations	C	D	%Recovery	%Mean recovery	%RSD
LQC	1023	1006	98.34	99.23	1.46
MQC	2728234	2763155	101.28		
HQC	4092857	4013865	98.07		
IS	2545698	2528133	99.31		

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

Table 4: Matrix effect of capmatinib

S. No	Low QC			High QC		
	Peak area with matrix	Peak area without matrix	Matrix factor	Peak area with matrix	Peak area without matrix	Matrix factor
1	297	312	95.37	4214350	4092000	102.99
2	343	336	102.34	3897220	4092000	95.24
3	290	308	94.39	3817017	4092000	93.28
4	343	351	97.82	4170975	4092000	101.93
5	344	334	103.07	3963920	4092000	96.87
6	337	347	97.25	3980288	4092000	97.27
Mean			98.37			97.93
± SD			3.58			3.79
%CV			3.64			3.87

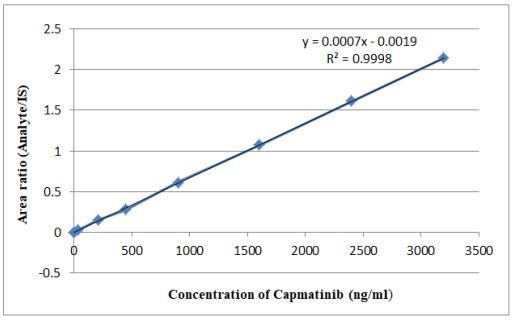

Figure 3: Linearity of capmatinib

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.[17,20] For capmatinib, the mean equations that were found were y = 0.0007x - 0.0019, and the r2 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.

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.[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.

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).[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%.

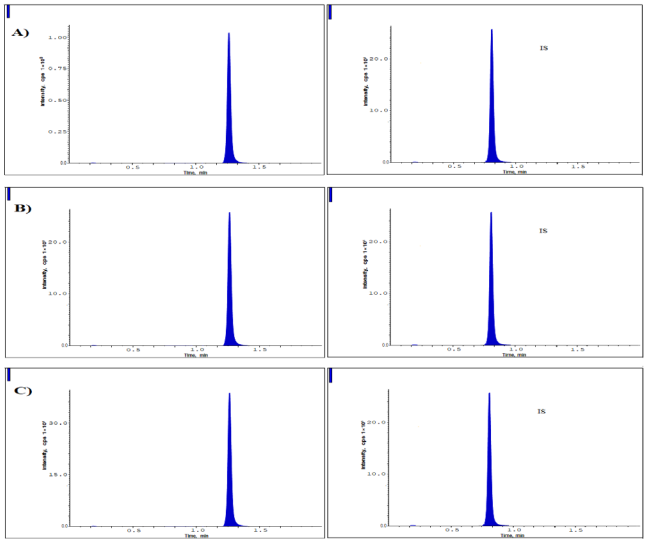

Figure 4: Capmatinib chromatograms at A) Low-QC B) Medium-QC and C) HQC level.

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).

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.

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

Table 5: Carry-over effect

Analyte	*Capmatinib*	
Sample ID	Peak Area	
	Drug	ISTD
Unextracted samples		
Mobile phase	0	0
Aqueous ULoQ	5456254	2545657
Mobile phase	0	0
Aqueous LLoQ	363	2544952
Extracted samples		
STD Blank	0	0
ULoQ	5457015	2545335
STD Blank	0	0
LLoQ	357	2545954

Table 6: Dilution integrity

S.No	*Back calculated concentration (ng/mL)*	
	1/10th Dilution (960 ng/mL)	*1/5th Dilution (1920 ng/mL)*
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
n	6	6
Mean	958.56	1921.75
SD	6.68	12.02
%CV	0.69	0.63
%Mean Accuracy	99.85	100.09

Table 7: Capmatinib stability findings

Stability study	*Concentration level*	*Comparison samples area mean*	*Stability samples area mean*	*%Mean stability*
	HQC	4084862	4063080	99.47
Long term stability	LQC	351	335	95.41
	HQC	4086959	4074689	99.69
Short term stability	LQC	349	337	96.47
	HQC	4085191	4116893	100.78
Bench top stability	LQC	355	337	94.93
	HQC	4085191	4097829	100.31
Freeze thaw stability	LQC	356	335	93.93
	HQC	4085191	4030468	98.66
Dry extract stability	LQC	349	333	95.37
	HQC	4085191	4146175	101.49
Stock solution stability	LQC	357	338	94.78
	HQC	4085191	4039762	98.88
Auto sampler stability	LQC	355	337	94.93

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.[20]

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. Canagliflogin 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 canagliflogin (IS), 445.15/267.12. The correlation between capmatinib concentrations and their respective peak proportions to canagliflogin 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.

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