RESEARCH ARTICLE

Fast and Sensitive Bioanalytical Method Development and Validation for Determination of Capmatinib in Human Plasma Using HPLC-MS/MS

Nameera Jabeen[1*], M Akiful Haque[2]

[1]*Department of Pharmaceutical Analysis, Anurag University, Venkatapur, Hyderabad, Telangana, India.*
[2]*Department of Pharmaceutical Analysis, Vishnu Institute of Pharmaceutical Education and Research, Medak, Telangana, India.*

Received: 22nd January, 2024; Revised: 16th March, 2024; Accepted: 27th May, 2024; Available Online: 25th June, 2024

ABSTRACT

The term "anticancer drugs" evokes memories of a time when the development of medications in that category was still necessary. Capmatinib, for example, is a kinase inhibitor that targets the C-Met receptor tyrosine kinase in the treatment of non-small cell lung cancer, which involves tissue repair and organ regeneration.[1] Thus, utilizing liquid chromatography-tandem mass spectroscopy (LC-MS/MS) in human plasma in accordance with United States Food and Drug Administration (USFDA) bioanalytical technique validation criteria, a quick, simple, specific, dependable, and sensitive approach was created using deucravacitinib as an internal standard. Capmatinib and the internal standard were separated using liquid-liquid extraction. The extracted sample run through a chromatographic system with an ACE-C18 column (4.6 × 100 mm, 5 µm); the mobile phase consisted of methanol and 2 mM ammonium formate in an 80:20 ratio at a flow rate of 1.00 mL/min. The system operates for three minutes in multiple reaction monitoring mode at the ABSCIEX API 4000 mass spectrometer using electron spray ionization. For capmatinib, the ion transitions are 413.10 to 382.10 and 426.30 to 358.20 for deucravacitinib, with a concentration range of 5.00 to 4000 ng/mL, the accuracy, precision, linearity, selectivity, and selectivity were validated and found to be within the acceptability limits.

Keywords: Capmatinib, Deucravacitinib, Liquid-liquid extraction, Human plasma, Methanol, Ammonium formate.

International Journal of Pharmaceutical Quality Assurance (2024); DOI: 10.25258/ijpqa.15.2.50

How to cite this article: Jabeen N, Haque MA. Fast and Sensitive Bioanalytical Method Development and Validation for Determination of Capmatinib in Human Plasma Using HPLC-MS/MS. International Journal of Pharmaceutical Quality Assurance. 2024;15(2):861-866.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Capmatinib is an anticancer medication that belongs to the heterodimeric receptor tyrosine kinase subfamily and is a strong ATP-competitive inhibitor of the prooncogene. Mesenchymal epithelial transition is employed in cellular and biological tests involving a variety of tumor types. Lung cancer is the second kind of cancer in the world to be the cause of mortality among all cancer types.[2] Lung cancer is classified as two types based on its histopathology is: Small lung cell cancer represents 10% of lung cancer and non-small cell lung cancer represents 90% of lung cancers with various subtypes of oncogenes.[3,4] The development of innovative cancer drugs has progressed very slowly, while molecular targeting methods have advanced significantly, improving patient prognoses significantly.[5]

Capmatinib is approved by United States Food and Drug Administration (USFDA) in the year 2020 for the treatment of non-small cell lung cancer (NSCLC), which is a highly MET inhibitor and also a promising drug with clinical outcomes[6]. Despite its high potentiality, there is much less research on bioanalytical quantification, so the present study will focus on the quantification of capmatinib in human plasma[7,8] by hyphenated technique, which makes it simple, fast, sensitive and specific method and validating it.

The main benefit of liquid chromatography combined with MS/MS detection is its excellent specificity and sensitivity, but its disadvantage is the lack of reliability in matrix effect-related results. To address the problems of ionization efficiency, injection volume, recovery, and fluctuations in matrix effect, an internal standard is useful tool for increasing performance. The selection of an appropriate internal standard should be based on the physicochemical characteristics of the target analyte in order to account for any matrix effects-related variations in ionization efficiency. Deucravacitinib is a free base with fundamental functional groups that can be extracted and

**Author for Correspondence: r.nameera@gmail.com*

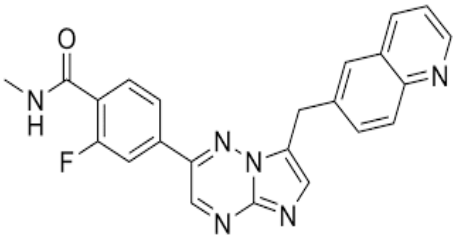

Figure 1: Structure of capmatinib

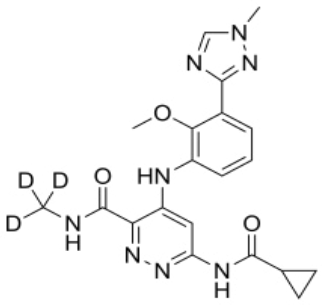

Figure 2: Structure of deucravacitinib

ionized; nevertheless, because of its non-polar nature, matrix effects are a potential issue. The medications capmatinib and deucravacitinib have a lipophilic structure which is shown in Figures 1 and 2.

MATERIALS AND METHODS

Instrumentation

The mass spectrometer used was the SCIEX API 4000 (from AB Sciex LLC, Framingham City, MA, USA), interfaced by Turbo ion spray with a Shimadzu prominence LC (software version: Analyst 1.6.3). The ACE C18 column (100 * 4.6 mm, 5 µm) was used to optimize the HPLC-MS/MS process.

Materials

Dr. Reddy's Laboratories in Telangana, India, provided gift samples of deucravacitinib, and capmatinib, which were purchased from Simson Pharma. Merck (Darmstadt, Germany) provided the HPLC grade methanol, the AR grade ammonium formate and, tertiary butyl methyl ether and the HPLC water. Human plasma was acquired from Om blood bank located in Pune, Maharashtra, India, using K2 EDTA as an anticoagulant.

Chromatographic Circumstances

The mobile phase was a mixture of 2 mM ammonium formate (80:20) and methanol, with a flow rate of 1.00 mL/min. Quantitation was carried out using a positive ion mode mass spectrometer. Using isocratic elution and a 2 µL injection volume, a quick and sensitive multiple-reaction monitoring methods was created and analyzed for the measurement of capmatinib in human plasma. The approach for liquid-liquid extraction is used in the method, and deucravacitinib is used as an internal standard. Methanol was used to prepare the 100 µg/mL sample in order to adjust the mass spectroscopy parameters.

Preparation of Stock Solutions

The capmatinib and deucravacitinib stock solution was prepared by combining 1-mg of standard in 1-mL of methanol to obtain 1-mg/mL stock and kept in the refrigerator. Further the quality control sample was prepared by using the standard stock solution.

Preparation of Working Dilutions

The samples were spiked with 0.980 mL of plasma with 0.02 mL dilution of analyte. The eight standards ranging from 5 to 4000 ng/mL was prepared to obtain a linear graph. The sample solutions were prepared by spiking human plasma 9.8 mL and a standard of 0.2 mL for accuracy and precision determinations. The final concentrations of QC samples of capmatinib were 5 ng/mL as LLoQ, 14 ng/mL as LQC, 1508 ng/mL as MQC and 2820 ng/mL as HQC.

Extraction of Capmatinib and Deucravacitinib from Human Plasma

Liquid-liquid extraction techniques were chosen for their simplicity, feasibility and recovery. The choice of solvent is based on the hydrophobicity of analytes. Non-polar samples were selected for extraction of aqueous plasma samples like hexane, tertiary butyl methyl ether, and cyclohexane, etc.., were used. The best solvent selected is methyl tertiary butyl ether. In the proposed method, liquid-liquid extraction was selected to isolate Capmatinib and Deucravacitinib from human plasma[8] 0.100 mL of K2EDTA plasma is aliquot into RIA (polypropylene) vials 0.050 mL of internal standard and 0.1 mL of 2mM ammonium formate was added to all samples except blank and vortexed. All samples were mixed with 1-mL of tertiary butyl methyl ether and, in addition, ammonium formate followed by vortexed. After that, all samples were vortexed for ten minutes at 2500 rpm, and the supernatant was added to new RIA vials. The samples were reconstituted with 0.5 mL of mobile phase and vortexed after being evaporated at 400°C under nitrogen gas until dry. For analysis, samples were moved to an autosampler vial. Then add 2 µL of the sample to the chromatographic system for examination.

HPLC-MS/MS Technique Validation

According to USFDA criteria for the development and validation of new bioanalytical methods, the validation of this technique was established by calculating the linearity, sensitivity, selectivity, accuracy, precision, recovery, matrix effect, and stability.[9,10]

Creation of an Innovative Method

Acetate and formate buffers with varying pH were used to run a number of trials. The ammonium formate and methanol ratio of 80:20 was selected as the mobile phase since the eluted peak showed good characteristics for both internal standard and capmatinib. According to USP criteria, this technique yields a peak with a retention time of 1.15 minutes for capmatinib observed in Figure 3 and 1.35 minutes for deucravacitinib,

Table 1: Optimizatized data for capmatinib and deucravacitinib using innovative method

Name of the sample	*Q1 - Mass*	*Q3 -Mass*	*Dwell*	*DP*	*EP*	*CE*	*CXP*
Capmatinib	413.100	382.100	200.00	100	10	34	12
Deucravacitinib	426.300	358.200	200.00	110	10	32	12

Table 2: Parameters

Parameter	*Value*
CAD (psi)	6.00
CUR (psi)	20.00
GS1 (psi)	40.00
GS2 (psi)	50.00
Ion spray voltage (V)	5500.00
Temperature (°C)	500.00
Scan type	MRM
Polarity	Positive

Table 3: Optimizatized method development parameters for capmatinib

S. No	*Parameters*	*Details*
1	Column	Ace C 18, 4.6*100 MM, 5 µm
2	Mobile phase	Mobile Phase (80: 20) of proportion A: Methanol B: Buffer (2 mM ammonium formate)
3	Injection volume	2.0 µL
4	Flow rate	1-mL/min
5	Column temperature	Ambient
6	Run time	3 minutes
7	Extraction procedure	Liquid liquid extraction
8	Detector	Tandem mass spectrometer

observed in Figure 4 and it satisfies all necessary features.[11] Table 1 and 2 represents the optimized data for innovative method.

Selectivity

Blank plasma from six different plasma lots was used to execute the selection process without spiking with capmatinib. Each blank plasma sample was subjected to an LLoQ test and analysis.

Linearity

Using a weighting factor of 1/X2, plasma samples with eight distinct concentrations ranging from 5 to 4000 ng/mL, were measured. Plotting the area ratios of each sample concentration against the capmatinib concentration was done. Less squares regression analysis was used to fit the points linearly.

Accuracy and Precision

The six repeated samples were injected to determine accuracy as well as intra- and inter-day precision.

Recovery

Six replicate injections of capmatinib were used to determine the extraction recoveries at LQC, MQC, and HQC levels.

Matrix Effect

Six distinct lots of human plasma were used, and each plasma LQC and HQC level's extracted post-extracted blanks were made and examined.

Stability

Two distinct QC standards, LQC and HQC, were used to determine the stabilities of the analyte following extraction from human plasma. The samples were maintained under autosampler normal circumstances for 24 hours at 10°C, for 12 hours at room temperature on the top of the bench, and for 25 hours in refrigerated storage at 2 to 8°C. This allowed for the stability of the standards to be acquired.

Chromatography

Measurement of capmatinib in human plasma was observed by comparing with LLoQ of plasma samples and deucravacitinib. The optimized parameters of capmatinib in human plasma were noticed in Table 3.

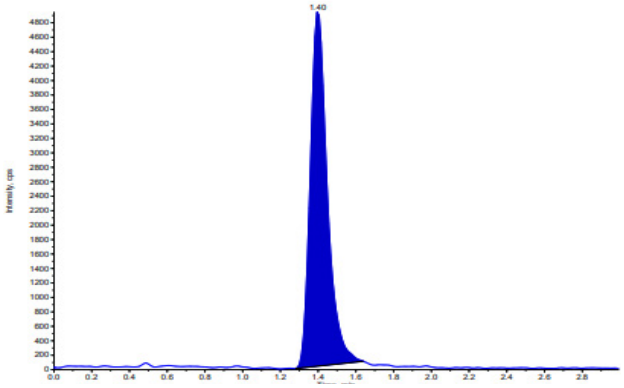

Figure 3: MS Peak of Capmatinib with Retention time

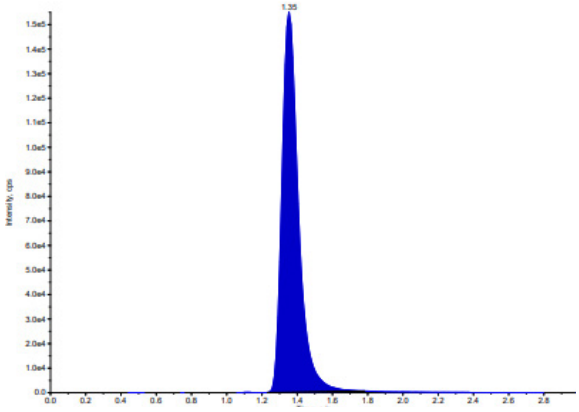

Figure 4: MS Peak of Deucravacitinib with Retention time

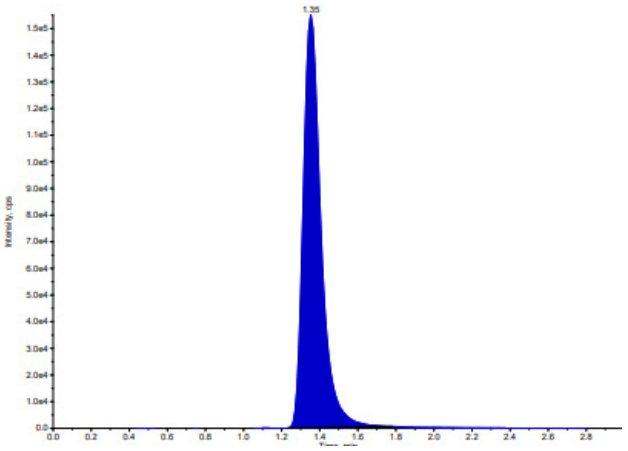

Figure 5: MS peak of deucravacitinib with retention time

Table 4 : Linearity table of capmatinib

S. No	*Concentration of capmatinib (ng/mL)*	*Peak: Area*
STD 1	5.033	0.008
STD 2	10.065	0.019
STD 3	30.969	0.054
STD 4	103.231	0.187
STD 5	448.830	0.785
STD 6	1496.101	2.566
STD 7	2992.2.03	4.619
STD 8	3740.253	5.836
Slope		0.00169
Intercept		
Correlation coefficient		
0.00033		
0.9985		

RESULTS AND DISCUSSION

Mass Spectrometry

Figure 5 shows the mass characteristics of the compound in positive ionization mode with excellent spectra. The ionization transition values of analyte and internal standard were 413.10 and 426.30 of Q1 mass and 382.10 and 358.20 were of daughter mass, respectively.

Specificity

The resolved analyte peaks verified the method's specificity, and there was no interference between the analyte peaks and the plasma constituents shown in Figure 6. Less than 20% of the corresponding LLoQ's area and 5% of the internal standard retention time were occupied by blank plasma sample interference during the analyte retention period.

Linearity

The method's linearity was confirmed by injecting eight calibration concentrations into the range of 5 to 4000 ng/mL shown in Table 4. The range of the calibration standards was between 85 and 115%. The obtained mean correlation coefficient was 0.997 represented in Figure 7. Therefore, capmatinib may be easily estimated within this concentration, and 5 ng/mL was established to be the lower limit of quantification (LLoQ) for capmatinib.

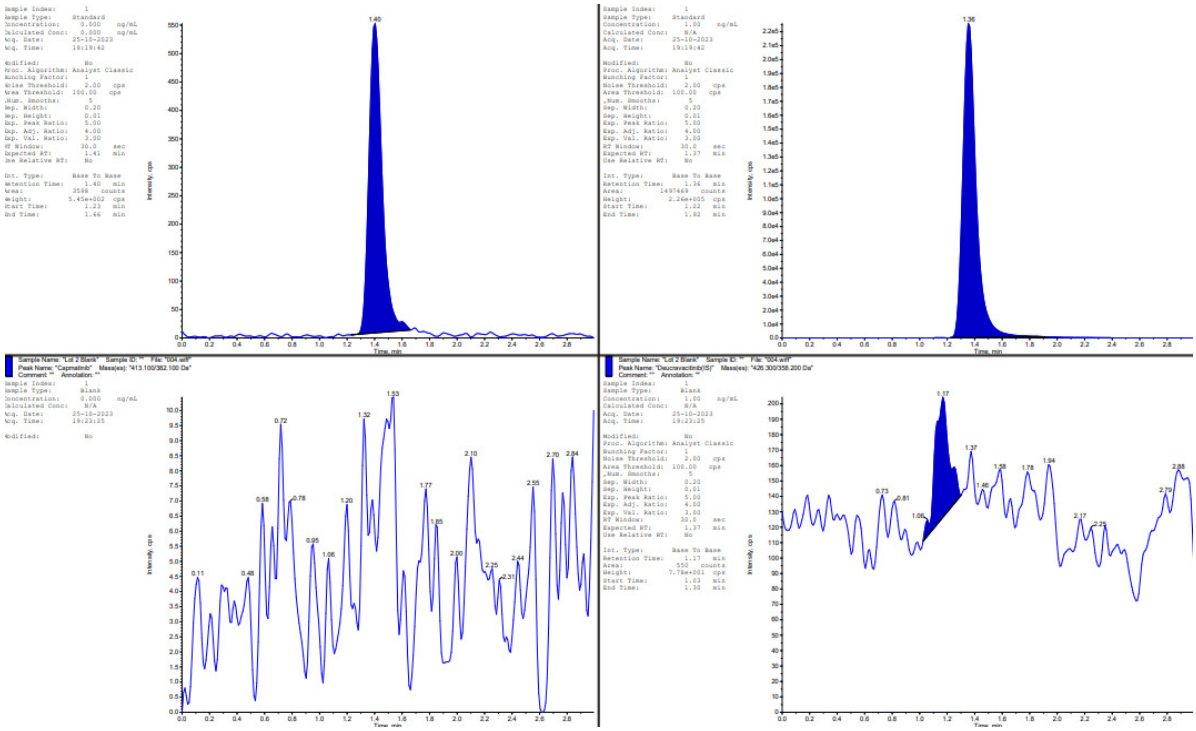

Figure 6: Selectivity chromatogram of capmatinib and deucravacitinib

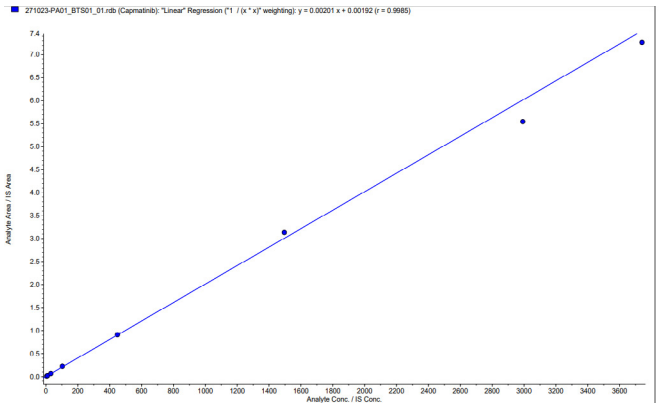

Figure 7: Calibration curve of capmatinib

Table 5: Accuracy and precision

Concentration	*LLQC (5.03 ng/mL)*	*LOQC (14.537 ng/mL)*	*MQC (1503.818 ng/mL)*	*HQC (2810.875 ng/mL)*
Accuracy	98.31	103.9	98.79	92.99
Intraday precision	101.46	108.09	100.88	101.02
Interday precision	100.14	106.71	103.22	108.88

Table 6: Recovery of capmatinib

S. No	*QC Samples*	*%Recovery*
1	LQC	83.02
2	MQC	64.85
3	HQC	64.00
4	Overall	70.623

Table 7: Matrix factor of capmatinib

Parameter	*Analyte*		*Internal standard*	
Concentration	LQC	MQC	LQC	MQC
Matrix Factor	1.07	1.42	1.01	1.00

Table 8: Stability of capmatinib under varied conditions

Standard (ng/mL)	*Bench top stability*	*Auto sampler stability*	*Refrigerator stability*
LQC	106.67	108.36	107.86
MQC	110.66	108.04	102.58

Accuracy and Precision

By injecting 12 replicate runs, the intra-day and inter-day precision and accuracy of this approach were developed. The findings range from 98.3 to 108.88%. Accuracy is defined as the ratio of the mean value to the true value collected and given as a percentage.

The sensitivity of capmatinib studied at LLQC is 5.03 ng/mL with 98.31% recovery is reasonable. The six sets of precision for three QC standards within the intraday) and interday and found to be reliable against the linear curve Both accuracy and precision data represented in Table 5.

Recovery

The percentage recovery for each QC samples was 64.85, 64.00, and 83.02%, while the overall recovery was determined to be 70.623% shown in Table 6. It was determined that the recovery was accurate, appropriate, and repeatable.

Matrix Effect

No significant matrix effect is observed At LQC and HQC levels and the matrix factor for internal standard was found to be from 0.96 to 1.50 represented in Table 7.

Stability

The concentration of capmatinib does not significantly decrease following auto sampler storage, short-term storage, or refrigerator stability. The results validated that capmatinib had reached its optimal stability shown in Table 8. Two distinct concentrations' recovery percentages were calculated.

CONCLUSION

The currently developed and validated a novel method for quantitating of capmatinib in human plasma by following the liquid liquid extraction technique where the concentration range is 5 to 4000 ng/mL. The developed method shows optimal selectivity and sensitivity with significant recovery with acceptable range, accuracy, and precision. Recovery for LQC, MQC and HQC have seemed to be appropriate, and the capmatinib is considered to be stable when it is subjected to the autosampler, refrigerator and stock solution stability. Within the short run time, the capmatinib shows exceptional precision, accuracy, sensitivity and recovery. All the multiple reaction conditions are properly maintained which is best suitable for method development to be carried out. The methods are less time-consuming, rapid analysis, high throughput, selective and can be used for routine analysis for same matrices.

ACKNOWLEDGMENTS

The Authors are thankful to Indiglobal Labs Pvt Ltd.., for their valuable support in carrying out the research work.

REFERENCES

1. Drug Bank Release, Version 5.1.9; 2022. Available online: https://www.drugbank.ca.
2. Bioanalytical method validation, European Medicines Agency, 2012,https://www.ema.europa.eu/en/bioanalytical-method-validation, accessed 4 November 2020.
3. Kim S, Kim TM, Kim DW, Kim S, Kim M, Ahn YO, Keam B, Heo DS, Acquired Resistance of MET-Amplified Non-small Cell Lung Cancer Cells to the MET Inhibitor Capmatinib, Cancer Research and Treatment , 2019 Jul;51(3):951-962. doi: 10.4143/crt.2018.052.
4. Torre LA, Siegel RL, Jemal A. Lung cancer statistics. Lung cancer and personalized medicine: current knowledge and therapies. 2016:1-9, doi.org/10.1007/978-3-319-24223-1_1 .
5. Chmielecki J, Foo J, Oxnard GR, Hutchinson K, Ohashi K, Somwar R, Wang L, Amato KR, Arcila M, Sos ML, Socci ND. Optimization of dosing for EGFR-mutant non–small

cell lung cancer with evolutionary cancer modeling. Science translational medicine. 2011 Jul 6;3(90):90ra59-, doi:10.1126/scitranslmed.3002356

6. Katayama R, Khan TM, Benes C, Lifshits E, Ebi H, Rivera VM, Shakespeare WC, Iafrate AJ, Engelman JA, Shaw AT. Therapeutic strategies to overcome crizotinib resistance in non-small cell lung cancers harboring the fusion oncogene EML4-ALK. Proceedings of the National Academy of Sciences. 2011 May 3;108(18):7535-7540., DOI: 10.1073/pnas.1019559108
7. Shenouda SK, Alahari SK. MicroRNA function in cancer: oncogene or a tumor suppressor?. Cancer and metastasis reviews. 2009 Dec;28:369-378., DOI: 10.1007/s10555-009-9188-5
8. Vansteenkiste JF, Van de Kerkhove C, Wauters E, Van Mol P. Capmatinib for the treatment of non-small cell lung cancer. Expert review of anticancer therapy. 2019 Aug 3;19(8):659-671, doi.org/10.1080/14737140.2019.1643239
9. Fan X, Yang G, Cui W, Liu Q, Zhang Z, Zhang Z. Development and full validation of an LC–MS/MS methodology to quantify capmatinib (INC280) following intragastric administration to rats. Biomedical Chromatography. 2020 Mar;34(3):e4768, DOI: 10.1002/bmc.4768
10. United State of America–Food and Drug Administration. Bioanalytical Method Validation Guidance for Industry. 2018. Available online: https://www.fda.gov/ucm/groups/fdagov-public/@fdagov-drugsgen/documents/document/ucm070107.pdf
11. Zhou C, Tian J, Lin P, Liu T, He A, Fang L, Sun L. Quantitation of capmatinib, a mesenchymal-epithelial transition factor inhibitor by UPLC–MS/MS in rat plasma and its application to a pharmacokinetic study. Bioanalysis. 2020 Mar;12(5):285-293, doi.org/10.4155/bio-2020-0011