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Development and Validation of Reverse-Phase High-Performance Liquid Chromatographic Method for Quantitative Estimation of Capmatinib in Tablet Dosage Form

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ABSTRACT

The work aims to develop a simple, accurate, precise, and reproducible Reverse Phase High-Performance Liquid Chromatographic (RP-HPLC) method for the quantitative determination of antineoplastic drug Capmatinibin bulk as well as in its tablet formulation. Chromatography was carried out on Zorbax Eclipse XDB- C18 (4.6 mm X 150 mm, 5 µm) column with a mobile phase consisting of phosphate buffer and methanol in the ratio 50:50 v/v in an isocratic mode was used. The detection was carried out at 255 nm using a UV detector, 20 µl injection volume was selected with a flow rate of 1 ml/min. The retention time was found around 6.5 minutes. The method was linear over the range of 5-45 µg/ml with a regression coefficient of 0.9997. The method was validated as per ICH guidelines. The developed method is superior in the terms of theoretical plates and it has got less tailing factor. The method can be applied for routine quality control analysis of Capmatinib in tablet formulation.

INTRODUCTION

Capmatinib (CAS 1029712-80-8) is an antineoplastic drug that is used for the treatment of patients with locally advanced or metastatic MET (Mesenchymal epithelial transition) exon 14 skipping (METex14) mutated non-small cell lung cancer (NSCLC). Capmatinib is an orally bioavailable inhibitor of hepatocyte growth factor receptor (HGFR) it selectively binds to c-Met, followed by inhibiting c-Met phosphorylation and disturbing c-Met signal transduction pathways. That may lead to cell death in tumor cells overexpressing c-Met protein [1-2]. The chemical name of Capmatinibis 2-fluoro-N-methyl-4-[7-(quinolin-6-ylmethyl)imidazo[1,2-b][1,2,4]triazin-2-yl]benzamide and its molecular formula is $C_{23}H_{17}FN_6O$. The molar weight of Capmatinibis 412.4 g/mol [3]. Capmatinib had Received its first approval on 6 May 2020 in the USA[4]. Structure of Capmatinib is shown in **Figure 1.**

Literature survey revealed LC-MS/MS and UPLC-MS/MS method to quantify Capmatinib, only one HPLC method was reported for metabolite pattern analysis during ADME study of Capmatinib in healthy male volunteers [5-7]. Hence attempts were made to develop a simple, rapid, precise, and accurate reverse phase chromatographic method to estimate Capmatinib in the tablet dosage form. The proposed method was optimized and validated as per ICH guidelines[8].

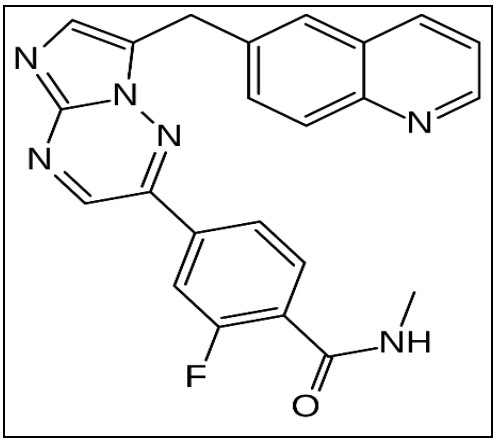

Figure No. 1: Structure of Capmatinib

The main objective is to give an overview of the mechanism of Reversed-Phase High-Performance Liquid Chromatography and to explain the basis of the retention mechanism and achieve high-speed separation without loss of reproducibility.

MATERIALS AND METHODS

Chemicals and Reagents

An analytically pure Capmatinib standard with defined potency 95.50 (as is basis) and Capmatinib (150 mg) tablets were obtained from Central Drug Testing Laboratory, Mumbai. Methanol HPLC grade, Ortho Phosphoric Acid, and Potassium dihydrogen orthophosphate from Molychem, Triethylamine (TEA) of analytical grade from Ranken, were used in the preparation of mobile phase. Ultra-purified HPLC grade distilled water obtained from the Milli-Q® system (Millipore, Milford, MA, USA) water purification unit was used to prepare all the required solutions.

Instrumentation

Perkin Elmer UV/VIS Spectrometer Lambda 25 connected to a computer loaded with software Perkin Elmer UV Win Lab was used for all the spectrophotometric measurements. The chromatography was performed on Thermo Scientific Ultimate 3000 system using chromeleon 7.4.2 software with LC instrument control. Precision parameter to be performed on the different instruments was done on Perkin Elmer Flexar HPLC using software Tc Nav/Ver 6.3.2. Zorbax Eclipse XDB- C18 (4.6 mm X 150 mm, 5 µm) column was used as a stationary phase. LAQUA, Horiba scientific PH meter was used to maintain the ionic concentration.

Determination of wavelength

The standard solution 10 µg/ml of Capmatinib was scanned in the range of 400-200 nm against diluent as a blank. Capmatinib showed maximum absorbance at 255 nm as shown in **figure 2.** So, the suitable wavelength selected for the HPLC analysis of Capmatinib was 255 nm.

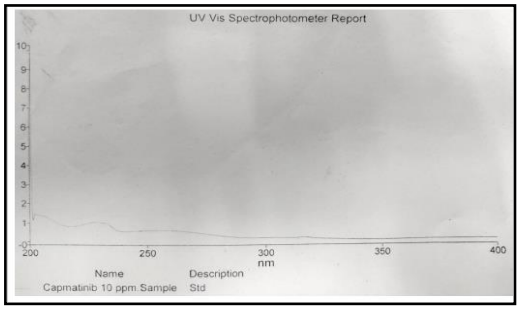

Figure No 2: UV scan of Capmatinib.

Mobile Phase preparation

Phosphate buffer (10 mM) and methanol in a ratio of 50:50 v/v were used as a mobile phase for the present study. Two different ports were used for running of mobile phase in isocratic form. Phosphate buffer was prepared by dissolving 1.3609 gm of KH_2PO_4 and 1 ml of triethylamine into 1000 ml HPLC grade water followed by adjustment of PH to 3 with orthophosphoric acid. The mobile phase was vacuum filtered through 0.45µm high flow nylon membrane filters and was sonicated and degassed using an ultra sonicator.

Diluent Preparation

A mixture of Methanol and distilled water in the ratio of 1:1 v/v was used for the preparation of standard and sample solutions.

Preparation of Standard Solution

A standard stock solution of 100 µg/ml was prepared by accurately weighing 10.0 mg of Capmatinib and transferring it into a 100.0 ml volumetric flask. To this add 50.0 ml of diluent and dissolve the drug properly by sonicating for about 5 min, make up the solution to 100 ml with the diluent. Working standard solution of concentration 30 µg/ml of Capmatinib was obtained by pipetting out 3.0 ml of standard stock solution and transferring it into 10.0 ml volumetric flask and making up to the mark with the diluent.

Preparation of Sample Solution

An amount of sample equivalent to 150 mg of Capmatinib in the tablet formulation was accurately weighed and transferred in 200.0 ml volumetric flasks followed by the addition of 100 ml of diluent. The mixture was then subjected to sonication for 15 min, frequently subjected to vortex for the complete dissolution of the drug. The solution was cooled to room temperature and further volume was made up to the mark with diluent. Sample solution of concentration 30 µg/ml of Capmatinib was obtained by pipetting out 4.0 ml of the above solution and transferring it into 100.0 ml volumetric flask and making up to the mark with the diluent. All sample solutions were passed through a 0.45 µ filter before analysis.

Chromatographic conditions

Zorbax Eclipse XDB- C18 (4.6 mm x 150 mm, 5µm), the column was used for analysis at 40°C column temperature and autosampler set at 10 ± 5 °C. The mobile phase consisted of PH 3 buffer and Methanol in the ratio of 50:50 v/v. The mobile phase was pumped through the column at a flow rate of 1.0 ml/min. The sample injection volume was 20 µl. The UV detector was set to 255nm for detection and the chromatographic runtime was 12 minutes.

Method optimization

Molecular structure and solubility data show that Capmatinib is a basic nonpolar molecule. Initial trials on HPLC were carried out using synchronis C18 (4.6mm X 250mm, 5 µ) column, with a mobile phase consisting of buffer (20 mM KH2PO4 with 1 ml/l Triethylamine at different PH ranging from 3.0 to 4.5) with varying proportion of methanol. In the chromatogram obtained by following the above conditions, poor peak shape and asymmetry were observed. Later trials were conducted on the same column with a mobile phase consisting of buffer (10mM KH2PO4 with 1 ml/l Triethylamine at PH ranging from PH 3 to 4.5) with varying proportions of methanol. In chromatogram recorded Capmatinib peak was obtained with unacceptable SST parameters. Further trials were conducted using Zorbax Eclipse XDB- C18 (4.6 mm x 150 mm, 5µ) with a mobile phase consisting of buffer (10mM KH2PO4 with 1 ml/l Triethylamine at PH ranging from PH 3 to 4.5) Chromatograms with accepted peak shape and SST Parameters were obtained on this column. Hence finally Zorbax Eclipse XDB- C18 (4.6 mm x 150 mm, 5µ) column and mobile Phase consisting of buffer (10 mM KH2PO4 with 1 ml/l Triethylamine at PH 3.0) was selected for the study.

Method Validation Studies

The method was validated for system suitability testing, specificity, linearity, precision, accuracy and recovery, LOD, LOQ, and robustness parameters according to ICH guidelines.

System Suitability Testing

System suitability parameters were evaluated and analyzed to check the system performance by injecting a working standard solution (six replicate) of a concentration of 30 µg/ml and blank preparation (single injection) into the HPLC. The chromatograms were recorded to

evaluate SST parameters like %RSD of Retention time, Tailing factor, Theoretical plates. Data of system suitability studies is summarized in **Table 1**.

Table No. 1: Data for System suitability testing and system precision study of Capmatinib.

Injection no	Area	Retention Time	Theoretical Plates	Tailing Factor
1	2315029	6.583	7889	1.09
2	2315262	6.579	7863	1.12
3	2314302	6.579	7967	1.11
4	2316243	6.579	7937	1.11
5	2315140	6.575	8036	1.13
6	2315534	6.579	7889	1.10
AVERAGE	2315251.667	6.579	7930.167	1.1
SD	636.628	0.0025298	64.04	0.01
% RSD	0.03	0.04	0.81	1.27
LIMIT	NMT 2.0%	NMT 1.0%	NMT 2.0%	NMT 2.0%

Specificity

For specificity solutions of blank, a standard of 30 µg/ml and a sample of 30 µg/ml were injected and their chromatograms were recorded as represented in **Figure 3,4,5** respectively.

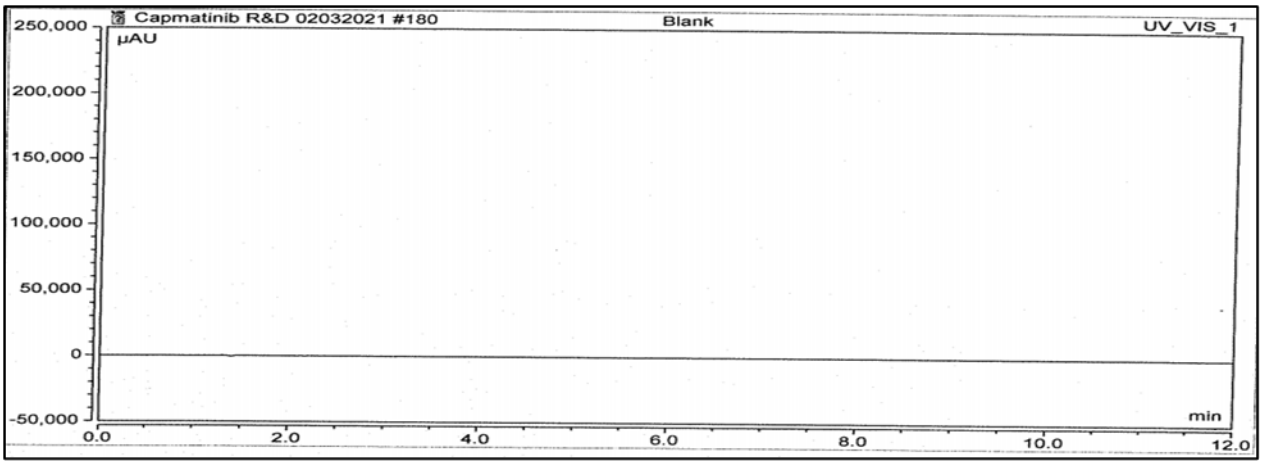

Figure No. 3: Chromatogram of blank solution.

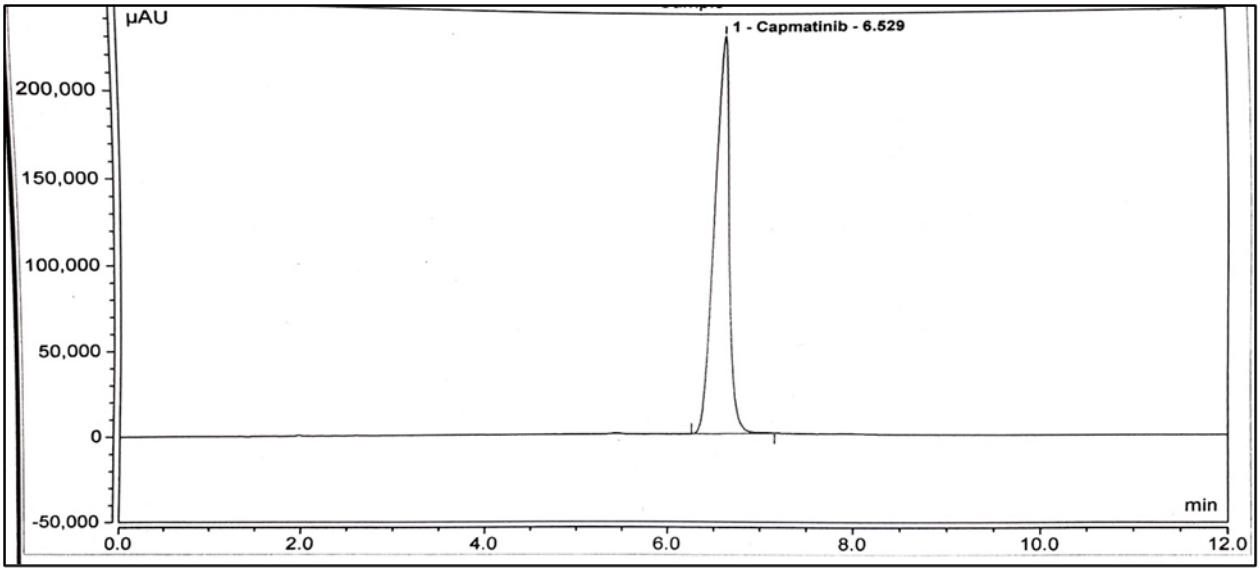

Figure No. 4: Chromatogram of standard solution of Capmatinib.

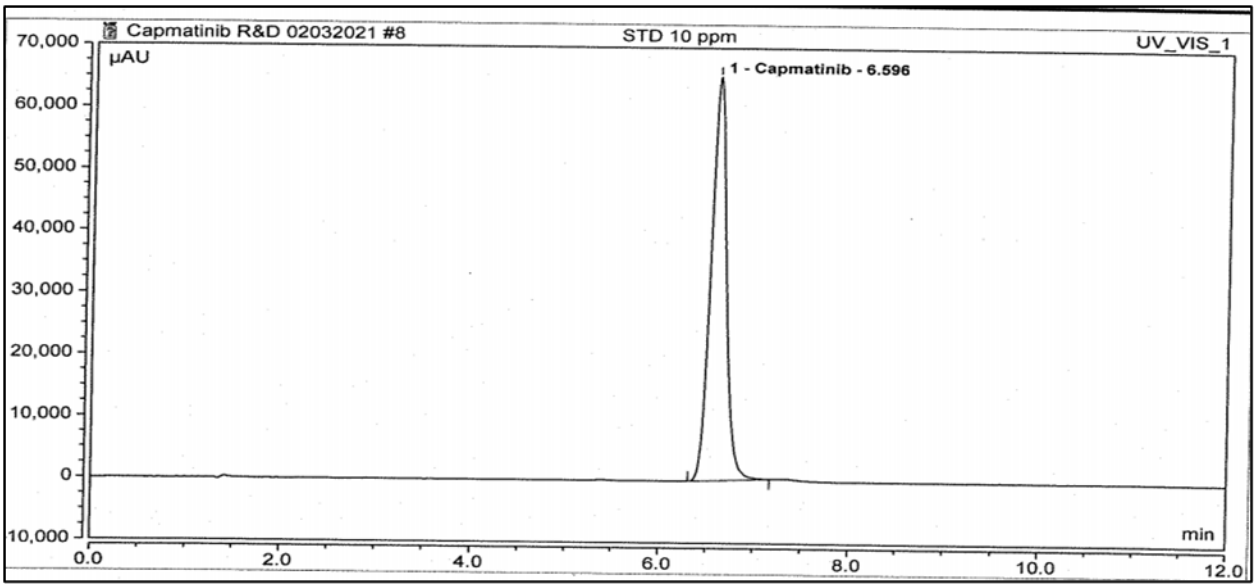

Figure No. 5: Chromatogram of sample solution of Capmatinib.

Linearity

The linearity of the method was obtained within the concentration range of 5-45 µg/ml for Capmatinib. The linearity graph was plotted by taking the concentration of the drug on the X-axis and the corresponding peak area on the Y-axis as shown in **Figure 6**. The linearity data is summarized in **Table 2**.

Table No. 2: Linearity data of Capmatinib.	
Concentration (µg/ml)	Area
5	408745.00
10	749801.33
15	1147328.33
20	1499691.33
25	1955295.33
30	2311714.67
35	2675267.67
40	3050589.67
45	3439717.67

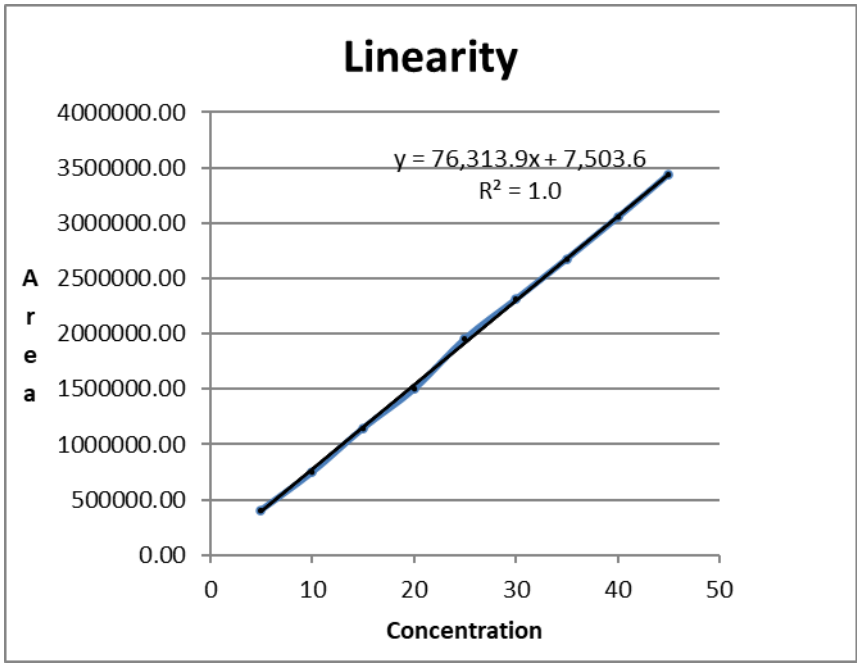

Figure No. 6: Linearity graph of Capmatinib.

Precision

"The precision of an analytical method is the degree of agreement among individual test results obtained when the method is applied to multiple sampling of a homogenous sample".

System Precision: This was performed by injecting six replicate injections of a standard solution (30 µg/ml). The average, SD, %RSD of an area in six replicate injections was calculated and reported. The results are summarized in **Table 1**.

Method Precision (Assay Repeatability): This was performed by injecting five replicate injections of standard solution (30 µg/ml) and six sample preparation of Capmatinib (30 µg/ml) in triplicates into the HPLC system. Its % Assay, average, SD, %RSD were calculated and reported. The mean assay percentage results are summarized in **Table 3.**

Intermediate Precision: This was performed on two different days, by two different analysts, and different HPLC instruments. Five replicates of standard solution (30 µg/ml) and three sample preparation (30 µg/ml) in triplicates were injected into the HPLC system. Its % Assay, average, SD, %RSD were calculated and reported. Results are summarized in **Table 4.**

Table No. 3: Method Precision (Assay Repeatability) data of Capmatinib.

Sample No.	% Assay
1	99.92
2	99.96
3	100.08
4	100.24
5	99.77
6	99.50
AVERAGE	99.91
SD	0.257
%RSD	0.26
Limit	NMT 2%

Table No. 4: Intermediate precision data of Capmatinib.				
Sample No.	% Assay Day-1 HPLC-1	% Assay Day-2 Analyst -1	%Assay Analyst-2	% Assay HPLC-2
1	99.92	99.81	100.05	100.21
2	99.96	99.64	99.94	100.45
2	100.08	99.78	100.07	100.07
AVERAGE	99.99	99.74	100.02	100.24
SD	0.08	0.09	0.071	0.19
%RSD	0.084	0.092	0.07	0.192
LIMIT	NMT 2%	NMT 2%	NMT 2%	NMT 2%

Accuracy and Recovery:

Accuracy is a measure of how close is the experimental value to the true value. Accuracy was determined by the method of standard addition method, by calculating of % mean recovery of the sample at Four different levels 100, 110, 120, 130%. At each level, three determinations were performed, the mg found, % recovery, and % RSD were taken into consideration. Accuracy results at various levels of concentration are summarized in **Table 5.**

LOD and LOQ:

Limit of detection (LOD) and limit of quantification (LOQ) of Capmatinib were determined from the calibration curve method using the following formulas:

$$LOD = 3.3 \times \propto/s,\ LOQ = 10 \times \propto/s$$

Table No. 5: Accuracy data of Capmatinib.						
% Level	mg Found	% Recovery	AVERAGE	SD	%RSD	%Mean Recovery
100	151.00	100.7	100.5	0.117	0.11	100.41
	150.79	100.5				
	150.65	100.4				
110	167.46	111.6	101.5	0.025	0.02	
	167.43	111.6				
	167.51	111.7				
120	180.67	120.4	100.2	0.141	0.14	
	180.36	120.2				
	180.87	120.6				
130	193.47	129.0	99.3	0.045	0.045	
	193.64	129.1				
	193.61	129.1				

Where ∝ is the Standard deviation of the response of the regression line and s is the slope obtained from the calibration curve After calculating, solutions of desired concentration for LOD and LOQ were prepared and injected. The chromatograms obtained were recorded as represented in **Figures 7, 8.**

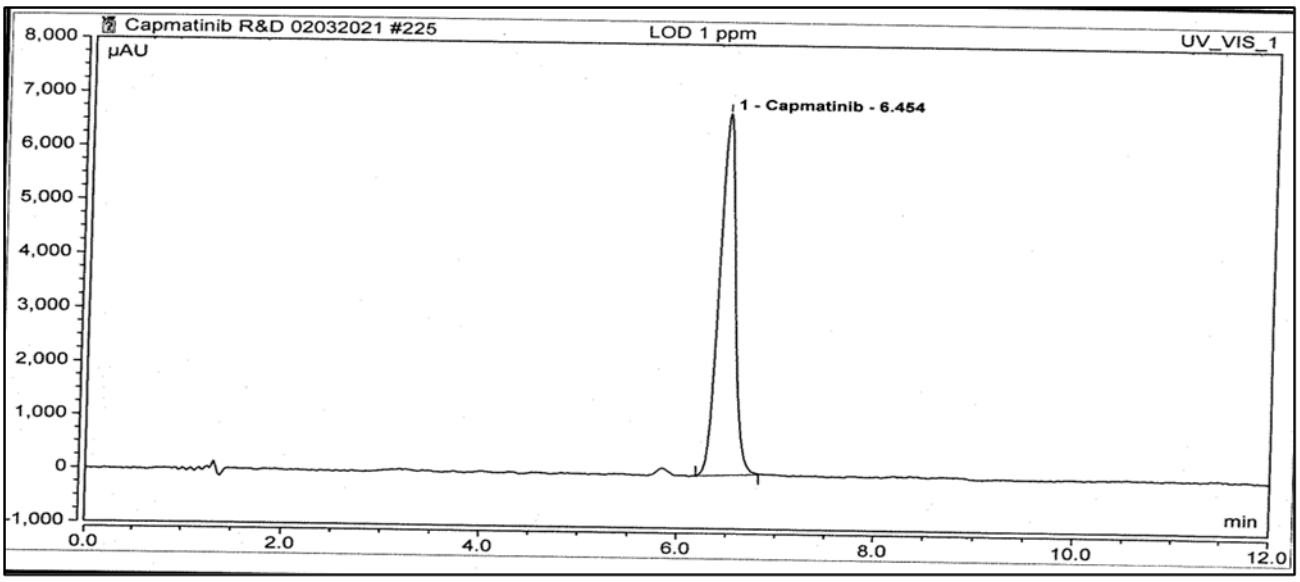

Figure No. 7: Chromatogram of LOD

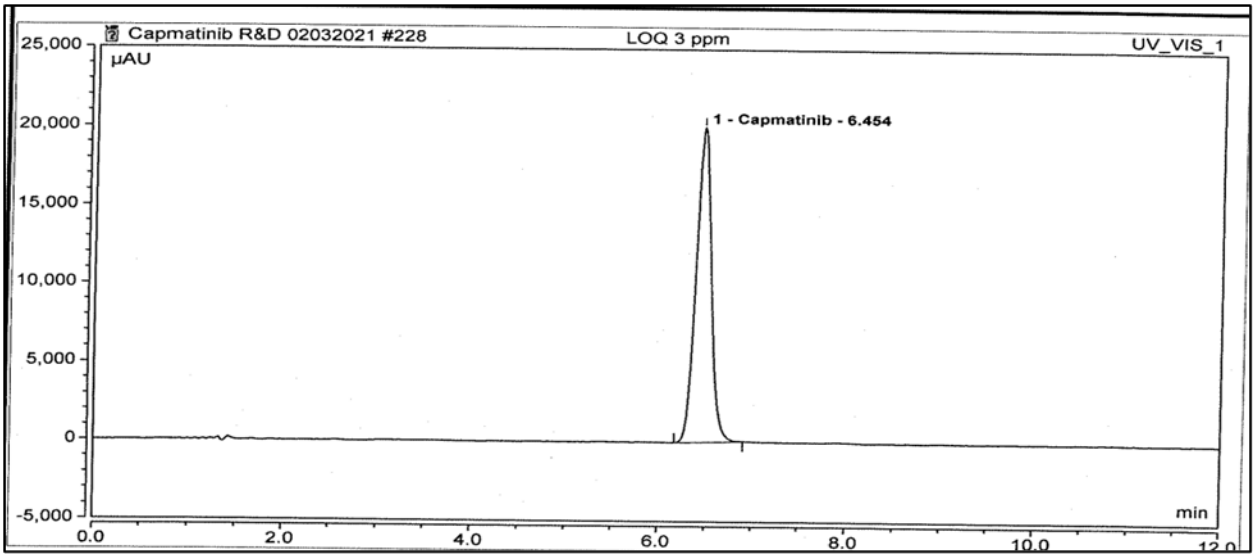

Figure No. 8: Chromatogram of LOQ

Robustness:

It is defined as a small or deliberate change in the parameter that should not affect any method. This was performed by a change in flow rate (± 0.2 ml/min), change the column in temperature (±5 °C), change in wavelength (±2 nm), Change in PH (± 0.2 nm), and change in column (Different column and HPLC instrument, Different column on same HPLC instrument). Three sample preparations of 30 µg/ml were prepared and injected in triplicate along with five replicate injections of a standard solution of 30 µg/ml under different chromatographic conditions. Its % assay, average, SD, %RSD were calculated and reported, results are summarized in **Table 6**.

Assay:

The optimized method was applied on tablets having a label claim of Capmatinib 150 mg. The assay was performed on the above solution five replicate injections of standard preparation 30 µg/ml and six sample preparation 30 µg/ml in triplicate were injected into the HPLC system. Its % assay, average, SD, %RSD were calculated and reported, results are summarized in **Table 7.**

Table No. 6: Robustness data of Capmatinib.

Parameter	Change in parameter (±)	% Assay Estimation	AVERAGE	SD	% RSD	LIMIT
Flow rate (±0.2 ml/min)	0.8	100.03	99.62	0.36	0.36	NMT 2%
	1	99.42				
	1.2	99.40				
Column temperature (±5°C)	25	100.67	100.7	0.29	0.29	
	30	100.37				
	35	100.95				
Wavelength (±2 nm)	253	100.12	100.13	0.03	0.03	
	255	100.12				
	257	100.16				
PH (±0.2 ml/min)	2.8	98.66	99.0	0.4	0.40	
	3	98.93				
	3.2	98.45				
Different column and HPLC instrument)	-	101.44	101.26	0.22	0.21	
	-	101.00				
	-	101.26				
Different Column and same HPLC instrument	-	101.31	100.03	1.12	1.12	
	-	99.21				
	-	99.57				

Table No. 7: Assay results of Capmatinib.

Sample No.	Weight of standard (mg)	Sample weight (equivalent to 150 mg of Capmatinib)	Mean Area of the standard at 255 nm	Area of a sample at 255 nm	% Assay
1	10.60	722.23	2320431	2530625	99.92
2		720.40		2525271	99.96
3		740.83		2599984	100.08
4		735.41		2585056	100.24
5		735.85		2574455	99.77
6		740.94		2585256	99.50
Mean					99.91
± SD					0.234
% RSD					0.23

RESULTS AND DISCUSSION

An RP-HPLC method for quantitative estimation of Capmatinib was developed and validated as per ICH guidelines. The results obtained indicate that the proposed method is rapid, accurate, selective, and reproducible. As there is no interference of blank at the retention time of Capmatinib hence method was specific. Linearity was observed over a concentration range of 5-45 µg/ml for Capmatinib. The correlation coefficient was found to be 0.99978.

The relative standard deviation for system suitability testing and system precision studies was found within a limit that is not more than 2%. Theoretical plates were found to be greater than 6000, also the tailing factor was reported to be less than 2. In Method precision (assay repeatability) studies of Capmatinib average assay percentage was found to be 99.91% which was within the limit i.e., between 98% to 102%. The relative standard deviation for all intermediate precision parameters was found to be within the limit.

The accuracy studies were shown as % recovery at 100% to 130% level for Capmatinib. The mean percent recovery was found to 100.41% which was within the limit. Hence the method was found to be accurate. The present method can detect and quantify the analyte at a lower concentration. Limit of detection and limit of quantification values were estimated as following LOD = 1 µg/ml, LOQ = 3 µg/ml where standard deviation($\propto=$ 23680.8) and

slope(s = 76313.9) values were obtained by calibration curve method. By analyzing robustness, resulted values were found to be within a limit that is less than 2%, thus the developed method was proved to be robust. The results obtained from the assay show that the percentage recoveries were high and SD values are very low, which confirms that the method is suitable for routine analysis of Capmatinib in its pharmaceutical preparation.

CONCLUSION

The RP-HPLC method development was found to be simple, precise, rapid, accurate for the quantification of Capmatinib in a liquid dosage form. The method was reliable in terms of system suitability, linearity, precision, accuracy and recovery, robustness, and assay. All the verification parameters were within the range according to ICH Q2A (R1) guidelines. Hence, we can conclude that the proposed RP-HPLC method can be used for routine analysis in the Pharmaceutical industry.

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