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NOVEL STABILITY INDICATING HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF CAPMATINIB IN BULK DRUG AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT
In the pharmaceutical industry, all manufactured products need to be of the highest quality to ensure the least risk to patients. To guarantee that goods pass certain standards, researchers, manufacturers and developers use various technical equipment and analytical techniques, including liquid chromatography, during the development process. Liquid chromatography is an analytical technique that is used to separate a certain sample into its individual components. HPLC is simple, specific, rapid, precise and accurate; it can be successfully and efficiently adopted for routine quality control analysis of drugs in bulk and pharmaceutical dosage form. In the present study a reverse phase high performance liquid chromatography method was developed and validated for the estimation Capmatinib in pharmaceutical formulations. To assess the effect of method parameters on chromatographic separation of the Capmatinib, statistically designed experiments were performed by varying different method parameters such as buffer concentration, pH of mobile phase, flow rate, and column temperature. The separation was performed on Spherisorb ODS C18 Column (250 x 4.6 mm and 5µm) at room temperature using methanol, acetonitrile and water in the ratio of 60:20:20 (v/v) in isocratic condition at a flow rate of 1.0 mL/min. The detection was performed by a ultraviolet detector (UVD) at 251 nm with total run time of 10 min. Calibration curves were linear in the concentration range of 5-35 µg/mL for with correlation coefficients of 0.9991. LOD and LOQ were found to be 0.033 µg/mL and 0.10 µg/mL proves the sensitivity of the developed method. The method can effectively separate the degradation compounds during the stress study and the standard drug Capmatinib was found to be stable in all the stress degradation conditions. The developed method was able to determine the contents of the Capmatinib commercial dosage forms and hence the method was used for the routine analysis of Capmatinib in bulk drug as well as in pharmaceutical formulations.

KEYWORDS: Capmatinib, HPLC analysis, Mesenchymal-epithelial transition, Method validation and Liquid chromatography.

INTRODUCTION
Pharmaceutical analysis is traditionally defined as analytical chemistry dealing with drugs both as bulk drug substances and as pharmaceutical products (formulations). However, in academia, as well as in the pharmaceutical industry, other branches of analytical chemistry are also involved, viz. bioanalytical chemistry, drug metabolism studies, and analytical biotechnology.[1,2] The development of drugs in the pharmaceutical industry is a long-term process, often in all these steps the amount of data generated is enormous.[3] Product analysis involves dealing with the various formulations used for toxicological studies, clinical studies, and marketing.[4] For both substances and formulations there is an increasing interest in the introduction of process analytical chemistry.[5] Biomolecules, i.e., macromolecules such as proteins or hormones, either produced by isolation from biological sources or by means of biotechnology, must also be subjected to careful analytical controlup.[6]

There are a number of regulations that have to be followed in the development of pharmaceuticals as well as in their production. Regulatory approval is required prior to each clinical trial and before marketing is licensed.[7,8] An important part of the development process is safety evaluation, primarily the toxicology tests, which run from 1 to 24 months in different species.[9] Quality is important in every product.[10] The methods of estimation of drugs are divided into physical, chemical, physico-chemical and biological ones.[11,12] The combination of mass spectroscopy with gas

chromatography is one of the most powerful tools available.[13] It is not only the moral responsibility of manufacturers to produce effective, safe and non-toxic forms but also their legal responsibility.[14-16] The importance of chromatography is increasing rapidly in pharmaceutical analysis.[17] HPLC is a special branch of column chromatography in which the mobile phase is forced through the column at high speed.[18]

Capmatinib is in a class of medications called kinase inhibitors. Capmatinib is used for the treatment of adults with metastatic non-small cell lung cancer (NSCLC) whose tumors have a mutation that leads to the exon 14 skipping of the MET gene. The chemical name is 2-Fluoro-N-methyl-4-[7-(quinolin-6-ylmethyl) imidazo [1,2b] [1,2,4] triazin-2-yl] benzamide hydrogen chloride. The molecular formula for capmatinib hydrochloride is $C_{23}H_{21}Cl_2FN_6O_2$. The relative molecular mass is 503.36 g/mol for the hydrochloride salt and 412.43 g/mol for the free base [19]. Its molecular structure was given in figure 1.

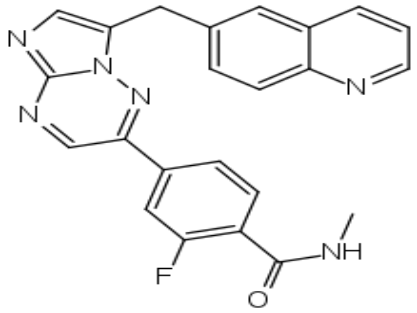

Figure 1: Molecular structure of Capmatinib.

Capmatinib is a kinase inhibitor targeting MET (mesenchymal-epithelial transition), including a mutant version formed by MET exon 14 skipping. METex14 skipping leads to a protein with a missing regulatory domain that decreases its negative regulation, resulting in increased MET downstream signaling.[20] Swelling hands/ankles/feet, tiredness, fever, nausea/vomiting, decreased appetite, weight loss, constipation, or diarrhea may occur.[21]

The literature survey for the available analytical methods for the analysis of Capmatinib confirms that there are very few analytical methods available for the estimation of Capmatinib in biological samples in single or in combination with its active metabolites in biological samples using LCMS or UPLC MS. There is no analytical method reported for the estimation of Capmatinib in pharmaceutical formulations.[22,23]

The aim of the study was to **develop and validate a simple, accurate**, precise and rapid method for estimation of **Capmatinib** pharmaceutical formulations using **RP-HPLC.**

Instrumentation

The author has attempted to develop a liquid chromatographic method for the simultaneous estimation of Capmatinib using isocratic Shimadzu HPLC equipment comprising of binary LC 10AT vp pumps, SIL 10AD vp Auto sampler, CTO 10A vp column oven, and Prontosil ODS C18 Column (250 x 4.6 mm and 5µm), and an SPD 10Avp UV-Visible detector. All the components of the chromatographic system were controlled using SCL-10A vp System controller. Data acquisition was done using LC Solutions version 1.23SP 1 software.

Chemicals and solvents

The working standard drug Capmatinib (99.17 % purity) along with the formulation dosage form (Tabrecta® - 200 mg) were obtained from Novartis Healthcare Pvt Ltd in Madhapur, Hyderabad. HPLC grade Methanol, Water and Acetonitrile were purchased form Merk chemicals private limited, Mumbai. The buffer solutions used for the study were AR Grade and purchased from Merck Specialties Private Limited, Mumbai, India.

Preparation of standard drug solution

Preparation of standard stock solution was the primary step prior to experimental work. A standard stock solution of 1000 µg/mL was prepared by weighing accurately 10 mg of the standard drug Capmatinib and was taken in a 10 mL volumetric flask having little amount of Methanol. Dissolve the drug in the solvent and make up to the mark. Then it was filtered through 45µ filter paper to remove un-dissolved particles or any solid substances. By diluting the standard solution with mobile phase, different concentrations (5, 10,15,20,25 & 30µg/mL) of standard solutions was prepared.

Preparation of formulation solution

Tablets of Tabrecta® brand containing 200 mg of Capmatinib was powdered using a sterile mortar and pestle. Then an amount of tablet powder equivalent to 50 mg of Capmatinib was accurately weighed and dissolved in 50 mL solvent using sonicator and filtered through 0.45 µ membrane filter. Then it was diluted while doing the formulation analysis.

HPLC Method Development

Selection of wavelength

- To select an appropriate monitoring wavelength, the standard solutions of 10µg /mL was prepared and scanned by the UV-Vis spectrophotometer. The obtained wavelength maximum was selected as suitable wavelength for the detection.

Selection of stationary phase

- Since the Upadacitinib is a Polar drug, a non-polar C18 column was selected for the separation of the drug. Different columns of different companies, manufactures and configurations were tested.

Method Development trails

Table 01: Method Development trails HPLC method development.

Trail.no	Parameter	Condition	Trail.no	Parameter	Condition
I	MP	Methanol: Acetonitrile 50:50 (v/v)	**IV**	MP	Acetonitrile: water 50:50 (v/v)
	Wavelength	251 nm		Wavelength	251 nm
	Stationary Phase	Kromosil ODS C18 Column (250 x 4.6 mm and 5µm)		Stationary Phase	Spherisorb ODS C18 Column (250 x 4.6 mm and 5µm)
	Flow Rate	1.0 mL/min		pH of MP	6.2
				Flow Rate	1.0 mL/min
II	MP	Methanol: Water 75:25 (v/v)	**V**	MP	Methanol: Acetonitrile 25:75 (v/v)
	Wavelength	251 nm		Wavelength	251 nm
	Stationary Phase	Sperbo Waters C18 Column (250 x 4.6 mm and 5µm)		Stationary Phase	Spherisorb ODS C18 Column (250 x 4.6 mm and 5µm)
	Flow Rate	1.0 ml/min		pH of MP	5.1
				Flow Rate	1.0ml/min
III	MP	Methanol: Acetonitrile 25:75 (v/v)	**VI**	MP	Methanol: Acetonitrile: Water 60:20:20 (v/v)
	Wavelength	251 nm		Wavelength	251 nm
	Stationary Phase	Prontosil ODS C18 Column (250 x 4.6 mm and 5µm)		Stationary Phase	Spherisorb ODS C18 Column (250 x 4.6 mm and 5µm)
	pH of MP	6.2		pH of MP	5.1
	Flow Rate	1.0 mL/min		Flow Rate	1.0 mL/min
				Pump Mode	Isocratic

Analytical Method Validation

The method was validated with respect to linearity, accuracy, precision, repeatability, selectivity, and specificity, according to the ICH guidelines. Validation studies were carried out by replicate injections of the sample and standard solutions into the chromatograph.

Specificity

Specificity of the method was checked by injecting the solution into the chromatograph. Specificity of the method was assessed by comparing the chromatogram of Capmatinib (standard), blank and sample solutions to those obtained for tablet solutions. Retention time of the Capmatinib in standard solution, and in the sample solution was compared to determine the specificity of the method.

System suitability

The system suitability was determined by making six replicate injections of the standard solution and analyzing Capmatinib for its peak area, peak USP tailing factor, and number of theoretical plates. The proposed accepted criteria are not more than 2% for RSD%, not less than 2 for resolution, not more than 2 for USP tailing factor, and not less than 2000 for the number of theoretical plates.

Sensitivity of the method

The limit of detection (LOD) and limit of quantization (LOQ) were defined as the lowest concentration of analyte in a sample that can be detected and quantified. The standard solutions of Capmatinib for LOD and LOQ were prepared by diluting them with suitable solvent. The LOD and LOQ were determined by the signal-to-noise (S/N) ratio for each compound through analyzing a series of diluted solutions until the S/N ratio yield 3 for LOD and 10 for LOQ, respectively.

Linearity and Range

The calibration curve in the developed method was constructed from LOQ concentration. Capmatinib standard stock solution of 1 mg/mL was used for preparation of subsequent aliquots. Sample solution was loaded and 20 µL was injected into column. All measurements were repeated for each concentration. The calibration curve of the area under curve versus concentration were recorded. Form the calibration curve, correlation and regression values were calculated for Capmatinib.

Precision

The precision studies were carried out by estimating response of Capmatinib six times at a standard concentration of 50 µg/mL and results are reported in terms of % RSD. The intra-day and inter-day precision studies were carried out by estimating the corresponding responses six times on same day for intraday and interday for three different days and it was expressed as the percentage relative standard deviation (% RSD) which was calculated as per the following expression

%RSD = (standard deviation / mean) x 100.

Accuracy/ Recovery

Accuracy of method was observed by recovery result from two placebos preparations accurately spiked with different concentration of Capmatinib. Recovery assessment was obtained by using standard addition technique which was by adding known quantities of pure standards at three different levels in 50%, 100% and 150% to the pre analyzed sample formulation. From the amount of drug found, amount of drug recovered and percentage recovery were calculated by using the formula.

%RSD = (standard deviation / mean) x 100.

Ruggedness

Two laboratory analysts carried out the precision of Capmatinib at a standard concentration of 50 µg/ml was prepared by different analysts in the laboratory conditions, the prepared solution were analyzed in the optimized conditions. Peak area that obtained was used for the determination of ruggedness of the method. Ruggedness was expressed in terms of %RSD which must be less than 2.

Robustness

Robustness of the proposed method included six deliberate variations to some chromatographic parameters. The modifications include different mobile phase ratios and different detector wavelengths and different percentage in the mobile phase (in the range of ± 5 of the nominal value and the normal %). The % change in each of the changed condition was calculated.

Formulation analysis

This proposed method was applied to the determination of Capmatinib in commercially combined tablets. The sample solution at a concentration of 50 µg/ml of Capmatinib was analyzed in the optimized conditions. Peak area of the resultant chromatogram was used for the estimation of assay using label clime recovery method. The % assay was calculated for Capmatinib using the standard calibration values.

Forced degradation study

Table 02: Methodology for forced degradation study.

S. No.	Degradation type	Experimental conditions	Time
1	Acid Hydrolysis	50mg of drugs were mixed with 50ml of 0.1N HCl solution. The solution was neutralized and diluted up to standard concentration (100 %) and was analyzed in the developed method condition	24 Hours
2	Base Hydrolysis	50mg of drugs were mixed with 50ml of 0.1N NaOH solution. The solution was neutralized and diluted up to standard concentration i.e 100% and was analyzed in the developed method condition	24 Hours
3	Oxidative Degradation	50mg of drugs were with 50ml of 3% Peroxide solution. The solution was neutralized and diluted up to standard concentration (100 %) and was analysed in the developed method condition	24 Hours
4	Photolytic Degradation	50mg of drug sample was kept in UV light [254nm]. After the selected time of light expose, the drug solution was prepared and was analysed	24 Hours
5	Thermal Degradation	50mg of drug sample was kept in oven at 60^0C. After the selected time of light expose, the drug solution was prepared and was analyzed	24 Hours

RESULTS AND DISCUSSION

The present work aimed to develop a simple and accurate HPLC method for the quantification of Capmatinib in pharmaceutical formulations. The mobile phase was optimized with Methanol: Acetonitrile: Water in the ratio of 60:20:20 (v/v). From the spectrum of Capmatinib, wavelength was selected, at 251. Maximum absorption of UV light was achieved at 251 nm with minimum interference of the excipients and solvents. All parameters of this proposed method were validated as per the ICH guidelines.

In The trail 6 condition, single sharp symmetric peak with acceptable system suitability was observed (Figure 2). Hence these conditions (From table 1) were found to be suitable and further valuation was carryout using these conditions.

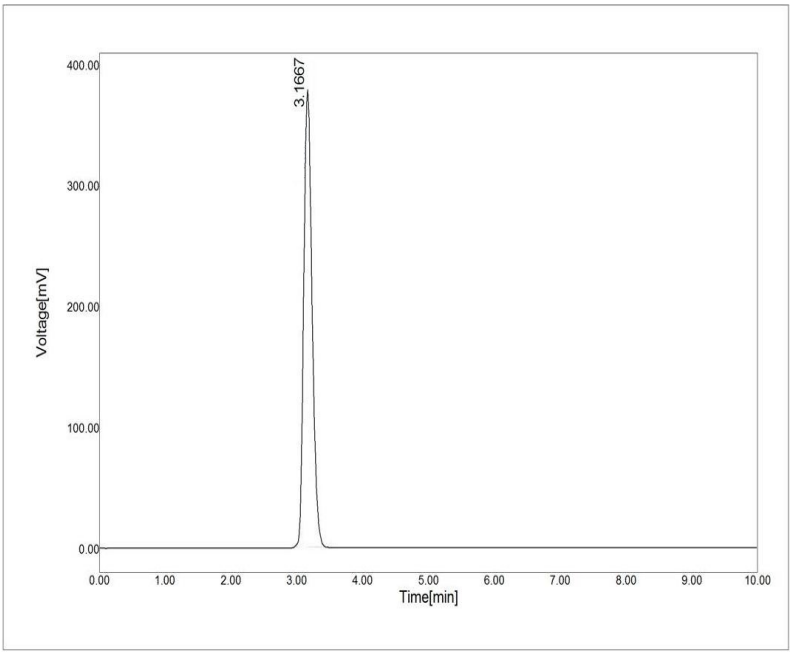

Figure 2: Optimized Chromatogram observed for Capmatinib.

Blank and Placebo solutions show no peak at the retention time of Capmatinib, hence proving the specificity of the method. A good linear relation was observed in the concentration range of 5-35 µg/mL with linear regression equation of y = 21601x + 10322 (R^2 = 0.9991) for Capmatinib in the developed method.

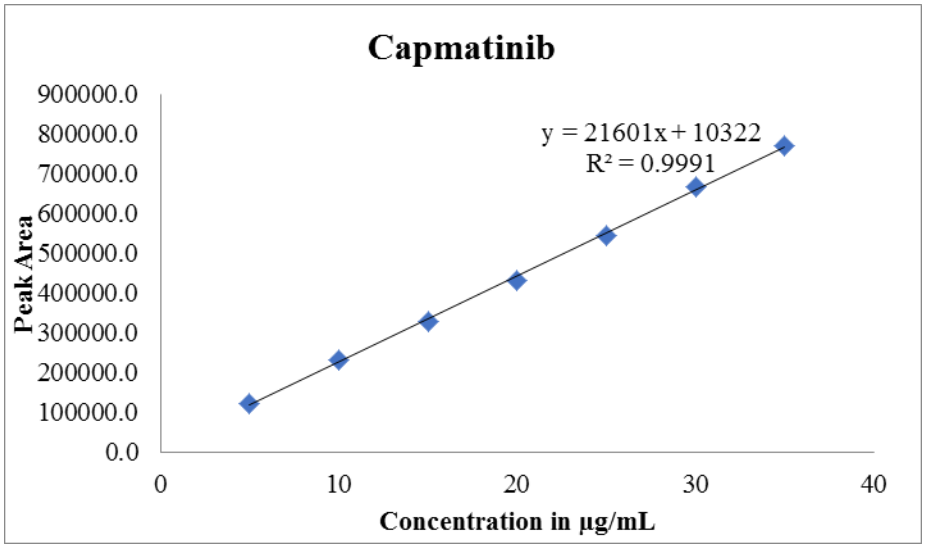

Figure 3: Linearity graph.

Recovery was carried out by standard addition method of 50%, 100% and 150% addition to standard, pre analyzed sample of 10 µg/mL concentration. The % recovery for each case was calculated and was found to be within the acceptance criteria of 98-102% and the % RSD of recovery in each spiked level was found to be within the acceptable limit of less than 2. This showed that the recoveries of Capmatinib in the proposed methods are satisfactory.

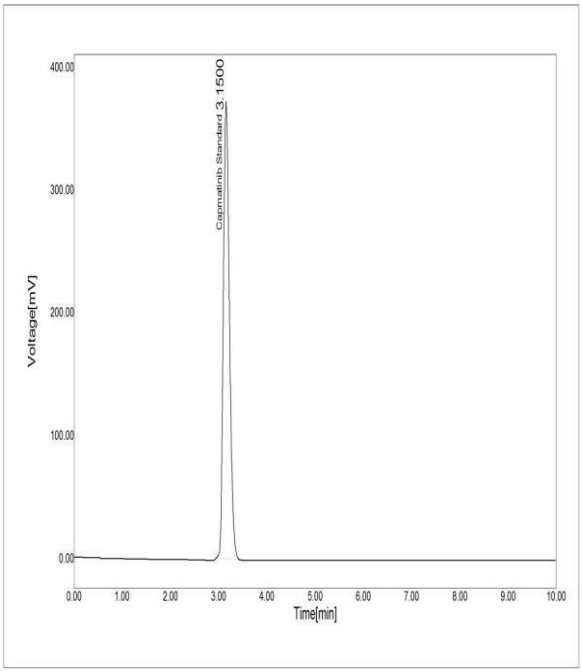

Figure 4: Chromatogram of Standard in the optimized conditions.

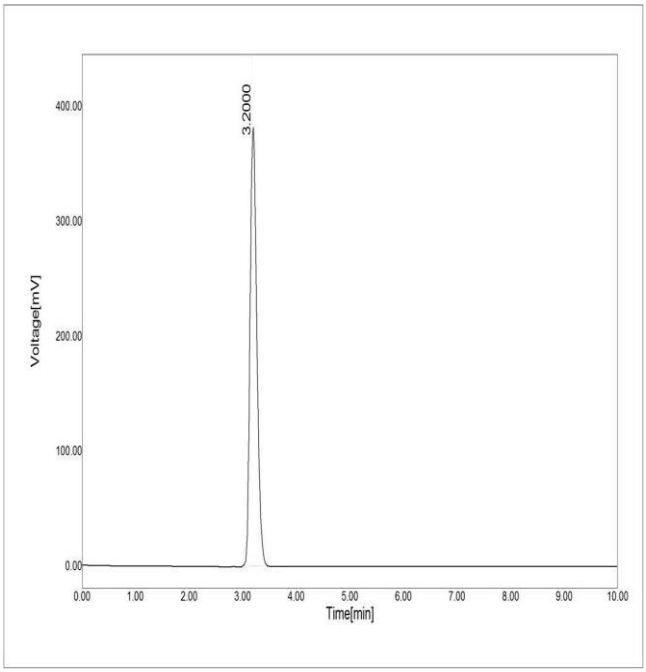

Figure 5: Chromatogram of Sample in the optimized conditions.

The precision was measured in terms of repeatability, which was determined by sufficient number of aliquots of a homogenous sample within the day (intraday) and next consequent three days for inter day precision. For each case % RSD was calculated and was found to be 0.10 and 0.38 in intraday precision and interday precision respectively. This was lying within the acceptable range of less than 2 %. This showed that the precision of the methods is satisfactory.

Ruggedness performed by using six replicate injections of standard solution of concentrations which were prepared and analyzed by different analyst on three different days over a period of one week. The percent relative standard deviation (% RSD) was calculated and it was found to be 0.68, which are well within the acceptable criteria of not more than 2.0. It was concluded that the analytical technique showed good repeatability.

LOD and LOQ values were found to be 0.03 µg/mL and 0.1 µg/mL respectively. This indicates that the proposed method is very sensitive. Small deliberate variation in the chromatographic condition does not show influence on the results indicates that the proposed method is robust. Results conforms that the method was accurate and doesn't affect the results to small change in the analytical conditions.

In all the stress degradation conditions i.e acidic, base, peroxide, thermal and UV light conditions, the standard drug Capmatinib was effectively separated, identified and quantified. The % assay of Capmatinib was found to be very high and the % degradation was found to be very less in the developed method. The degradation products were found to be 2, 3, 1, 1 and 3 in acidic, base, peroxide, thermal and UV light conditions respectively. All the degradation products were effectively separated and there is no overlap of degradation compounds with the standard drug. Hence the developed method was found to be stability indicating.

Table 3: Forced degradation study results.

S No	Condition studied	No of degradation compounds separated	% Assay	% Degradation
1	Acid	2	93.51	8.71
2	Base	3	96.73	5.63
3	Peroxide	2	97.48	4.28
4	Thermal	1	96.95	3.21
5	UV light	3	91.45	6.89

The validated method was applied for the assay of commercial tablets of Capmatinib i.e Tabrecta®-200mg. The % assay in formulation analysis was found to be 98.60 for Capmatinib in the developed method. More than 98% assay was observed in the developed method.

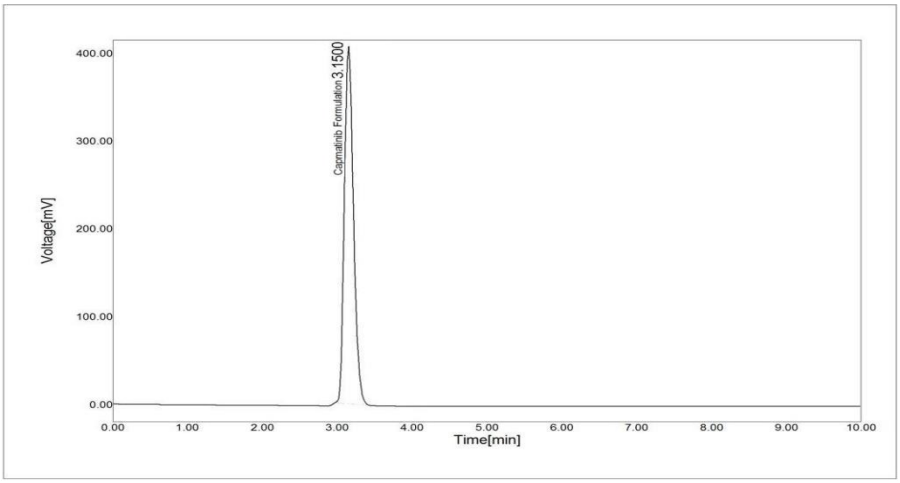

Figure 6: Formulation chromatogram.

Table 4: Formulation results.

S. No	Drug	Brand	Label claim	Concentration prepared	Concentration found	% assay
1	Capmatinib	Tabrecta®	200 mg	20 µg/mL	19.72 µg/mL	98.60

Hence the method was found to be suitable for the routine analysis of Capmatinib in bulk drug as well as formulations. The summary results observed method development, validation and application of the analytical method developed for the analysis of Capmatinib in formulations was given in table 5.

Table 5: Summery results achieved in the method developed for analysis of Capmatinib.

Study	Parameter	Results
Method Developed	Elution	Isocratic
	Mobile Phase	Methanol: Acetonitrile: Water in 60:20:20 (v/v)
	pH	5.1
	Column	Spherisorb ODS C18 Column (250 x 4.6 mm and 5µm)
	Wavelength	251 nm
	Flow	1.0 mL/min
	Runtime	6 min
	Temperature	Ambient
Method validation	Retention Time	3.2 min
	Tailing factor	1.05
	Theoretical plate	8215
	Resolution	--
	Linearity range	5 to 35 µg/mL
	Slope	21601
	Intercept:	10322
	r^2 (correlation coefficient)	0.9991
	Intraday Precision	0.10
	Interday Precision	0.38
	Ruggedness	0.68
	Recovery	98.48 to 99.73
	% change in Robustness	0.14 to 1.51
	LOD	0.03 µg/mL
	LOQ	0.10 µg/mL
Method Application	% Degradation in Acidic Base Peroxide Thermal UV Light	 8.71 5.63 4.28 3.21 6.89
	Formulation assay	98.60

CONCLUSION

The simple, sensitive, and inexpensive isocratic RP-HPLC method was developed and validated for the estimation Capmatinib in bulk drug and pharmaceutical formulations. The method was successfully developed and validated according to the ICH guidelines. The validation results showed that this method was specific, sensitive, linear, precise, accurate, and robust. In the developed method there is no other co-eluting peak with main peaks and the method is specific for the determination of Capmatinib tablet formulation. The developed method enables rapid quantification of many samples in routine analysis. So, it can be concluded that the developed stability indicating RP-HPLC method can be successfully applied to the quantification of Capmatinib in tablet dosage form.

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