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International Journal of Current Research and Innovation in Pharma Sciences

Journal homepage: https://www.ijcrips.com/

Research Article

# STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF CAPMATINIB IN BULK AND TABLET DOSAGE FORM

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**ARTICLE INFO**

***Article history:***
Received: 10-06--2022
Accepted: 18-06-2022
Available online: 25-06-2022

***Keywords:***
Capmatinib,
RP-HPLC,
Method Development,
Validation

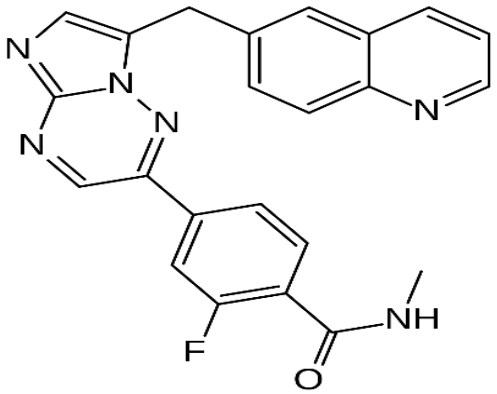
**ABSTRACT**

*The stability indicating method was developed and validated for estimation of Capmatinib. The mobile phase was consisting of Acetonitrile : methanol (60:40).The linearity range of Capmatinib was found to be 2-10µg/ml. The calibration curve was plotted and regression equation of Capmatinib was found to be y = 791970x + 4780.7 with correlation coefficient (r 2 ) of 0.9993. Detection was done at 252 nm and the retention time of Capmatinib was found to be 3.8 min with the flow rate of 1.0 ml/min. From accuracy study % recovery of Capmatinib was found in the range of 98.89-100.29 % which is in the limits accordingly the ICH guidelines. The method was found to be simple, linear, rapid, accurate, precise, reproducible and robust. The % RSD was found within limit as per ICH guidelines. The result showed that proposed chromatographic method was suitable for the accurate, precise and rapid determination of Capmatinib in its bulk form and pharmaceutical dosage form.*

## INTRODUCTION:

Capmatinib is a small molecule kinase inhibitor targeted against c-Met (a.k.a. hepatocyte growth factor receptor [HGFR]), a receptor tyrosine kinase that, in healthy humans, activates signaling cascades involved in organ regeneration and tissue repair. Aberrant c-Met activation - via mutations, amplification, and/or overexpression - is known to occur in many types of cancer, and leads to overactivation of multiple downstream signaling pathways such as STAT3, PI3K/ATK, and RAS/MAPK. Mutations in MET have been detected in non-small cell lung cancer (NSCLC), and the prevalence of MET amplification in epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI)-naive patients with NSCLC has been reported to be 1.4% - 21%. This co-occurrence has made c-Met a desirable target in the treatment of NSCLC.[1]

Aberrant activation of c-Met has been documented in many cancers, including non-small cell lung cancer (NSCLC). Mutations that result in the skipping of _MET_ exon 14 lead to the formation of a mutant c-Met with a missing regulatory domain - these mutant proteins have a reduced ability to negatively regulate, leading to a pathological increase in their downstream activity. Capmatinib inhibits the phosphorylation of both wild-type and mutant variants of c-Met triggered by the binding of its endogenous ligand, hepatocyte growth factor - in doing so, it prevents c-Met-mediated phosphorylation of downstream signaling proteins, as well as the proliferation and survival of c-Met-dependent tumor cells.[2-4]




*Structure: Capmatinib*

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[Doi: 10.1000/ijcrips/2022/05]

## MATERIALS AND METHODS:

**Chemicals:**

Ansalytical HPLC grade solvents were used in all experiments, including Acetonitrile, Water, Ammonium Acetate, Glacial Acetic acid, Methanol.

**Equipments:**

Analytical weighing balance make Shimadzu, Ultra Sonicator. Make- Bio-Technics India, Make- pH Meter: Make-Hanna Instruments, Italy High Performance Liquid Chromatography Make: Analytical Technologies Ltd, UV Spectrophotometer. Make- Shimadzu.

**Chromatographic System:**

**Determination of Wavelength**

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is one that gives good response for the drugs that are to be detected. For good response, optimization of wavelength was done at different wavelengths by UV detector. In the present study, drug solutions of 100 µg/mL of Capmatinib were prepared in Acetonitrile. After observing UV spectra of the drug, wavelength by spectra was found at 252 nm and selected for further study.[5]

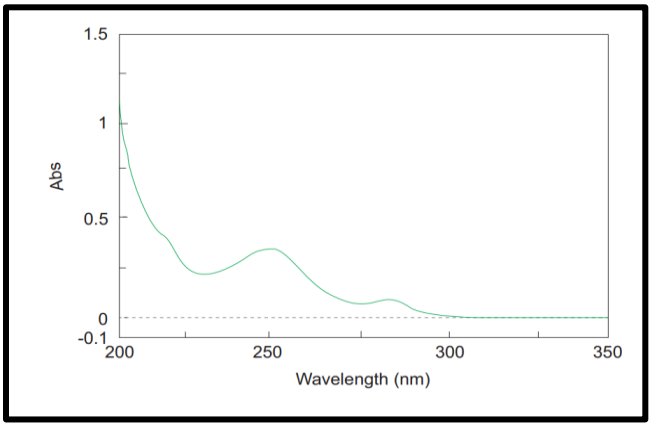


*Figure 1: UV Spectra of Capmatinib Showing λmax 252 nm*

**Preparation of Mobile Phase:**

Prepare mobile phase by taking methanol and ACN in various proportion ACN : methanol (60: 40). Mobile phase was filtered through 0.45µm membrane filter and degassed by sonication for 20 min.[6]

**Preparation of Diluent :**

Dilute with the mixture of Methanol and Acetonitrile 40:60 v/v, Mix well, Sonicate to degas.

**Solution preparation:**

**Preparation of standard stock solutions:**

Accurately 100.0 mg weighed quantity of Capmatinib was transferred to 100.0 mL volumetric flask. That was dissolved by adding 50.0 mL mobile phase and then the drug solution was diluted up to the mark with mobile phase to get the stock solution of 1000 µg/mL of Capmatinib. The working standard solutions of these drugs were obtained by appropriate dilution of the respective stock solution with mobile phase. [7, 8]

**Preparation of Sample Solution**

20 Tablet of Capmatinib marketed formulation were taken, and crushed. The average amount of powder equivalent to 10 mg of capmatinib were taken and dissolved in 100 ml solvent. Sonicate for 10 min with occasional swirling. Withdraw 0.6 ml and transferred to 10 mL volumetric flask & diluent was added to make up the volume. Sonicate for 10 min with occasional swirling. The above solution was filtered through 0.45µm membrane filter, The prepared solution is of 6 µg/ml.[9-11]

*Table 1: Chromatographic Condition*

| | |
|---|---|
| **Mobile Phase** | Acetonitrile: Methanol (60:40 v/v) pH3 |
| **Column** | Cosmosil C18 (4.6 mm x 290mm, Particle size 5 µm) |
| **Flow rate** | 1.0 ml/min |
| **Injection volume** | 20µl |
| **Wavelength** | 252 nm |
| **Run time** | 3.8 min |
| **HPLC Make** | Analytical Technologies Ltd |

## RESULTS AND DISCUSSION:

**Method Validation:**

The method was validated for Linearity, Accuracy, Precision, Robustness, Ruggedness, LOQ, LOD, Specificity, System Suitability, and degradation studies parameter according to ICH guidelines.[12]

**System suitability:**

System suitability parameters were measured to verify the system, method and column performance. Standard solution of Capmatinib was injected in to the system for six times and system suitability parameters were checked.[13]

*Table No.2: Data for System suitability study*

| Sr. No. | conc. (µg/ml) | Retention Time (min) | Theoretical plates | Asymmetry Factor |
|---|---|---|---|---|
| 1 | 6 | 3.81 | 8264 | 1.15 |

| | | | | |
|---|---|---|---|---|
| 2 | 6 | 3.8 | 8132 | 1.14 |
| 3 | 6 | 3.78 | 8645 | 1.15 |
| 4 | 6 | 3.82 | 8123 | 1.14 |
| 5 | 6 | 3.85 | 8046 | 1.15 |
| 6 | 6 | 3.76 | 8231 | 1.15 |
| **Mean** | | 3.80 | 8240.17 | 1.15 |
| **SD** | | 0.03 | 213.39 | 0.01 |
| **%RSD** | | 0.83 | 2.59 | 0.45 |

**Specificity:**

Excipients and impurities were not interacting with the standard drugs. Hence method is specific. Results of specificity are shown in Table 3.[14]

*Table No.3: Data for specificity study of Capmatinib*

| Drug conc. (µg/ml) | Excipients (µg/ml) | Total conc. (µg/ml) | Area | Mean | SD | % RSD |
|---|---|---|---|---|---|---|
| 2 | 4 | 6 | 1564842 | 1564462 | 9951.44 | 0.64 |
| 2 | 4 | 6 | 1574218 | | | |
| 2 | 4 | 6 | 1554326 | | | |
| 4 | 4 | 8 | 3256485 | 3250908.333 | 17267.15 | 0.53 |
| 4 | 4 | 8 | 3264698 | | | |
| 4 | 4 | 8 | 3231542 | | | |
| 6 | 4 | 10 | 4695415 | 4675041 | 22102.21 | 0.47 |
| 6 | 4 | 10 | 4678165 | | | |
| 6 | 4 | 10 | 4651543 | | | |

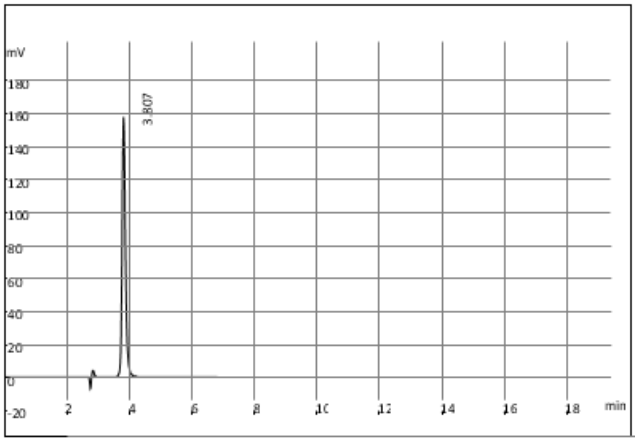


*Figure. No. 2: A Chromatogram of Specificity of 6µg/ml Capmatinib*

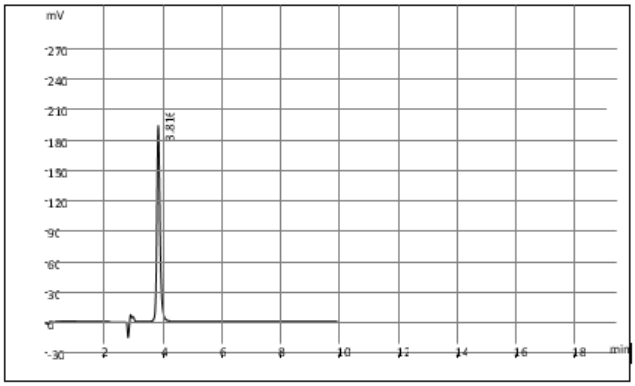


*Fig. No. 3: A Chromatogram of Specificity of 8µg/ml Capmatinib*

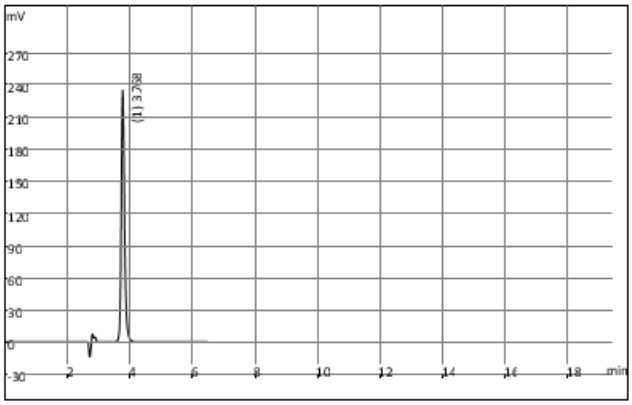


*Fig. No. 4: A Chromatogram of Specificity of 8µg/ml Capmatinib*

**Linearity:**

Capmatinib was found to be linear in the concentration range of 2-10 µg/ml. Results obtained are shown in Table 4.

*Table 4: Linearity graph*

| Sr. No. | Conc. (µg/ml) | Area |
|---|---|---|
| 1 | 2 | 1548794 |
| 2 | 4 | 3278497 |
| 3 | 6 | 4689741 |
| 4 | 8 | 6316448 |
| 5 | 10 | 7949516 |

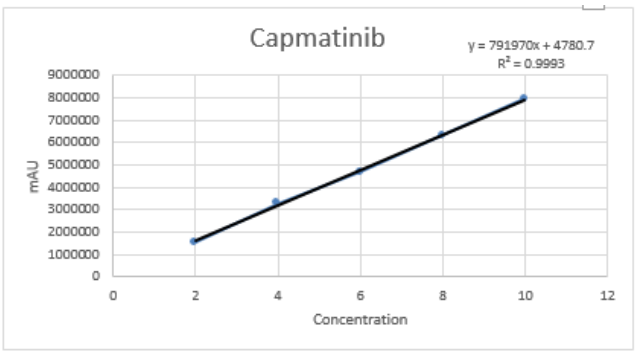


*Fig. No. 5: Calibration curve for Capmatinib*

**Accuracy:**

Accuracy was studied by standard addition method and % recovery found was within acceptable limit. 3 determinations were performed in every level. The individual recovery and mean recovery was greater than 99%. As a result the accuracy of proposed method was developed and shows to the smart recovery values. Results of recovery study are shown in Table no.15 and statistical validation is shown in Table 5. [15-19]

*Table No.5: Data for recovery study of Capmatinib*

| Level of addition | Standard added (mg) | Drug in tablet (mg) | Final Conc. (µg/ml) | Area obtained* | Std Area | Drug recovered (µg/ml) | %Recovery |
|---|---|---|---|---|---|---|---|
| 50% | 2 | 4 | 6 | 4694512 | 4689741 | 75.08 | 100.10 |
| | 2 | 4 | 6 | 4701526 | | 75.19 | 100.25 |
| | 2 | 4 | 6 | 4680215 | | 74.85 | 99.80 |
| 100% | 4 | 4 | 8 | 6288595 | 6316448 | 99.56 | 99.56 |
| | 4 | 4 | 8 | 6334572 | | 100.29 | 100.29 |
| | 4 | 4 | 8 | 6274381 | | 99.33 | 99.33 |
| 150% | 6 | 4 | 10 | 7956404 | 7949516 | 125.11 | 100.09 |
| | 6 | 4 | 10 | 7915478 | | 124.46 | 99.57 |
| | 6 | 4 | 10 | 7861493 | | 123.62 | 98.89 |

*Table No.6: Statistical validation of Capmatinib*

| Level of addition | % Mean recovery* | SD | % RSD |
|---|---|---|---|
| 50% | 100.05 | 0.23 | 0.23 |
| 100% | 99.73 | 0.50 | 0.50 |
| 150% | 99.52 | 0.60 | 0.60 |

**Precision**

Intraday and inter-day precision assures the repeatability of test results. The % RSD found was below 2 for Capmatinib. Result of intraday and inter-day precision was shown Table.

The % RSD found below 2, Hence results complies as per guidelines.

*Table No.7: Data for intraday precision of Capmatinib*

| Sr. No. | Conc. (µg/mL) | Area | Mean | SD | %RSD |
|---|---|---|---|---|---|
| 1 | 2 | 1536452 | 1532613.33 | 15785.03 | 1.03 |
| 2 | 2 | 1546125 | | | |
| 3 | 2 | 1515263 | | | |
| 4 | 6 | 4661450 | 4678603.33 | 33133.98 | 0.71 |
| 5 | 6 | 4679846 | | | |
| 6 | 6 | 4694514 | | | |
| 7 | 10 | 7931457 | 7933687.67 | 20073.17 | 0.25 |
| 8 | 10 | 7954783 | | | |
| 9 | 10 | 7914823 | | | |

*Table No.8: Data for inter-day precision of CAPMATINIB*

| Sr. No. | Conc. (µg/mL) | Area | Mean | SD | %RSD |
|---|---|---|---|---|---|
| 1 | 2 | 1553149 | 1553344.00 | 11429.75 | 0.74 |
| 2 | 2 | 1542013 | | | |
| 3 | 2 | 1564870 | | | |
| 4 | 6 | 4681493 | 4684764.67 | 16334.12 | 0.35 |
| 5 | 6 | 4670314 | | | |
| 6 | 6 | 4702487 | | | |
| 7 | 10 | 7978419 | 7966449.67 | 26186.01 | 0.33 |
| 8 | 10 | 7984512 | | | |
| 9 | 10 | 7936418 | | | |

**Robustness**

Robustness was studied by different deliberate variations in the chromatographic conditions i.e. Change in flow rate and wavelength. From robustness study % RSD was found to be within limit of 2 % for the Capmatinib. Hence it is robust and complies as per ICH guidelines. Results are shown in Table 9.

*Table No.9: Data for Robustness study of Capmatinib*

| Sr. No | Parameter | Condition | Area | Mean | SD | %RSD |
|---|---|---|---|---|---|---|
| 1 | Change in Flow rate (ml/min) | 0.9 | 4689715 | 4704808 | 16955.3 | 0.36 |
| 2 | | 1 | 4701554 | | | |
| 3 | | 1.1 | 4723154 | | | |
| 1 | Change in Wavelength (nm) | 250 | 4661542 | 4667653 | 19883.2 | 0.43 |
| 2 | | 252 | 4689874 | | | |
| 3 | | 254 | 4651542 | | | |

**Ruggedness**

Ruggedness was studied by different analyst. From ruggedness study % RSD was found to be within limit of 2 % for the Capmatinib. Hence it is complies as per ICH guidelines. Results obtained are shown in Table 10.

*Table No.10: Data for ruggedness study of Capmatinib*

| Sr. No | Analyst | Conc. (µg/ml) | Area | Mean area* | SD | % RSD |
|---|---|---|---|---|---|---|
| 1 | Analyst-I | 6 | 4694845 | 4675133.33 | 17071.34 | 0.37 |
| | | | 4665413 | | | |
| | | | 4665142 | | | |
| 2 | Analyst-II | 6 | 4698551 | 4677181.66 | 23850.33 | 0.51 |
| | | | 4651452 | | | |
| | | | 4681542 | | | |

**Limit of Detection and limit of Quantitation**

The results of LOD and LOQ are presented in Table

| Drugs | LOD (µg/ml) | LOQ (µg/ml) |
|---|---|---|
| **CAPMATINIB** | 0.047 | 0.1443 |

**% Assay of Marketed formulation**

The % Assay of Rahika Capmatinib 200 mg marketed formulation of Nutricharge was calculated and given in Table 11.

*Table No 11: % Assay of Marketed Formulation*

| Sr. No. | Marketed Formulation | Area Obtained* | Area of Standard | % Assay |
|---|---|---|---|---|
| 1 | Rahika Capmatinib 200 mg | 4635384 | 4689741 | 98.84 |

**Degradation Studies:**

Stress testing of the drug substance can help to identify the likely degradation products, the stability and specificity of the analytical procedure. Degradation studies were performed on solutions containing 6µg/ml of Capmatinib. Results of the forced degradation studies are summarized in Table

*Table No. 12: Results of Forced Degradation Studies for Capmatinib*

| | Acid stress | Alkali stress | Peroxide stress | Thermal stress | Photolytic stress |
|---|---|---|---|---|---|
| **% Recovered** | 85.09 % | 89.52 % | 80.57 % | 95.67 % | 98.30 % |
| **% Degradation** | 14.91 % | 10.48 % | 19.43 % | 4.33 % | 1.70 % |

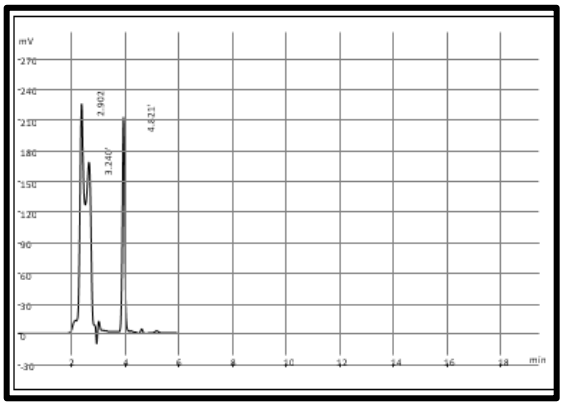


*Fig.No. 6: Chromatogram of Acid Stressed Standard Capmatinib*

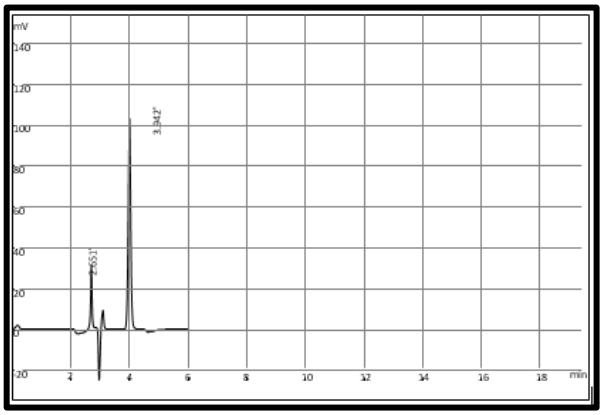


*Fig. No. 7: Chromatogram of Alkali Stressed Standard Capmatinib*

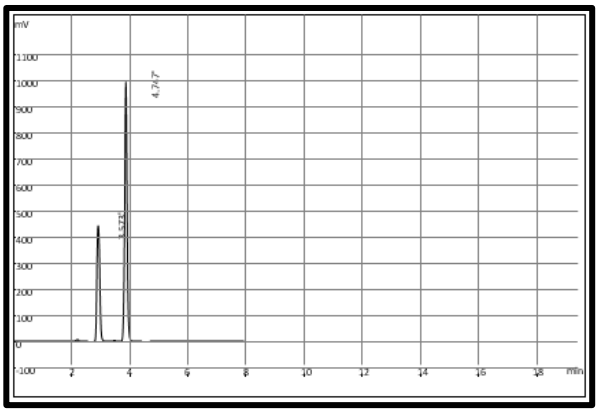


*Fig. No. 8: Chromatogram of Peroxide Stressed Standard Capmatinib*

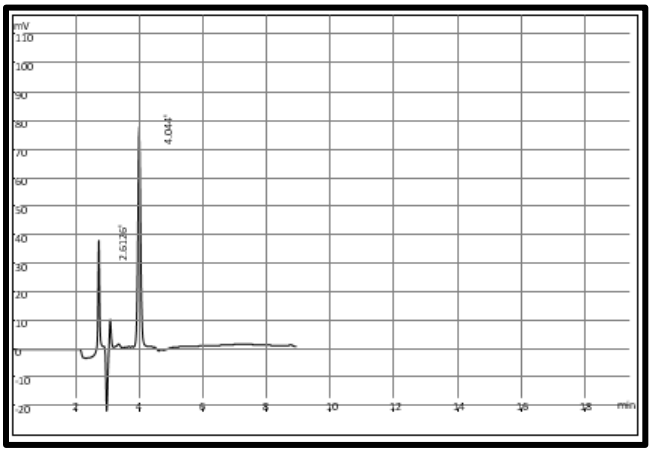


*Fig. No. 9: Chromatogram of Thermal Stressed Standard Capmatinib*

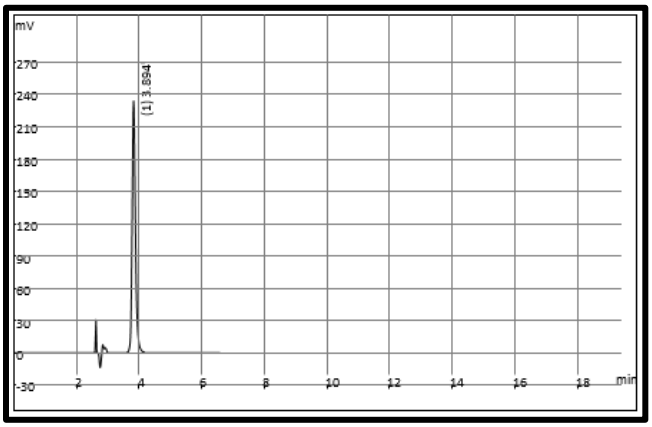


*Fig. No. 10: Chromatogram of Photolytic Stressed Standard Capmatinib*

## CONCLUSION:

In the present research work, a successful attempt was made for determination of Capmatinib in Bulk and dosage form using HPLC. The method was developed by experimentation, based on literature survey. The simplicity, rapidity, reproducibility and economy of the proposed method completely fulfill the objective of this research work.

The stability indicating method was developed and validated for estimation of Capmatinib. The mobile phase was consisting of Acetonitrile: methanol (60:40).The linearity range of Capmatinib was found to be 2-10µg/ml. The calibration curve was plotted and regression equation of Capmatinib was found to be y = 791970x + 4780.7 with correlation coefficient (r 2 ) of 0.9993. Detection was done at 252 nm and

the retention time of Capmatinib was found to be 3.8 min with the flow rate of 1.0 ml/min. From accuracy study % recovery of Capmatinib was found in the range of 98.89-100.29 % which is in the limits accordingly the ICH guidelines. The method was found to be simple, linear, rapid, accurate, precise, reproducible and robust. The % RSD was found within limit as per ICH guidelines. The result showed that proposed chromatographic method was suitable for the accurate, precise and rapid determination of Capmatinib in its bulk form and pharmaceutical dosage form. Degradation studies were performed on solutions containing 6µg/ml of Capmatinib. The force degradation study were employed on five conditions i.e.Acidic, Alkaline, Oxidative, Thermal and Photolytic degradation. The degradation products produced during the stability study were well separated from the pure drug signifying the specificity of developed procedure. The major degradation of drug was found to be in acidic and peroxide stress condition.All the analyzed validation parameters showed acceptable data with

satisfactory correlation co-efficient and lower % RSD as per the ICH guidelines. The developed Stability indicating RP-HPLC method can be utilized by industry for quantitative estimation of Capmatinib as bulk and in tablet dosage form.

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**Cite this article:** Dhone Suresh, S.K.Parjane, Mayur Bhosale, Stability Indicaing RP-HPLC Method Development and Validation of Capmatini In Bulk and Tablet Dosage Form. *International Journal of Current Research and Innovation in Pharma Sciences, 2022;1(1):29-36*