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# Method Development and Validation for the Estimation of Tepotinib in Pharmaceutical Dosage Forms by RP-HPLC

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**ABSTRACT**

The estimate of Tepotinib using RP-HPLC technology was established using a straightforward, precise method. The stationary phase is used in chromatography. Ascentis 150 mm by 4.6 mm by 2.7 µm, mobile phase Acetonitrile: 0.1% OPA in a 50:50 mixture pumped with a flow rate of 1 ml/min, a detection wavelength of 310nm, a column temperature of 30°C, and mobile phase as the diluent. Conditions were set as the best course of action. Method 0.9 and 1.2 were found to be the intermediate accuracy values. LOQ and LOD are, respectively, 0.85g/ml and 0.28g/ml. Using the a forementioned technique99.15% of the formulation used in marketing passed the test. Tepotinib degradation investigations were conducted; under all circumstances, the purity threshold was higher than the impurity angle and in the appropriate range.

## INTRODUCTION:

Tepotinib is an oral tyrosine kinase inhibitor targeted against MET for the treatment of metastatic non-small cell lung cancer in patients exhibiting MET exon 14 skipping mutations. Tepotinib is a MET tyrosine kinase inhibitor intended to treat a variety of MET-overexpressing solid tumors. It was originally developed in partnership between EMD Serono and the University of Texas M.D. Anderson Cancer Center in 2009 and has since been investigated in the treatment of neuroblastoma, gastric cancers, non-small cell lung cancer, and hepatocellular carcinoma.

Tepotinib was first approved in Japan in March 2020 for the treatment of non-small cell lung cancers (NSCLC) with MET alterations, and was subsequently granted accelerated approval by the US FDA in February 2021, under the brand name Tepmetko, for the treatment of adult patients with metastatic NSCLC and MET exon 14 skipping alterations.[7,9] It is the first oral MET-targeted tyrosine kinase inhibitor to allow for once-daily dosing,[9] an advantage that may aid in easing the pill burden often associated with chemotherapeutic regimens. In February 2022, tepotinib was approved for use in Europe. MET is a desirable target in the treatment of certain solid tumors as it appears to play a critical role, both directly and indirectly, in the growth and proliferation of tumors in which it is overexpressed and/or mutated.

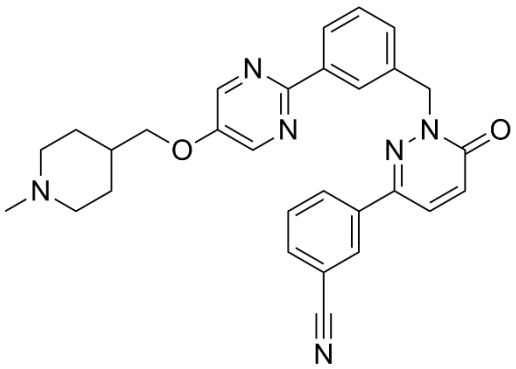


**Figure no 1: Structure of Tipotinib**

The Literature survey indicates that there are no methods for the Estimation of Tepotinib. Therefore an attempt was made to develop and validate a simple and economical RP-HPLC method as per ICH guidelines for the estimation of Tepotinib pharmaceutical dosage forms.

## MATERIALS AND METHODS:

### CHEMICALS

Sun Pharma Limited, Hyderabad has provided the tepotinib pure API drugs. Renkem India provided all the chemicals and buffers utilized in this method. All the solvents used for work are HPLC-grade chemicals.

### Instrumentation and chromatographic condition

AGILENT HPLC, model G4-286b-HPLC system with photodiode array detector was used for the development and method validation with an automated sample injector. Ascentis (150mm x 4.6mm, 2.7mm) column was used for the separation. Acetonitrile and 0.1% OPA are used as mobile phase (50:50). Analysis was carried out in isocratic mode with a flow rate of 1.0ml/min and injection volume were 10 µL. The column temperature was 30 °C; the run time was 5 min. The data acquired was at 310nm. The output signal was monitored and integrated using Empower2 software.

### Preparations of solutions

### Diluent

The diluent used was acetonitrile and water in a ratio of 50:50.

**Preparation of Standard stock solutions:** Accurately weighed 11.25mg of Tepotinib transferred 50ml and volumetric flasks, 3/4th of diluents was added and sonicated for 10 minutes. Flasks were made up of diluents and labeled as Standard stock solution (225µg/ml of Tepotinib)

**Preparation of Standard working solutions (100% solution):** 1ml of Tepotinib from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with a diluent. (22.5µg/ml of Tepotinib)

**Preparation of Sample stock solutions:** 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50ml of diluents were added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters. (2250 µg/ml of Tepotinib)

**Preparation of Sample working solutions (100% solution):** 0.1 ml of filtered sample stock solution was transferred to a 10 ml volumetric flask and made up with a diluent. (22.5µg/ml of Tepotinib).

**Preparation of buffer:**

0.1N Ammonium Formate.

**Method validation**

The method validation of HPLC was carried out for the estimation of Tepotinib drug substance as per ICH guidelines to demonstrate that the method is proposed for routine analysis.

**RESULTS AND DISCUSSION**

**System suitability**: The system suitability parameters were determined by preparing standard solutions of Tepotinib (22.5ppm) the solutions were injected six times and the parameters like peak tailing, resolution, and USP plate count were determined. The % RSD for the area of six standard injection results should not be more than 2%. System suitability parameters are shown in figure 2 and values and mentioned in Table 1.

**Table 1: System suitability results**

| **Sr. no** | **Tepotinib** | | |
|---|---|---|---|
| Inj | RT(min) | USP Plate Count | Tailing |
| 1 | 2.617 | 5554 | 1.10 |
| 2 | 2.619 | 5621 | 1.12 |
| 3 | 2.621 | 5832 | 1.16 |
| 4 | 2.623 | 5779 | 1.18 |
| 5 | 2.625 | 5545 | 1.13 |
| 6 | 2.626 | 5586 | 1.13 |

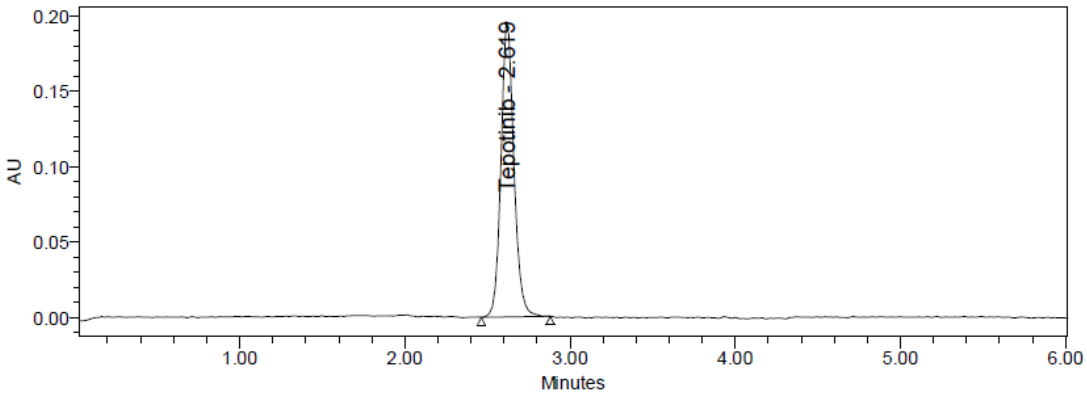


**Figure no: 2 System suitability Chromatogram**

According to ICH guidelines plate count should be more than 2000, the tailing factor should be less than 2 and the resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

**Table no: 2 Linearity for Tepotinib**

| Linearity Level (%) | Concentration (ppm) | Area |
|---|---|---|
| 0 | 0 | 0 |
| 25 | 5.625 | 256844 |
| 50 | 11.25 | 561587 |
| 75 | 16.875 | 787888 |
| 100 | 22.5 | 1070717 |
| 125 | 28.125 | 1329213 |
| 150 | 33.75 | 1577146 |

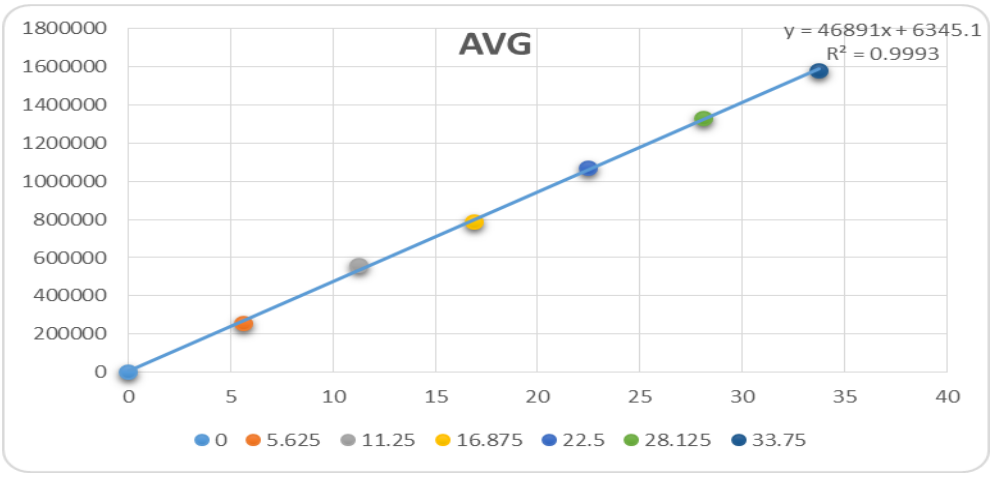


**Figure no 3: Tepotinib calibration curve**

To demonstrate the linearity of the assay method, injected 6 standard solutions with concentrations of about 12.5ppm to 75ppm of Tepotinib were. Plot a graph to concentration versus peak area. The slope obtained was 21101 Y-Intercept was 6345 and the Correlation Co-efficient was found to be 0.999 and Linearity plot was shown in Fig 3.

**Accuracy:** Three Concentrations of 50%, 100%, and 150% are Injected in a triplicate manner, and %Recovery was calculated as:

**Table no 3 Accuracy data**

| **% Level** | **Amount Spiked (µg/mL)** | **%Amount found** | % **RECOVERY** | Mean %Recovery |
|---|---|---|---|---|
| 50% | 11.25 | 11.335 | 100.75 | 99.40% |
| | 11.25 | 11.149 | 99.10 | |
| | 11.25 | 11.089 | 98.57 | |
| 100% | 22.5 | 22.065 | 98.07 | |
| | 22.5 | 22.189 | 98.62 | |
| | 22.5 | 22.191 | 98.62 | |
| 150% | 33.75 | 33.905 | 100.46 | |
| | 33.75 | 33.779 | 100.08 | |
| | 33.75 | 33.849 | 100.29 | |

Three levels of Accuracy samples were prepared by the standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.40% for Tepotinib respectively.

**Table no 4: System precision data of Tepotinib**

| Sr.No | Peak Area |
|---|---|
| 1 | 1057172 |
| 2 | 1065288 |
| 3 | 1040589 |
| 4 | 1041964 |
| 5 | 1055581 |
| 6 | 1049619 |
| AVG | 1051702 |
| STDEV | 9510.1 |
| %RSD | 0.9 |

The Above %RSD for the peak areas of Tepotinib was obtained from six replicate injections of standard solutions which were within the range of the limit (<2%).

From a single volumetric flask of working standard solution, six injections were given and the obtained areas were mentioned above. Average area, standard deviation, and % RSD were calculated. % RSD obtained as 0.9% respectively for Tepotinib. As the limit of Precision was less than "2" the system precision was passed in this method.

**Table no 5: Robustness data for Tepotinib**

| Parameter | %RSD |
|---|---|
| Flow Rate(-) 0.9ml/min | 1.7 |
| Flow Rate(+) 1.1ml/min | 0.9 |
| Mobile phase(-)65B:35A | 1.3 |
| Mobile phase(+)55B:35A | 0.2 |
| Temperature(-) 25°C | 2.8 |
| Temperature(+) 35°C | 3.2 |

Robustness conditions like flow minus (0.9ml/min), flow plus (1.1ml/min), mobile phase minus (50B:50A), mobile phase plus (60B:40A), temperature minus (25°C) and temperature plus (35°C) were maintained and samples were injected in a duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

**Table no 6: % Assay of Teponitib**

| Drug name | Label claim | % Assay |
|---|---|---|
| Tepotinib | 11.25mg | 99.15 |

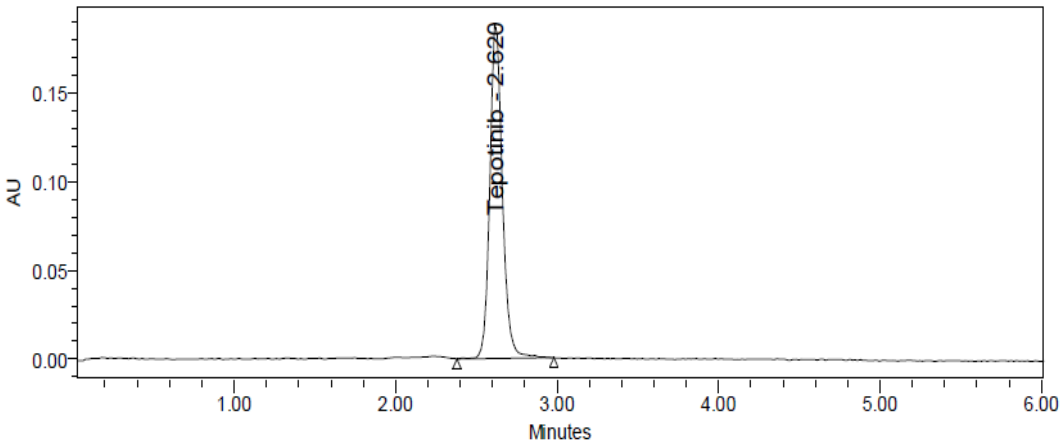


**Figure no 4: % Assay of Tepotinib**

**Table no 7: LOQ and LOD data**

| Drug | LOD | LOQ |
|---|---|---|
| **Tepotinib** | 0.28 | 0.85 |

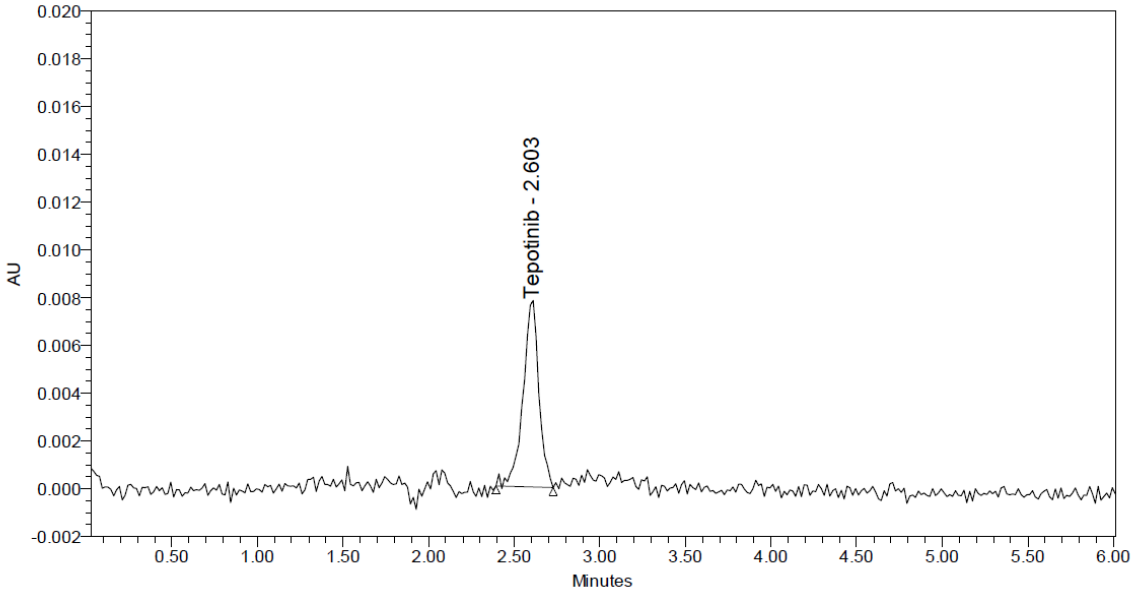


**Figure no 5: LOD chromatogram of standard**

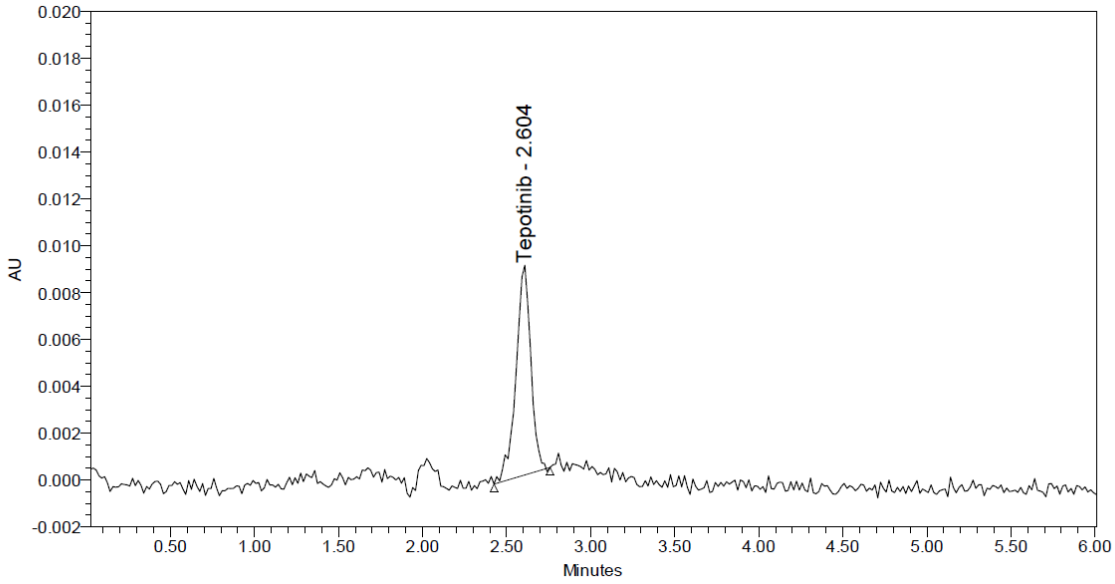


**Figure no 6: LOQ chromatogram of sample**

## SUMMARY

Chromatographic conditions used are stationary phase Ascentis 150mm x 4.6 mm, 2.7µm., Mobile phase0.1% OPA: Acetonitrile in the ratio of 50:50 and flow rate was maintained at 1ml/min, detection wavelength was 310nm, column temperature was set to 30$^{o}$C and diluent was mobile phase conditions were finalized as an optimized method. System suitability parameters were studied by injecting the standard six times and the results were well under the acceptance criteria. A linearity study was carried out between 25% to 150 % levels, $R^2$ value was found to be 0.999. Method Precision was found to be 0.9 and 1.2 for intermediate precision.LOD and LOQ are 0.28µg/ml and 0.85µg/ml respectively By using the above method assay of the marketed formulation were carried out 99.15% was present. Degradation studies of Tepotinib were done, and in all conditions purity threshold was more than the purity angle and within the acceptable range. The full-length method was not performed; if it is done this method can be used for routine analysis of Tepotinib.

## CONCLUSION

A new stability-indicating RP-HPLC technique was developed and validated for the estimation of Tepotinib in pharmaceutical dosage forms. The developed method was precise, accurate, with higher resolutions, shorter retentions with various degradants, and economical. Hence, this method can be used for in-process evaluation in pharmaceutical firms and quality control of these in-drug testing.

## ACKNOWLEDGEMENT

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## ETHICAL APPROVAL

This study does not involve experiments on animals or human subjects.

## REFERENCES

1. Inai T, Mancuso M, Hashizume H, Baffert F, Haskell A, Baluk P, Hu-Lowe DD, Shalinsky DR, Thurston G, Yancopoulos GD, McDonald DM: Inhibition of vascular endothelial growth factor (VEGF) signaling in cancer causes loss of endothelial fenestrations, regression of tumor vessels, and appearance of basement membrane ghosts. Am J Pathol. 2004 Jul;165(1):35-52.
2. Rugo HS, Herbst RS, Liu G, Park JW, Kies MS, Steinfeldt HM, Pithavala YK, Reich SD, Freddo JL, Wilding G: Phase I trial of the oral antiangiogenesis agent AG-013736 in patients with advanced solid tumors: pharmacokinetic and clinical results. J Clin Oncol. 2005 Aug 20;23(24):5474-83.
3. Rini BI: SU11248 and AG013736: current data and future trials in renal cell carcinoma. Clin Genitourin Cancer. 2005 Dec;4(3):175-80.
4. Gilbert JA, Adhikari LJ, Lloyd RV, Halfdanarson TR, Muders MH, Ames MM: Molecular markers for novel therapeutic strategies in pancreatic endocrine tumors. Pancreas. 2013 Apr;42(3):411-21.
5. B. Jala Chandra Reddy and 2N. C. Sarada* et al., Development and validation of Stability Indicating RP-HPLC Method for the Determination of Tepotinib in Bulk and its Pharmaceutical Formulations , Der Pharmacia Lettre, 2016, 8 (11):97-106.
6. Achanta Suneetha1 *, Sharmila Donepudi2 et al., HPLC method development and validation for the estimation of Tepotinib in rabbit plasma, Braz. J. Pharm. Sci. 2017;53(3):e00012.
7. Gorja Ashok1*, Sumantha Mondal2 et al., Development and Validation of Stability indicating method for the estimation of Tepotinib in tablet dosage forms by UPLC, Indian J. Pharm. Biol. Res. 2017; 5(3):1-6.
8. Sunil Pingili Reddy,[1,2] K. Sudhakar Babu,[2] Navneet Kumar,[1] andY. V. V. Sasi Sekhar[1] et al.,Development and validation of stability indicating the RP-HPLC method for the estimation of related compounds of guaifenesin in pharmaceutical dosage forms,Pharm Methods. 2011 Oct-Dec; 2(4): 229–234.