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Development and Validation of RP-HPLC Method for the Determination of Tepotinib in Pure and Pharmaceutical Dosage Forms

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ABSTRACT

The main objective of the proposed study was to develop an accurate, sensitive, precise, quick, and isocratic reverse phase HPLC (RP-HPLC) method and validate it for the quantification of Tepotinib in bulk and pharmaceutical tablet dosage form. Quantitative HPLC is carried out on a waters 2996 high-performance liquid chromatography with a PDA detector module, which included an automated injector with a 5 µl injection volume and a quadra-pump. The column utilized is a Reverse Phase XTerra RP18 C_{18} column (250mmx4.6 mm internal diameter with particle size 5µm) at the ambient temperature at a mobile phase flow rate of 1.0 ml/min under isocratic conditions. The organic modifier used is acetonitrile, while the mobile phase used is 0.03M Sodium Dihydrogen Orthophosphate in water: acetonitrile (25:75 v/v).UV detection at 210 nm is used as the wavelength of detection with a PDA detector. The Retention time of Tepotinib was 4.77 minutes. With a correlation coefficient of about 0.99, peak-response was obtained as function of concentration over the range of 40 to120 µg/ ml for Tepotinib. Tepotinib was shown to have a percentage assay of 109.73 %. Tepotinib had a limit of detection of 2.5 µg/ ml and a limit of quantification (LOQ) of 7.5 µg/ ml. The presence of excipients in the formulation had no effect on the assay method. This method can be used for regular analysis in formulation QC-studies and allows a straightforward, selective, sensitive, and specific assessment of Tepotinib since it is economical and precise.

Keywords: Tepotinib, Tepmetko, RP-HPLC, Isocratic, Acetonitrile

INTRODUCTION

Tepotinib is an oral tyrosine kinase inhibitor targeted against Mesenchymal-epithelial transition factor MET for the treatment of variety of MET-overexpressing solid tumors [1]. 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. 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 [2]. Tepotinib was first approved in Japan in March 2020 for the treatment of metastatic non-small cell lung cancers (NSCLC) with MET alterations, and was subsequently granted accelerated approval by the US FDA on 3rdFebruary 2021, under the brand name Tepmetko, for the treatment of adult patients with metastatic NSCLC and MET exon 14 skipping mutations [3]. It is the first oral MET-targeted tyrosine kinase inhibitor to allow for once-daily dosing, an advantage that may aid in easing the pill burden often associated with chemotherapeutic regimens [4]. In February 2022, Tepotinib was approved for use in Europe. Tepotinib inhibits MET phosphorylation and subsequent downstream signaling pathways in order to inhibit tumor cell

proliferation, anchorage-independent growth, and migration of MET-dependent tumor cells [5]. Tepotinib has also been observed to down-regulate the expression of epithelial-mesenchymal transition (EMT) promoting genes (e.g. MMP7, COX-2, WNT1, MUC5B, and c-MYC) and upregulate the expression of EMT-suppressing genes (e.g. MUC5AC, MUC6, GSK3β, and E-cadherin) in c-MET-amplified gastric cancer cells, suggesting that the tumor-suppressing activity of Tepotinib is driven, at least in part, by the negative regulation of c-MET-induced EMT [6]. It has also been shown to inhibit melatonin 1B and nischarin at clinically relevant concentrations, though the relevance of this activity in regards to Tepotinib's mechanism of action is unclear.

Molecular formula and molecular weight of Tepotinib is $C_{29}H_{28}N_6O_2$ and 492.6 g/ mol respectively. It has an IUPAC Name 3-[1-[[3-[5-[(1-methylpiperidin-4-yl) methoxy] pyrimidin-2-yl] phenyl] methyl]-6-oxopyridazin-3-yl] benzonitrile [7].

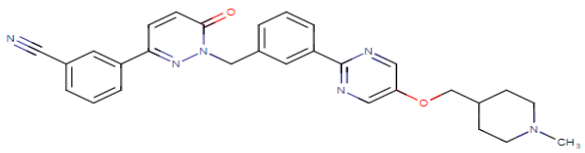

Fig 1: Structure of Tepotinib

The Literature survey indicates that there are very few methods reported for the Estimation of Tepotinib [8-14]. Furthermore, no official or preliminary monograph on this analyte has been published in any of the compendial pharmacopoeias. The goal of this study was to develop an accurate and efficient RP HPLC method to estimate the Tepotinib drug in unit dosage form for oral administration. The validation of the devised approach is also addressed in this study, as per ICH standards [15,16].

MATERIALS AND METHODS

Chemicals and Reagents

Tepotinib, 99% pure was acquired from Sun Pharma Pvt Ltd. HPLC-Grade solvents water and acetonitrile were obtained from Merck Specialities Pvt Ltd, India. Water was deionised and further purified by means of Milli-Q plus water purification system, Millipore Ltd (U.S.A). AR grade Sodium Dihydrogen Orthophosphate was obtained from Ranchem Pharmaceuticals India Ltd.

Instrumentation

Quantitative HPLC is carried out on a waters 2996 high-performance liquid chromatography with a PDA detector module, which included an automated injector with a 5 µl injection volume and a quadra-pump. The column utilized is a Reverse Phase XTerra RP18 C_{18} column (250mmx4.6 mm internal diameter with particle size 5µm). Empower Software is installed on the HPLC equipment. The column temperature is controlled to ambient and eluted over 20.0 minutes at a mobile solvent speed of 1.0 ml/ min under isocratic conditions. The organic modifier used is acetonitrile, while the mobile phase used is 0.03M Sodium Dihydrogen Orthophosphate in water: acetonitrile (25:75 v/v). It is degassed and filtered via 0.45-m Nylon membrane filters before use. For the analyte Tepotinib, UV detection at 210 nm is used as the wavelength of detection with a PDA detector. Acetonitrile is used as the diluent to make the standard dilutions. Tepotinib is found to elute at 4. 77 minutes.

Chromatographic condition and measurement procedure

Preparation of the primary standard drug solution:

To make the primary standard stock solution, 500mg of Tepotinib was dissolved in a 100ml volumetric flask with 20ml of diluent acetonitrile, sonicated for 15 minutes, and then brought up to 100ml with diluent to get the primary standard stock solution containing 5000µg/ml of Tepotinib.

Preparation of working standard drug solution:

After adding 5 ml of the primary working standard solution to the 50-ml volumetric flask, the flask was filled with 50 ml of acetonitrile. This resultant solution, which includes 500 ug/ml of Tepotinib, is suitable for use as a working standard solution. The stock solutions were kept in a cool, dark place that was controlled at a temperature of 4˚C.

Sample preparation:

After measuring the weight of each individual tablet, we were able to calculate the average weight of twenty Tepmetko® pills prepared inhouse. The tablets were crushed into a powder form and a sample weight equivalent to 500-mg of pure Tepotinib was taken. This was shifted to a 100ml pre-calibrated-measuring flask, and dissolved in acetonitrile. After being sonicated in the diluent, it was strained via Whattman#41 filter paper, the resultant primary working sample solution contained 5000 µg/ml of Tepotinib. 5ml of the filtrate was quantitatively transferred to a 50-ml pre-calibrated-measuring flask, the diluent acetonitrile was added to bring the volume of the solution to 50 ml. This resulting solution serves as a working testing solution containing 500 µg/ml of Tepotinib. The stock solutions were kept in a dark place at 4 degrees centigrade.

Optimized chromatographic conditions

The chromatographic conditions were optimized finally using 0.03M Sodium Dihydrogen Orthophosphate in water: Acetonitrile (25:75 v/v) as the eluting solvents in isocratic mode at a flow rate of 1ml/min with an injection volume of 5 µL. Run time was 20 minutes, at an ambient column oven temp with acetonitrile (sonicated and degassed) as diluent in a XTerra RP18 C_{18} column (250mm x 4.6 mm internal diameter with particle size 5µm). The detection was done by Photo diode array (PDA) detector at a wavelength of 210nm. The retention time was found to be 4.77mins.

Linearity: Aliquots of Tepotinib drug working stock solutions were placed in various 10ml volumetric flasks and made the volume up to the 10ml with the mobile phase, yielding in final strengths of 200-600 µg/ml (Table 2). The peak areas and retention times of the drug solutions (loaded at 5µL) were measured thrice in the column. Using a PDA-detector set at 210 nm, a linearity-graph was generated by plotting peak areas-vs-Tepotinib concentration in µg/ ml.

Accuracy: The approach's accuracy was found by evaluating the drugs' recovery using the standard-spiking method. To assess if the analyte contained in the formulation caused positive or negative interventions, known amounts of the drug equivalent to 10 percent standard drug solution was added to 80 percent, 100 percent, and 120 percent of the target test concentration of formulation. Each set-of-addition was replicated thrice at each dilution level. The results were compared to a competent reference standard after extraction of sample preparation. The percentage of analyte recovered by the assay was used to assess the accuracy. Table 3 shows the results of accuracy investigations on standard solution and process-related impurity; recovery measurements suggest that the procedure was accurate.

Precision: Quality-control samples in 100 % (w/v) dilution were used to assess intraday and inter-day precision. On the same day, six replicates of the target concentrations were examined for intra-day variation, and six replicates were examined for inter-day variation on three different days. The method's repeatability is indicated by the low RSD value (1%). (Table 4)

Limits of detection and quantification: The method's LOD was set at the lowest concentrations of active pharmaceutical components with a signal-to-noise (S/N) ratio of around 3. (LOD). The lowest active therapeutic medication concentrations that can be assessed with acceptable precision and accuracy while maintaining a signal-to-noise (S/N) ratio of roughly 10 (LOQ).

Method applicability: The newly created method was evaluated by applying it to pharmaceutical tablets for the estimation of Tepotinib.

RESULTS AND DISCUSSION:

The purpose of this research was to create a chromatographic technique for the quantifiable determination of unit-dose Tepotinib.

Optimization of chromatographic conditions: An isocratic RP- HPLC procedure for assaying the active ingredient was developed due to lack of an easy, reproducible, and quick-to-use method for the determination of Tepotinib concentration in formulary matrices. The effect of various HPLC technique variables on the result of the study was examined to optimize the chromatographic parameters. Various proportions of CH_3CN: O-H_3PO_3, CH_3CN-H_2O, and CH_3CN-KH_2PO_4 buffer were tested. After several early investigatory tests, 0.03M Sodium Dihydrogen Orthophosphate in water: Acetonitrile (25:75 v/v) was chosen over other mobile phases because it resulted in improved resolution of active component. This procedure gives the good estimation of analyte after multiple exploratory & investigatory trail runs. The active pharmaceutical analyte had excellent UV sensitivity and was interference-free at 210 nm. The analyte peak was highly defined and without any incidence of tailing under these conditions. The set of conditions previously noted in this article were chosen for additional validation after considering the entire body of data acquired from this extensive study.

Method validation tests [11, 12]**:** Method precision (RSD, percent), method accuracy (recovery percent & %RSD,), linear range (r^2), and LOD & LOQ were explored as recommended method validation characteristics.

Linearity and Range: With a correlation coefficient of 0.99, the graph of chromatographic-peak areas of analyte versus respective concentrations was shown to be linear in the band of 200-600 µg/ml for Tepotinib (Table 3). The least square fit data of linear regression analysis was derived from the measurements and is given in Table 1. For Tepotinib it is y= 10755x. Table 1 presents the regression parameters for this technique that include slope, intercept, and % RSD. These findings suggest that there was a significant correlation.

Accuracy: Individual recovery of the analyte at 80 %-dilution level on w/v basis, 100 %-dilution level on w/v basis and 120 %-dilution level on w/v basis of prescribed concentrations was 85.8 percent to 89.6 percent, for Tepotinib demonstrating the method's accuracy. The RSD was usually less than 1% in these data, demonstrating that the technique seems to be very accurate and generates consistent results (Table 3).

Precision: Table 4 summarizes the intraday and inter day fluctuation in precision analysis. The method's repeatability is indicated by the low RSD value (less than-1%). These results show that the approach has a high level of precision and repeatability, both within a single analytical run and across multiple runs (Table 4).

Limit-of-detection & limit-of-quantifications: Tepotinib has a limit of detection of 2.5 µg/ml and a limit of quantification (LOQ) of 7.5 µg/ml, respectively. These numbers illustrate the method's high sensitivity, which is essential in most investigations, as well as the fact that it can be used to detect and quantify the analyte over a wide concentration range.

Specificity: The retention time for was determined to be 4.7 mins, according to the representative chromatogram given in Figure 1. When the pharmaceutical tablet matrices were evaluated, no indication of excipient interference signals was observed in the respective retention time of the chromatogram. It indicates that the analyte was not disturbed of probable merging peaks. As a result, this technique can be employed with certainty.

Table 1: Regression analysis & Operating-System Suitability Results:

Study-Parameter	Tepotinib
Retention Time (min)	4.7
Peak areas	5413036
Percentage of peak areas	99.94
USP-Tailing	1.07
Theoretical Plates	8070.87
Resolution	4.59
Linear range (µg/ml)	200-600
Limit-of-Detection (µg/ml)	2.5
Limit-of-Quantification (µg/ml)	7.5
Correlation-Coefficient (r^2)	0.99
Assay-in-Percentage (%)	99.93

Table 2: Summary of the standard calibration Curve for Linearity experiment

Calibration Standard Dilution Level	Concentration of Tepotinib (µg/ml)	Peak Area
40 %	200	2619915
60 %	300	3427024
80%	400	4093358
100 %	500	5259835
120 %	600	6339714

Table 3: Accuracy evaluation by Spike-analysis method

Accuracy study at 80% target level	Injection Number	Tepotinib (Tepmetko)	
		Standard Soln.	Spiked Soln.
Tepmetko-® tablet dosage form solution at 80% level was spiked with 10% of standard solution of Tepotinib	1	4303571	4831206
	2	4406026	4866728
	3	4364677	4852163
	Mean area	4352293	4849277
	Std. Dev	54362	16875
	% RSD	1.2	0.6
	%Recovery		85.8
80% of the target concentration is equivalent to Tepotinib 400 µg/ml in acetonitrile as diluent.			
Accuracy study at 100% target level	Injection Number	Tepotinib (Tepmetko)	
		Standard Soln.	Spiked Soln.
Tepmetko-® tablet dosage form solution at 100% level was spiked with 10% of standard solution of Tepotinib	1	5528868	6126795
	2	5468750	6031967
	3	5509341	6056675
	Mean area	5495133	6071593
	Std. Dev	29917	58916
	% RSD	0.8	0.98
	% Recovery		98
100% of the target concentration is equivalent to Tepotinib 500 µg/ml in acetonitrile as diluent.			
Accuracy study at 120% target level	Injection Number	Tepotinib (Tepmetko)	
		Standard Soln.	Spiked Soln.
Tepmetko-® tablet dosage form solution at 120% level was spiked with 10% of standard solution of	1	6523087.679	7199304
	2	6450775.882	7036699
	3	6560129.648	7004284
	Mean area	6505522.5	7034853

Tepotinib	Std. Dev	78365.00	132815
	% RSD	1.19	1.8
	%Recovery		89.60
120% of the target concentration is equivalent to Tepotinib 600 µg/ml in acetonitrile as diluent.			

Table 4: Evaluation of precision with-in-day and day-to-day analysis

Intra-Day Precision study of 100% standard dilution containing 500µg/ ml of Tepotinib			Inter-Day Precision study of 100% standard dilution containing 500µg/ ml of Tepotinib	
S. No	Tepotinib		Tepotinib	
	Ret. time	Peak area	Ret. time	Peak area
1	4.766	5473169	4.772	5473169
2	4.760	5424649	4.765	5424649
3	4.770	5507732	4.758	5507732
4	4.772	5479818	4.757	5479818
5	4.760	5440818	4.771	5440818
6	4.756	5507974	4.767	5507974
Average	4.764	5465391	4.765	5465391
Std. Dev	0.007	44902	0.006	44902
% RSD	0.14	0.60	0.14	0.60

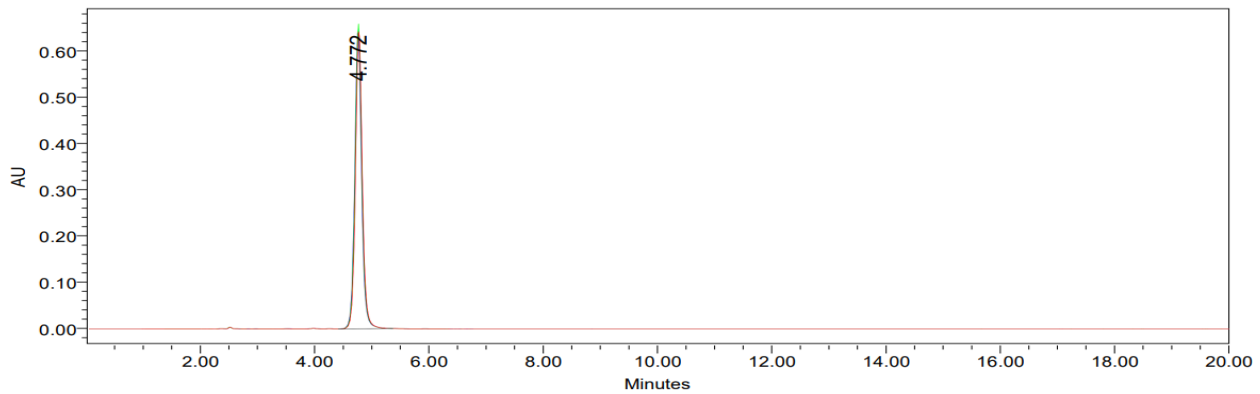

Figure 2: Chromatogram of Tepotinib 500µg/ml analyzed by optimized Isocratic RP-HPLC method.

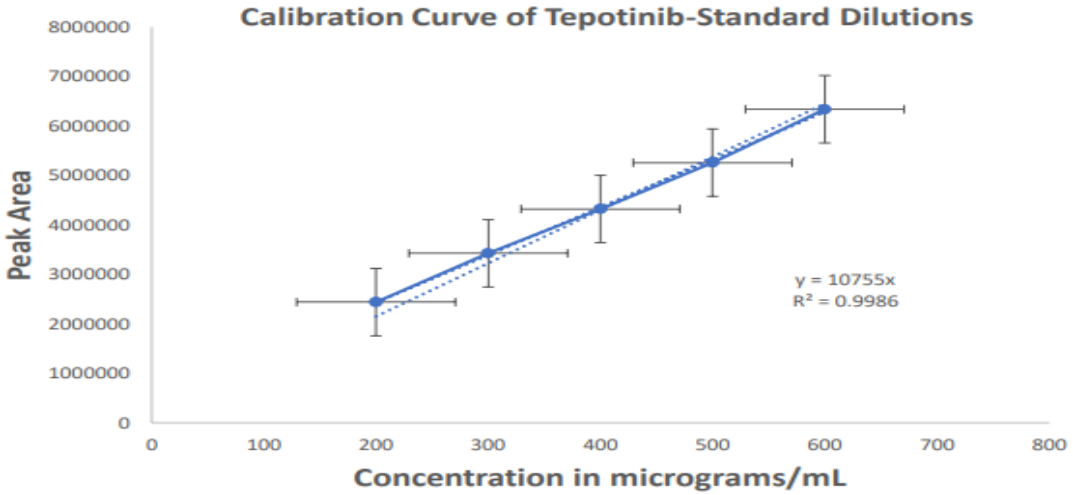

Figure 3: Linearity graph of Tepotinib dilutions of standard solutions:

CONCLUSION

In this study, an efficient and commonly available HPLC method for the analysis of Tepotinib in pharmaceutical matrices was devised. This method's key advantages are its significantly reduced run times, ease of use, and ease of operation. All these features are critical in operation, especially when analyzing a large number of samples. The validation experiments demonstrated that the procedural approach has a large calibration concentration range, adequate precision & accuracy, and practically reliable sensitivity. The method can be used for regular analysis in formulation QC-studies and allows for a straightforward, selective, sensitive, and specific assessment of Tepotinib.

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AUTHORS CONTRIBUTIONS

I, declare that it's an original research work which was carried out by the author.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Sung-HwaSohn, Hee Jung Sul, Bohyun Kim, Bum Jun Kim, Hyeong Su Kim and Dae Young Zang. Tepotinib Inhibits the Epithelial–Mesenchymal Transition and Tumor Growth of Gastric Cancers by Increasing GSK3β, E-Cadherin, and Mucin 5AC and 6 Levels. Int J Mol Sci. 2020 Sep; 21(17): 6027.
2. Witthawat Ariyawutyakorn, Siriwimon Saichaemchan, and Marileila Varella-Garcia. Understanding and Targeting MET Signaling in Solid Tumors - Are We There Yet? J Cancer. 2016; 7(6): 633–649.
3. Alisha Desai and Sandra Cuellar. The Current Landscape for METex14 Skipping Mutations in Non–Small Cell Lung Cancer. J Adv Pract Oncol. 2022 Jul; 13(5): 539- 544.
4. https://go.drugbank.com/salts/DBSALT003143.
5. Guifang Jing, Fang Yu, and Huandong Xue. Tepotinib suppresses proliferation, invasion, migration, and promotes apoptosis of melanoma cells via inhibiting MET and PI3K/AKT signaling pathways. Oncol Lett. 2022 Jun; 23(6): 170.

6. Sung-Hwa Sohn, Hee Jung Sul, Bohyun Kim, Bum Jun Kim, Hyeong Su Kim and Dae Young Zang. Tepotinib Inhibits the Epithelial–Mesenchymal Transition and Tumor Growth of Gastric Cancers by Increasing GSK3β, E-Cadherin, and Mucin 5AC and 6 Levels. International Journal of Molecular Sciences 21(17):6027. DOI:10.3390/ijms21176027.
7. https://pubchem.ncbi.nlm.nih.gov/compound/ Tepotinib.
8. J.Nikhil, C. Parthiban, M. Sudhakar, K. Vijaya Sri. Method Development and Validation for the Estimation of Tepotinib in Pharmaceutical Dosage forms by RP-HPLC. IJPPR. Human, 2022; Vol. 26 (1): 468-477.
9. Monika Shirwar, Shubhangi Birajdar, Shruti Garad, Smita Kumbhar. Development and validation of novel UV-visible Spectrophotometric Method for Estimation of Tepotinib in Bulk and in Pharmaceutical Formulation. IJPPS, 2023; Vol 5 (9): 32-36.
10. Sk. Mastanamma, G. Geethika, G. Raja Chakravarthy and S. Harika. RP-HPLC method development and validation for the estimation of Tepotinib in presence of its impurities in a tablet dosage form, IJPSR, 2024; Vol. 15(5): 1506-1516.
11. Dr. S. Srinivasa Rao, Dr. Subhas Sahoo, K. Kavitha. Stability indicating method development and validation for the estimation of tepotinib in api and tablet dosage form by RP-HPLC. JIDPTS. Volume:6, Issue:10, Oct:2023: 14-17.
12. Mohamed W. Attwa, Gamal A.E. Mostafa, Haitham Al Rabiah and Adnan A. KadI. An LC–MS/MS Analytical Method for Quantifying Tepotinib in HumanLiver Microsomes: Application to In Vitro and In Silico Metabolic Stability Estimation. Separations 2023, 10, 330.
13. Smita T Kumbhar, Pratima S Kokare, Pradip B Digge. Development of Novel RP-HPLC Method for Estimating Tepotinib in Bulk and Pharmaceutical Dosage Form IJPQA, Volume 14 Issue 4, October - December 2023. 1188-1193.
14. Dr. Meruva Sathish Kumar, Mrs. S. Marakatham Dr. Phani Deepika Polampalli, Dr. S. Siva Prasad. Development and Validation of UV Spectrophotometric Method for Determination of Tepotinib in Bulk and Pharmaceutical Dosage Forms. Research Square. March 2024, 1-16
15. ICH guidelines validation of analytical procedures; Text & Methodology Q2 R1; 2005.
16. ICH Harmonizes Tripartite guidelines, validation of analytical procedures; Text & Methodology Q2 (R1); current step 4 version. 2005, 1- 13.