RESEARCH ARTICLE

Development of Novel RP-HPLC Method for Estimating Tepotinib in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

Objective: The study's goal is to develop a highly appropriate, fast, precise, and validated reverse-phase high-performance liquid chromatography (RP-HPLC) approach that indicates stability for the estimation of tepotinib in pharmaceutical dosage form and bulk, in accordance with ICH recommendations.

Method: The components were separated by chromatography using a Phenomenex Kinetex XB-C18 (150×4.6 mm, 5µ) column. The absorbance was measured at 272 nm, and the flow rate was 1.0 mL/min. The International Council for Harmonisation (ICH) states that checks were made for linearity, precision, accuracy, system appropriateness, specificity, and protocol robustness.

Results: Tepotinib was shown to have a retention period of 3.4 minutes. The results showed that the linearity ranged from 5 to 25 µg/mL ($r^2 = 0.9993$), and the percentage mean recoveries for tepotinib's accuracy and precision fell within the range of (%RSD< 2). It was discovered that the Limit of Detection (LoD), and Limit of Quantitation (LoQ) were, respectively, 0.429 and 1.3 µg/mL.

Keywords: Tepotinib, Verification, Indicating Stability, ICH guidelines for reverse-phase high-performance liquid chromatography.

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INTRODUCTION

For patients with MET exon 14 skipping mutations who have metastatic non-small cell lung cancer, tepotinib is an oral tyrosine kinase inhibitor that is prescribed. The chemical name 3-[1-[3-[5-[(1-methyLpiperidin-4-yl) methoxy] pyrimidin-2-yl] phenyl] methyl] is used to refer to temopinib. benzonitrile [6-oxopyridazin-3-yl].[1,2] Its molecular weight is 492 g/mol. It has the chemical formula $C_{29}H_{28}N_6O_2$. Figure 1 depicts the structure of temotinib. A MET tyrosine kinase inhibitor called tematinib is used to treat a variety of solid tumors that overexpress MET. Tepotinib binds to MET tyrosine kinase preferentially and inhibits MET signaling pathways, perhaps leading to death in cancer cells that overexpress this kinase.[2] Tepotinib is soluble in water, methanol, ethanol, and acetonitrile. Methanol was therefore employed as a diluent for the task.

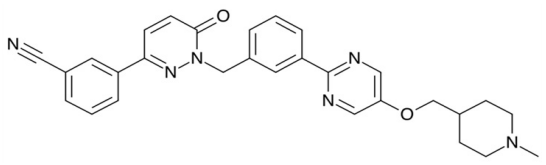

Figure 1: Structure of tepotinib

MATERIALS AND METHODS

Materials

The reference standard, tecunitinib API, was obtained from Spectrum Pharma Research Solutions Pvt Ltd in Hyderabad. The India Mart app was used to acquire Tepmetko pills. We bought water, acetonitrile, and HPLC-grade methanol from Merck Pvt. Ltd. in Mumbai.

Instrumentation

The Shimadzu AY220 analytical balance, the Oscar Microclean 103 ultrasonicator, and the Agilent 1260 Infinity II autosampler are examples of high-performance liquid chromatography (HPLC) equipment.

Optimized Chromatographic conditions[3-5]

Optimized chromatographic conditions are shown in Table 1.

Designing the tepotinib API standard stock solution

A total of 10 mL of tepotinib was precisely weighed and then deposited into a volumetric flask containing 10 mL. A total of 5 mL of methanol was then added to the tepotinib and under ultrasound for 10 minutes until the volume reached 10 mL with methanol. Take 1-mL of the aforesaid solution, transfer

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it to a 10 mL volumetric flask, and add methanol to bring the volume up to 10 mL.[6,7]

Arrangement for tepotinib tablet sample solution

The tablet powder containing 10 mg of tepotinib was added to a 10 mL volumetric flask. After adding 5 mL of methanol and sonicating for 10 minutes, the volume was adjusted to the desired level. 0.1 mL of the sample was transferred from the aforementioned solution to a volumetric flask containing 10 mL. After adding 5 ml of sonicated methanol for 10 minutes, the volume was adjusted to the desired level.[8,9]

Wavelength selection for tepotinib

The composition of the mobile phase for the manufacture of tepotinib was obtained, and using a UV-vis spectrophotometer, the UV range 200–400 nm was scanned. The absorption maxima was identified as 272 nm from the observed UV spectrum.[9,10]

Method Validation

Linearity and range

For the linearity studies of tepotinib the concentration was determined over the range 5 to 25 µg/mL. The calibration curve was plotted by taking the peak area verses concentration in µg/mL. From the calibration curve the values of the coefficient of regression, slope, and Y-intercept were calculated.

Accuracy

Accuracy of 80, 100, and 120% of the three distinct levels were chosen for this procedure. In a 10 mL volumetric flask, the pure medication was added to the previously tested sample solution and diluted to the mark with methanol. To measure the peak area, the corresponding solutions were injected into an HPLC system after being filtered via a 0.45 µm syringe filter. The accuracy was calculated using the tepotinib recovery values.[11]

Precision

Through the introduction of six tepotinib solution samples (µg/mL) on two separate days. %RSD of the response was used to calculate the method's precision.[12,13]

Table 1: Optimized conditions

S. No.	*Parameters*	*Optimized conditions*
1	Column	Kinetex XB-C18 Phenomenex (150×4.6mm, 5µ)
2	Column temperature	30ºC
3	Mobile phase	Acetonitrile: Water
4	Mobile phase ratio	80:20 V/V
5	Diluents	Methanol
6	Flow rate	1 mL/min
7	Injection volume	10 µL
8	Wavelength	272 nm
9	Retention time	3.4 min
10	Run time	10 min

Robustness

To test the system's resilience, chromatographic parameters including flow rate and detection wavelength were purposefully altered. Tepotinib solution was injected into the chromatographic system to verify the test findings after each modification, and the outcomes were compared to the initial results obtained under the original chromatographic settings.[14]

System suitability

Tepotinib solution was injected after a blank mobile phase, in order to meet the system appropriateness criteria. These criteria were used to check the parameters, which included resolution of a peak, number of theoretical plates, capacity factor, and tailing factor (Table 2).[15]

Limit of Detection and Limit of Quantitation

The term "limit of detection" refers to the lowest quantity of a sample that, under specified chromatographic circumstances, can be detected but not necessarily measured. The quantity of material that can be quantified is the limit of quantification. The formulas for calculating LoD and LoQ are 3.3 and 10 σ/S, respectively. In this calculation, σ represents the standard deviation of response and S is the slope of the calibration curve.[16-18]

Studies on forced degradation

Analysis of all forced degradation trials was done at the concentration level of 10 µg/mL. The drug solution was treated with 1-mL of 0.1N HCl for acid degradation, 1-mL of 1N NaOH for alkaline degradation, and 1-mL of 10% H_2O_2 for oxidative degradation. The solution was then neutralized and diluted using diluents after being left at room temperature for 10 minutes. 10 mg of the medication were degraded with dry heat by being stored in a hot air oven at 80ºC for five hours. A 0.45 µm syringe filter was used to filter the sample solution (const. of Tepotinib-10 µg/mL) of tepotinib before it was injected into a chromatographic machine to measure the peak area.[19,20]

RESULTS

Physical Characterization of Tepotinib

Tepotinib powder's physical characteristics, such as color and solubility, were examined. Additionally, the medication was scanned using a UV spectrophotometer. Using methanol as the solvent, the absorption maxima for tectinib was discovered to be 272 nm (Figure 2).

Method Validation

Linearity

The value of correlation coefficient (r2) was 0.9993 which is well within acceptance limit (r2<1). The Tepotinib solution chromatogram, Calibration curve graph and Linearity overlay of Tepotinib are shown in Figure 3-5, respectively.

Range

The range between 5 to 25 µg/mL was selected for conducting the analysis.

Table 2: System suitability results

Parameter	*Values*
Wavelength	272 nm
Retention time	3.4 minutes
Theoretical plates	2390
Tailing factor	1.65
Resolution	1.7

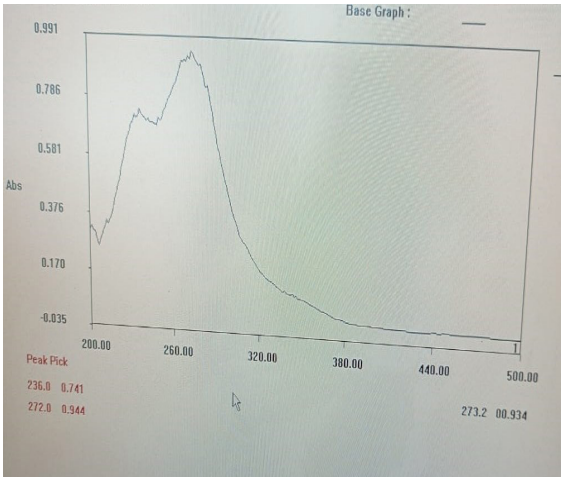

Figure 2: UV spectrum of tepotinib

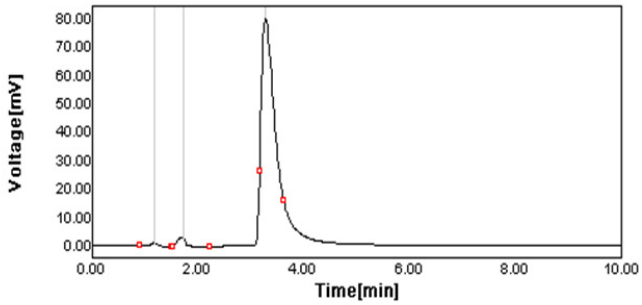

Figure 3: Tepotinib solution chromatogram

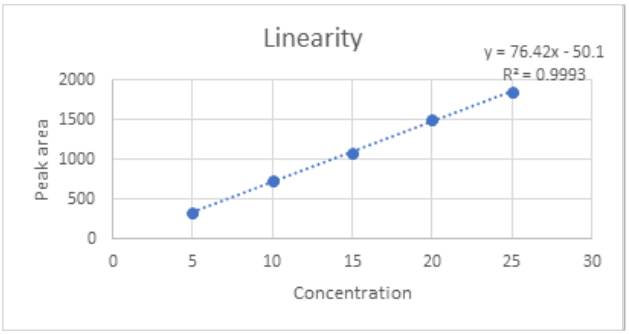

Figure 4: Calibration curve of tepotinib

Accuracy

The percentage recoveries (Table 3) of the present procedure for the tepotinib solution carried at different levels, respectively indicate that the recoveries are well within the acceptance range (RSD<2), therefore the method is accurate.

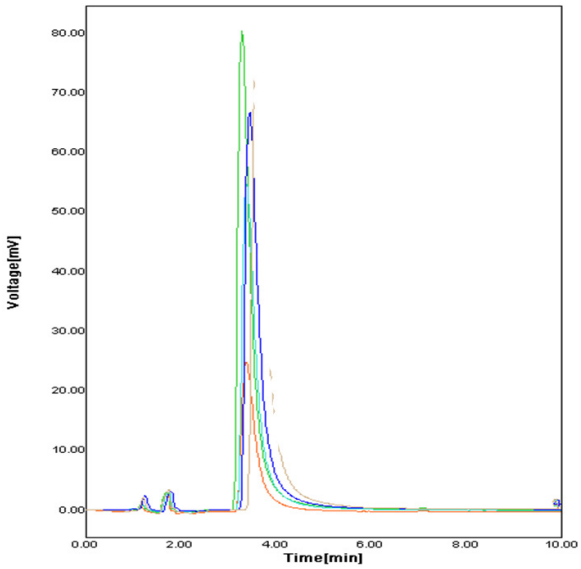

Figure 5: Linearity overlay of Tepotinib

Table 3: Accuracy results

S. No	*% Level*	*Amount spiked*	*Amount recovered*	*% Recovery*
1	80	18	18.3	101.7
2	100	20	20.04	100.2
3	120	22	21.83	99.26

Table 4: Precision results

Readings	*Conc.*	*Peak area*		*Statistical analysis*	
		Day 1	*Day 2*	*Day 1*	*Day 2*
1		1123	1119	Mean = 1114.66	Mean = 1115
2		1106	1100	SD = 10.269	SD = 9.591
3	15 (µg/mL)	1128	1126	%RSD = 0.921	%RSD = 0.860
4		1118	1108		
5		1112	1115		
6		1101	1122		

*Values given in the table are mean ± SD, n = 6 responses, %RSD: Relative standard deviation

Table 5: Robustness (Concentration. of Tepotinib- 15 µg/mL)

S. No.	*Parameter*	*Optimized*	*Used*	*Peak area*	*% Recovery*
1	Flow rate (± 0.1 mL)	1.0 mL/min	0.9 mL/min	1119	99/6
			1.1 mL/min	1110	98
2	Detection wavelength (± 1 nm)	272 nm	271 nm	1126	100.2
			272 nm	1118	99.5

Precision

The precision was calculated by repeatability. Table 4 shows the results of precision.

Robustness

Robustness is shown in Table 5.

Forced degradation studies

Tepotinib was found more sensitive to base degradation; the assay value was decreased to 85.32%. It had been conjointly susceptible to acidic, oxidative and dry heat degradation yielding % degradation values of 6.70, 6.74 and 3.03. Table 6 shows results of Forced Degradation Studies. Figures 6-9 shows the acid, Base, Oxidative and dry heat Degradation Chromatogram of Tepotinib

Specificity

When data from the drug solution before and after spiking were compared, it was found that the blank solution's contribution to Tepotinib recovery was less significant, indicating that the approach was selective, results shown in Table 7. Chromatogram of mixture Blank, working solution, Drug Product solution shown in Figure 10.

Table 6: Forced degradation studies

Parameters	*Area*	*Assay*	*% Degradation*
Acid degradation	3185	93.21	6.70
Base degradation	4763	85.32	14.59
Oxidative degradation	3183	93.17	6.74
Degradation with dry heat	3390	96.89	3.03

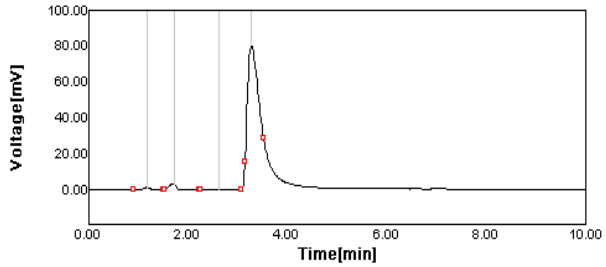

Figure 6: Acid degradation chromatogram of tepotinib

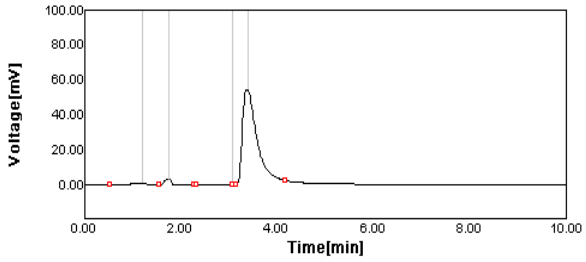

Figure 7: Base degradation chromatogram of tepotinib

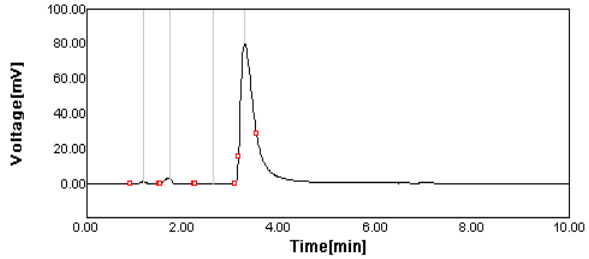

Figure 8: Oxidative degradation chromatogram of tepotinib

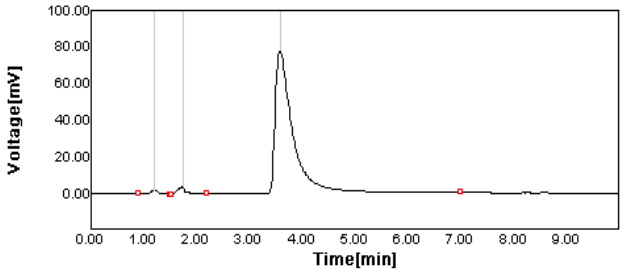

Figure 9: Dry heat degradation chromatogram of tepotinib

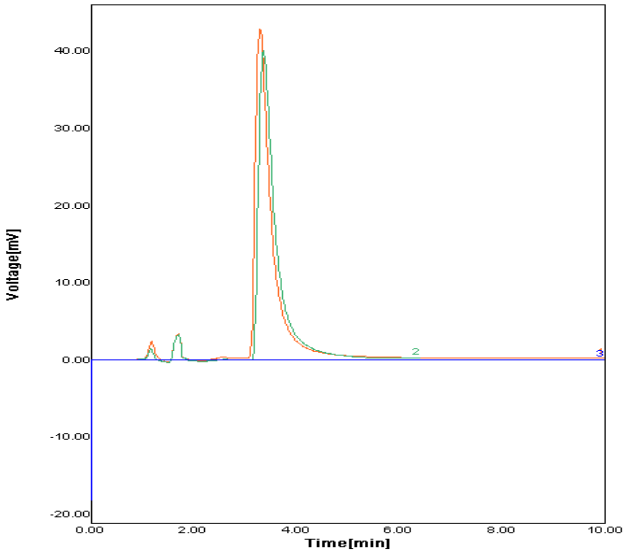

Figure 10: Chromatogram of mixture blank, working solution, drug product solution

Table 7: Specificity results

Sample	*Retention time (min)*	*Area*	*% Assay*
Blank	–	–	–
Working solution	3.4	1094	–
Drug product	3.31	1190	99.90

Table 8: Summary of validation parameter

Parameter	*Values*
Maximum wavelength (nm)	272
Range (µg/mL)	5-25
Regression equation	Y = 72.46x-50.1
Precision (%RSD)	Intra-day-0.9213244
	Inter-day-0.860239
LoD (µg/mL)	0.429
LoQ (µg/mL)	1.3
Robustness	Robust

Assay

The marketed formulation's analysis was carried out using the intended RP-HPLC method in order to determine the tepotinib content. The tepotinib tablet's average assay percent was found to be 98%. Summary of validation parameter given in Table 8.

DISCUSSION

In the current work, we have designed and validated the reverse phase liquid chromatographic method for the measurement of tepotinib in bulk and formulation, taking into account the criteria provided by ICH.[21-23] Methanol of HPLC grade was utilized as the diluent. The mobile phase for the chromatographic separation is an 80:20 mixture of acetonitrile and methanol. Using the UV-vis spectrophotometer, the tepotinib solution (consisting of 10 µg/mL) was scanned. At 272 nm, the absorbance was measured. The calibration curve for different concentrations of tepotinib were plotted. From the calibration curve data regression equation was found to be Y = 76.42-50.1 and the correlation coefficient was noted as R^2 = 0.9993. Linearity was found to be within the concentration 5-25 µg/mL.

Percentage recovery is the technique used for the calculation of accuracy. The range between 99.26-101.7% was found for accuracy. The results were within the limit. Hence, the method was found to be accurate. Repeatability was used to study precision. It was discovered that the percentage RSD for both intra-day and inter-day precision was 0.92 and 0.86, respectively. Little adjustments to the chromatographic equipment were made to test the method's robustness. %recoveries were discovered to be within the bounds. Thus, the approach is reliable. The suggested method worked well for tepotinib stability-indicating investigations in pharmaceutical dose form. For the routine analysis of the tepotinib in bulk and formulation the proposed method can used to get accurate, precise results quickly.

CONCLUSION

A stability-indicating high-performance liquid chromatography method was used to design and validate a straightforward, accurate, precise, repeatable, and sensitive approach for the measurement of tepotinib in bulk and formulation. It was also discovered that the intended approach was incredibly quick, economical, and efficient. Compared to other strategies mentioned in the literature review, the approach is innovative. Since buffered solutions were typically used in previous procedures, we used HPLC-grade solvents in the current work to carry out the chromatographic separation. Another development was the creation of a new work for the assessment of tepotinib, the stability-indicating approach. This is an appropriate method for their estimate in the pharmaceutical formulation, as confirmed by the validation parameter results. This approach can be used to regularly analyze the quality of tepotinib in pharmaceutical formulation and bulk. Keeping in mind the ICH guidelines, the procedure was designed.

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