Method Development and Validation of Tepotinib by Using Reverse Phase Liquid Chromatography in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

Method Development and Validation of Tepotinib By Using Reverse Phase Liquid Chromatography in Bulk and Pharmaceutical Dosage Form Chromatographic separation was achieved on a agilent eclipse C18 column (150x4.6mm, 3.5 μ) using isocratic elution with a mobile phase containing buffer and acetonitrile within the ratio of 50:50 as mobile phase with a flow of 1 ml/min at ambient temperature and UV detection was carried at 254 nm. Analysis was achieved within 6 min over an honest linearity within the concentration range from 22.5-337.5 μ g/ml of Tepotinib. By injecting the standard six times, system suitability parameters were studied and the outcomes were well under the acceptance criteria. Precision and recovery study results were found to be within the suitable limit. By using the above technique assay of marketed formulation was performed and found to be within the limit. Degradation studies were carried out on Tepotinib, with a purity threshold greater than purity angle in all conditions and within the acceptable range. The above mentioned technique was validated according to ICH guidelines.

KEY WORDS: HPLC, TEPOTINIB, DEVELOPMENT, VALIDATION, DEGRADATION STUDIES.

INTRODUCTION

Tepotinib, sold under the brand name Tepmetko, is a medication for the treatment of adults with metastatic non-small cell lung cancer (NSCLC) whose tumors have a mutation that leads to mesenchymal-epithelial transition (MET) exon 14 skipping. It is a c-Met inhibitor, a type of tyrosine kinase inhibitor. The most common side effects seen in clinical trials were edema, fatigue, nausea, diarrhea, muscle aches, and shortness of breath. Like capmatinib, tepotinib can also cause interstitial lung disease and liver damage, and is toxic to a developing fetus.

MATERIAL AND METHODS / EXPERIMENTAL DETAILS

2.1 Materials: Acetonitrile and Tri ethyl amine, water (HPLC grade) were purchased from Merck (India) Ltd, Worli, Mumbai, India. API of Tepotinib as reference standard was procured from Laurus Labs Pvt. Ltd., Hyderabad.

2.2 Tools: An HPLC system (Waters alliance e2695 model) consisting of quaternary pump, PDA detector-2998 was used. Data processing was performed with Empower 2.0 software.

Figure 1: Chemical structure of Tepotinib

2.3 Chromatographic Conditions: Chromatographic separation was carried out in isocratic mode at room temperature using agilent eclipse C18 column (150x4.6 mm, 3.5 μ). A mixture of acetonitrile and 0.1% tri ethyl amine (TEA) in 50:50 v/v at a flow of 1 ml/min was used as mobile phase. The injection volume was 10 μ l and the run time was 6.0 min.

2.4 Preparation Of Buffer: 1 ml of tri ethyl amine is dissolved in 1 lt of HPLC grade water and filtered through 0.45 μ filter paper.

2.5 Diluent: Acetonitrile was used as diluent.

2.6 Preparation Of Standard And Quality Control Samples: Accurately weigh and transfer 225 mg of Tepotinib in 100 ml volumetric flask and add app. 70 ml of diluent, sonicate to dissolve it for 30 min. and made up to the mark with diluent. Further dilute 5 ml of the above solution to 50 ml with diluents.

2.7 Preparation Of Sample Solution: Accurately weigh and transfer weight equivalent to 225 mg of Tepotinib sample in 100 ml of volumetric flask and add 70 ml of diluent. Sonicate to dissolve and dilute up to the mark with diluent. Take 5 ml of the above solution and diluted to 50 ml and filtered through 0.45 μ nylon syringe filter.

2.8 Method Validation

2.8.1 System Suitability: System suitability parameters were measured to verify the system performance. The parameters including USP plate count, USP tailing and % of RSD are calculated and found to be within the limit.

2.8.2 Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of other components, which may be expected to be present in the sample and standard solution. It was checked by examining the chromatograms of blank samples and samples spiked with Tepotinib.

2.8.3 Accuracy: Accuracy is the closeness of the test results obtained by the method to the true value. It was assessed by the recovery studies at three different concentration levels. In each level a minimum of three injections were given and amount of the drug present, percentage recovery and related standard deviation were calculated.

2.8.4 Precision: Precision of the analytical method is the degree of agreement among individual test results. It was studied by analysis of multiple sampling of homogeneous sample. The precision of the present method was assessed in terms of repeatability, intraday and inter-day variations. It was checked by analyzing the samples at different time intervals of the same day as well as on different days.

2.8.5 Linearity: Linearity of an analytical method is its ability to obtain results directly proportional to the concentration of the analyte in the sample within a

definite range. The six series of standard solutions were selected for assessing linearity range. The calibration curve was plotted using peak area versus concentration of the standard solution and the regression equations were calculated. The least squares method was used to calculate the slope, intercept and correlation coefficient.

2.8.6 Stress Degradation: Stress degradation should be no interference between the peaks obtained for the chromatogram of forced degradation preparations. Stress degradation studies were performed as per ICH guidelines Q1 (A) R2. The degradation peaks should be well separated from each other and the resolution between the peaks should be at least 1.0 and the peak purity of the principle peaks shall pass. Forced degradation studies were performed by different types of stress conditions to obtain the degradation of about 20%.

2.8.7 Robustness: Robustness of an analytical procedure is a measure of its ability to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness study was performed by injecting standard solution into the HPLC system and altered chromatographic conditions such as flow rate (± 0.2 ml/min), organic content in the mobile phase ($\pm 10\%$). The separation factor, retention time and peak asymmetry were calculated by determining the effect of the modified parameters.

Table 1. Optimized chromatographic conditions

Parameter	Condition
Stationary phase	Agilent eclipse C18 (150x4.6 mm, 3.5 μ)
Mobile phase	0.1% TEA: Acetonitrile (30:70)
Injection volume	10 μ l
Flow rate	1.0 ml/min
Column temperature	25°C
Wave length	254 nm
Run time	6.0 min
Retention time of Tepotinib	3.714 min
*TFA : Tri ethyl amine nm: Nanometer	

RESULTS AND DISCUSSION

The current study was designed to develop a simple, precise and rapid analytical RP-HPLC method, which can be used for the analysis of assay method for the estimation of Tepotinib in bulk and pharmaceutical dosage form. The chromatographic conditions were optimized in order to provide a good performance of the assay. To optimize mobile phase, various combinations were tried for Tepotinib. The final working mobile phase is 0.1% tri ethyl amine and acetonitrile in the composition of 50:50 v/v. Detection was carried out in

several wavelengths in order to obtain enough sensitivity for the two APIs in smaller proportion (Tepotinib). At last the wave length 254 nm was selected at which the drug showed good absorbance. The flow rate was 1.0 ml/min. The retention time for Tepotinib was 2.423 min respectively. The proposed method is validated in accordance with the ICH guidelines with all of the results within the limits. The detection was carried out with a total runtime of 6.0 min. Optimized chromatographic conditions were shown in table 1.

Figure 2: Chromatogram of system suitability

Figure 3: Chromatogram of blank

System Suitability: The system suitability was performed by injecting standard solution containing 225 µg/ml of Tepotinib in six replicates. The results indicate that the system suitability parameter is within the limit. The standard chromatogram was shown in figure 2.

Table 2. Results of linearity

S. No	Tepotinib Concentration (µg/ml)	Area
1	22.50	184880
2	56.25	459113
3	112.50	896209
4	168.75	1256347
5	225.00	1834505
6	281.25	2178517
7	337.50	2655617

Specificity: There was no interference from blank at the retention time of Tepotinib. Figure 3 represents the blank chromatogram.

Figure 4: Calibration plot of Tepotinib

Table 3. Results of method precision

S. No.	Area of Tepotinib
1	1900847
2	1897995
3	1903999
4	1907151
5	1903482
6	1902710
Mean	1902697
Std. dev	3088.07
% RSD	0.162

Linearity: Linearity was determined by plotting a calibration curve of peak area against their respective concentration. From this calibration curve it was found that the curve was linear in the range of 22.5-337.5 µg/ml of Tepotinib. The regression equation for calibration curve of Tepotinib was $Y = 7831.06x + 4819.23$ ($R^2 = 0.9991$), the results were shown in table 2 and the calibration plot was shown in figure 4.

Table 4. Results of Intermediate precision

Area of Tepotinib	Relative standard deviation
1900654	0.17
1897351	
1903262	
1905471	
1903031	
1902356	

Precision: Precision of this method was assessed in terms of intraday (repeatability) and (intermediate precision) variations. The intraday studies were determined by performing six repeated analysis of the sample solution of Tepotinib on the same day under the same experimental conditions. The intermediate precision of the method was carried out in the same laboratory by studying the analysis with different analyst and different instrument. The method is highly precise as %RSD values were found to be <2%. Good recoveries of the drug

were obtained at each added concentration, indicating that the method was accurate. Table 3 gives the method precision results and the table 4 gives the intermediate precision results.

Accuracy: The accuracy of the method was performed by calculating the recovery experiments at three levels (50%, 100% and 150%). APIs with concentration 112.5, 225, 337.5 µg/ml of Tepotinib was prepared. The test solution was injected three times for each spike level and assay was performed as per the test method. The recovery results were close to 100% and also the RSD values were less than $\pm 2\%$. The percentage recovery, mean and relative standard deviation were calculated. Recovery values demonstrated that the method was accurate within desired range. Accuracy results were shown in table 5.

Table 5. Results of accuracy

Accuracy	Amount of Tepotinib	% Recovery
50	5	99.1
100	10	100.8
150	15	100.4

Table 6. Results of robustness

Parameter	% RSD of Tepotinib
Flow (0.8 ml/min)	0.22
Flow (1.2 ml/min)	0.31
Organic phase (45:55)	0.18
Organic phase (55:45)	0.06

Table 7. Results of forced degradation

Stress parameter	% of degradation Tepotinib
Acid degradation (1N HCl+ reflux+ 24 hrs)	16.2
Alkali degradation (1N NaOH+ reflux+ 24 hrs)	16.6
Peroxide degradation (30% Peroxide+ reflux+ 24 hrs)	
Thermal degradation (Sample + heat for 6 hrs) 17	14.3
Hydrolysis degradation (1 ml HPLC water + reflux + 24 hrs)	15

Robustness: Robustness of the chromatographic method was determined by varying flow rate and mobile phase composition. % RSD was found to be within

the acceptable limit. Robustness results were shown in table 6.

Forced Degradation: The proposed method can be used for release and stability studies for effective evaluations and can be considered as stability indicating method. The forced degradation study was carried out according to the ICH requirements include acid, base, oxidation, thermal, hydrolysis degradation. From the chromatograms it is evident that the selected drugs were stable under the applied stress conditions though the degraded peaks were observed. Forced degradation study results were shown in table 7.

The devised approach was validated in accordance with ICH requirements and found to be very precise, quick, simple, cost-effective, and sensitive to the specific pharmaceutical dosage form.

CONCLUSION

In this study a novel, rapid, economical, sensitive and easily available HPLC method was developed for the determination of Tepotinib in bulk and pharmaceutical dosage form. In this method shorter run time, low price, accessibility, sensitivity, reliability and reproducibility. These properties are important when a large number of samples are to be analyzed. The validation of all the parameters like linearity, accuracy, specificity, robustness, method precision were done and found to be within the acceptable limit. The RSD values for all the parameters were found to be less than 2%, which indicates the validity of the method and the results obtained by this method are in fair agreement. So the proposed method could be easily applied for the routine analysis and the pharmaceutical formulations of Tepotinib in quality control laboratories without any preliminary separation.

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Authors' Contributions: Autor 1 designed the study, performed the statistical analysis, wrote the protocol, and wrote the manuscript. Autor 2 to check the work and review the manuscript.

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