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Development and Validation of a Robust Stability-Indicating RP-HPLC Method for Quantitative Analysis of Tepotinib in Pharmaceutical Formulations

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Abstract:
A highly accurate and reliable Reverse Phase -High Performance Liquid Chromatography technique was designed and validated for the quantitative analysis of Tepotinib in drug products. Separation was achieved on a Luna® C18 column (3 µm, 150 mm × 5 mm I.D.) using an isocratic mobile phase consisting of 80% phosphate buffer (0.01 N K_2 HPO_4 , adjusted to pH 3.5) and 20% acetonitrile at a flow rate of 1.0 mL/min. Detection took place at 270 nm, and Tepotinib was observed at a retention time of 2.235 mins. The method met ICH validation standards, demonstrating selectivity, excellent linearity over the range of 10–50 µg/mL (correlation coefficient $R^2 > 0.999$), and accuracy (recovery between 99.26–101.7%). Precision was confirmed, with intra-day and inter-day relative standard deviations of 0.92% and 0.86%, respectively. Test recovery underscored the method's accuracy, and deliberate adjustments to parameters like temperature, flow, and mobile phase composition established its robustness. This protocol is well-suited for quality control and stability testing of Tepotinib formulations.
Keywords: RP-HPLC, Tepotinib, Method development, non-small cell lung cancer (NSCLC)

Introduction

Non-small cell lung cancer (NSCLC) comprises a spectrum of histopathological subtypes, most notably adenocarcinoma , squamous cell carcinoma (SCC), and large cell carcinoma (LCC). Adenocarcinoma, the predominant variant, arises most frequently in individuals with minimal or no smoking history and typically manifests in the peripheral parenchymal regions of the lung. Squamous cell carcinoma, constituting approximately 30% of NSCLC cases, is intimately linked to tobacco exposure and generally originates within the central bronchial airways. Large cell carcinoma, comprising roughly 3–9% of diagnoses, is distinguished by undifferentiated large neoplastic cells that lack the definitive cytological features of the subtypes, rendering it principally a diagnosis of exclusion.[1-5]

Occupationalexposures including asbestos, beryllium, cadmium, crystalline silica, vinyl chloride, nickel, chromium compounds, coal derivatives, mustard gas, chloromethyl ethers, and diesel exhaust particulates constitute salient etiological determinants for the development of lung carcinoma. The diameter of inhaled asbestos fibers serves as a pivotal biomarker for predicting lung cancer mortality, with finer fibers showing greater pathogenicity. Notably, beryllium exposure, especially within aerospace and electronics manufacturing, is prominently implicated in pulmonary carcinogenesis, reflecting the profound carcinogenic potential of select occupational agents in industrial sectors[7-10,14-16]

Only a few HPLC-based analytical methods have been reported for the determination of Tepotinib (fig 1), whether in bulk form, as a stand-alone dosage, or in combination with other therapeutic agents, despite a thorough literature review. The current study aims to close this gap by creating a novel, quick, simple, accurate, repeatable, and stability-indicating RP-HPLC approach for Tepotinib analysis. Additionally, the method is validated in accordance with ICH requirements, namely Q1A(R2) for stability evaluation and Q2(R1) for method validation.

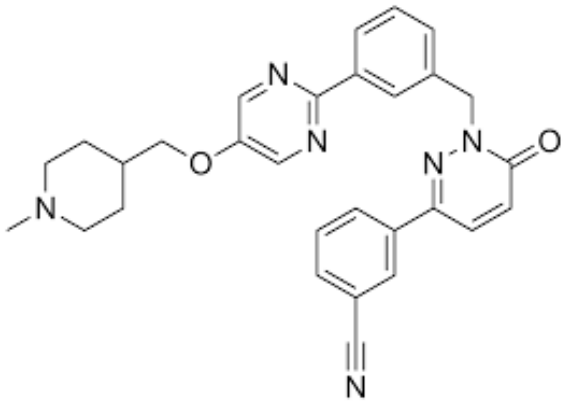

Fig.1: Structure of Tepotinib

Materials and Methods

Chemicals and Reagents:

The pure active pharmaceutical ingredient (API) of tepotinib, possessing a stated purity of 99.98%, was procured from Glenmark India. The tepotinib tablets, marketed under the name TEPMETKO, were supplied by an India Mart pharmacy through

Grow Fast, located in West Bengal. All reagents, solvents, and buffer solutions utilized in the analytical procedure were sourced from LICHROSOLV (MERCK), India.

Instrumentation

Analytical determinations were conducted using a Waters Alliance high-performance liquid chromatography (HPLC) system (Waters, USA), outfitted with a quaternary pump (model M00SM4493M), an autosampler (model M17VSM029N), a column oven (model B18HLP981G), and a photodiode array detector (model 2998PDA).

2.1 Methods

General Preparations

Preparation of Potassium hydrogen phosphate: 0.01 N potassium hydrogen phosphate (K_2HPO_4) buffer, accurately weigh 6.8 g of potassium hydrogen phosphate and dissolve it in 1000 mL of Milli-Q ultrapure water. The solution is then degassed by sonication to eliminate dissolved gases, followed by precise pH adjustment to 3.5 using 0.1% orthophosphoric acid to achieve an optimal buffering environment.

Preparation of stock Solutions:

A standard stock solution of tepotinib was prepared by accurately weighing 25 mg of the compound and dissolving it in 25 mL of a suitable diluent composed of acetonitrile and phosphate buffer. The mixture was subjected to sonication for 10 minutes to ensure complete solubilization, followed by dilution to the final volume. Subsequently, this stock solution was serially diluted to yield working standard solutions within the concentration range of 10–50 µg/mL for analytical applications.

Preparation of mobile phase: From the above buffer solution transferring of 800 ml into 1000 ml container and 200 ml of acetonitrile and mix well than filter it.

Method development:

Optimized Method:

Multiple method development trials were performed utilizing various chromatographic conditions with the Phenomenex Kinetex XB-C18 column (3 µm particle size, 70nm × 5nm pore structure), systematically evaluating distinct mobile phase compositions including both 1% orthophosphoric acid: acetonitrile (70:30v/v) and acetonitrile:0.1% OPA (50:50 v/v) as well as varying the column temperature to 35°C and experimenting with different injection volumes. Ultimately, the finalized and optimized chromatographic parameters are detailed in Table 1 and optimized chromatogram given as figure no 2.

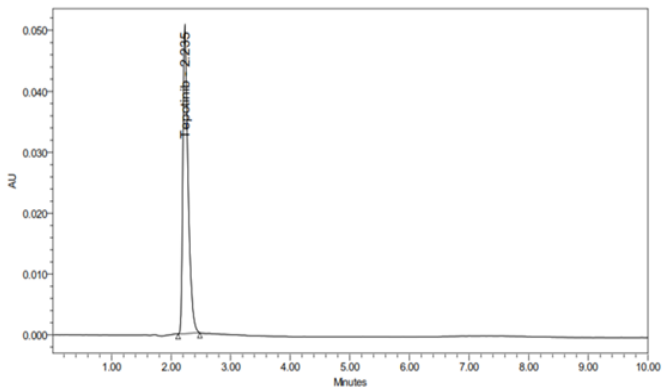

Fig.2: Optimized Chromatogram

Table 1: Optimised Chromatographic Conditions

Peak Name	Retention time	Area	Height	USP Plate count	USP Tailing
Tepotinib	2.235	197829	201931	5219	1.2

Standard solution: A standard stock solution of tepotinib was prepared by accurately weighing 25 mg of the compound and dissolving it in 25 mL of a suitable diluent composed of acetonitrile and phosphate buffer. The mixture was subjected to sonication for 10 minutes to ensure complete solubilization, followed by dilution to the final volume. Subsequently, this stock solution was serially diluted to yield working standard solutions within the concentration range of 10–50 µg/mL for analytical applications.

Chromatographic Conditions	
Column	Luna® C18 (3 µm, 150 mm × 5 mm I.D.)
Mobile phase	0% 0.01 N K_2HPO_4 buffer (pH 3.5) and 80% acetonitrile
Flow rate	1.0 mL/min
Column temperature	30°C
Injection volume	20 µL
Retention time (min)	2.235
Run time (min)	10.0
Detection wavelength	270 nm

Method Validation: The validation of the HPLC methodology was performed in strict compliance with the International Council for Harmonisation (ICH) guidelines for the concurrent quantification of tepotinib substance, thereby demonstrating the method's appropriateness for routine analytical applications.[30]

System Suitability:

For system suitability studies theoretical plates should be > 2000, tailing factor must be < 2 and resolution between peaks should be > 2.

Specificity (Selectivity):

Specificity was ascertained through systematic analysis of blank, placebo, and tepotinib samples. No extraneous or co-eluting peaks were detected at the characteristic retention time of tepotinib, thereby confirming the method's selectivity and unequivocal ability to discriminate the analyte from potential interferences.

Linearity and Construction of Calibration Curve:

Linearity was assessed across a concentration range of 10 to 50 µg/mL. The calibration curves demonstrated an exceptional degree of correlation, with a coefficient of determination (R^2) exceeding 0.999. The resulting regression equation was established as y = 6305.2x + 3804.1. Results are displayed in Table 3.

Accuracy: Accuracy was evaluated employing the standard addition technique at three distinct concentration levels 50, 100, and 150 µg/mL with each level analyzed in triplicate. The mean recovery achieved was 99.59%, thereby substantiating the method's precision and accuracy and results shown int table 4.

Precision:

System Precision:

Six replicate injections of the standard solution produced percentage relative standard deviation (%RSD) values that remained within the prescribed acceptable limits, thereby

confirming the method's precision and repeatability. Results of peak area are summarized in Table no 5

Method Precision:

Six independently prepared sample aliquots were analyzed, resulting in a percentage relative standard deviation (%RSD) of 0.7%, thereby demonstrating excellent method reproducibility and precision. Results of peak area are summarized in table no 5 The % RSD of method precision study was within the limit for Tepotinib.

Robustness Parameter:

The method robustness was analyzed by altering flow rate (±0.05 mL/min) and organic phase ratio (±0.5%). All changes resulted in %RSD < 2%, confirming the method's robustness. Results of peak area are summarized in table no 6

Stress Stability Studies:

Stress studies were carried out as per ICH guidelines under different acid, base, oxidative, photolytic, and thermal conditions.[30]

Forced Degradation Studies:

The analytes were exposed to a range of stress conditions in accordance with ICH Q1A(R2) in order to demonstrate the method's stability-indicating assessment.

Acid Hydrolysis

Heat 1 mL of stock solution with 1 mL of 1N HCl for one hour at 60°C. Following 1N NaOH neutralization, diluent was added to the volume and filtered.[13]

Alkali Hydrolysis: Stock solution 1mL is mixed with 1mL of 1N NaOH was heated at 60°C for 1 hour. After neutralization with 1N HCl, it was diluted, filtered, and analysed[14]

Oxidative Degradation

Stock solution 1 mL was added to 1 mL of 10% H_2O_2 was heated at 60°C for 1 hour. The sample was cooled, diluted, filtered, and injected.

Thermal Degradation

A solid material was taken in a petri dish and heated for three hours to 105°C. After dissolving the deteriorated material in diluent, it was added. Solid substance was taken in a petri plate and heated at 105°C for 3 hours. The degraded substance was dissolved in diluent and introduced.

Photolytic Degradation: Solid samples were exposed to UV light in a photo stability chamber for 3 hours. The samples were then diluted and analyzed.

Result and Discussion

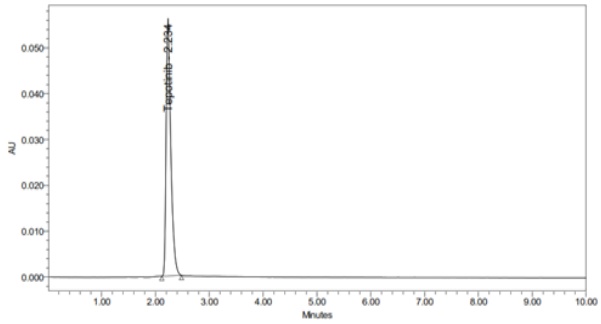

Fig.3: System Suitability Chromatogram of Tepotinib

System Suitability:

System suitability was evaluated through the injection of six replicate aliquots of a tepotinib standard solution at a concentration of 30 µg/ml. Critical chromatographic parameters, including peak tailing factor, resolution, and theoretical plate count, were meticulously assessed. The percentage relative standard deviation (%RSD) for peak area was determined to be below 2%, thereby affirming the adequacy and reliability of the chromatographic system for analytical purposes.

Table 2: System Suitability Results

S.No	Tepotinib		
Injects	RT (min)	USP Plate Count	Tailing
1	2.243	3415	1.13
2	2.243	3425	1.14
3	2.264	3427	1.13
4	2.214	3424	1.12
5	2.275	3425	1.13
6	2.245	3425	1.12
Mean	2.2473		
SD	0.02096		
C.V	0.9		

Specificity (Selectivity):

Tepotinib exhibited a retention time of 2.234 minutes. Employing this methodology, no interfering peaks were detected in the blank and placebo samples at the corresponding retention time of the analyte. This outcome substantiates the specificity of the analytical approach.

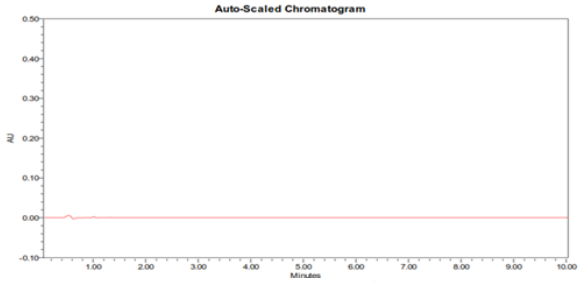

Fig.4: Blank Chromatogram of Tepotinib

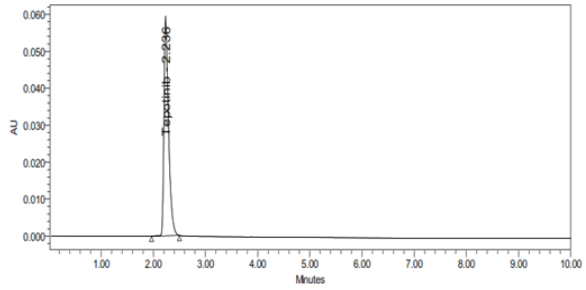

Fig.5: Specificity Chromatograms of Tepotinib

Linearity:

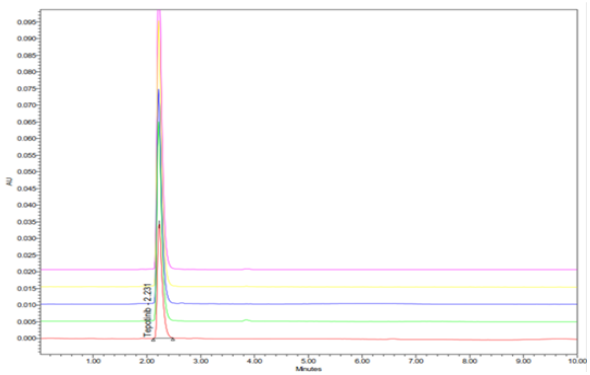

Fig.6: Linearity Chromatograms of Tepotinib

Five distinct linear concentrations of tepotinib, spanning from 10 to 60µg/mL, were injected in duplicate. The mean peak areas obtained are as previously indicated. The linearity equation derived for tepotinib was expressed as y = 6305.2x + 3804.1. A correlation coefficient (R^2) of 0.999 was achieved, confirming the excellent linear relationship for both compounds.

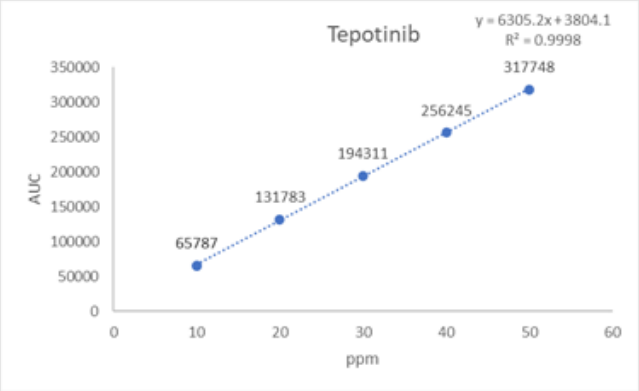

Fig.7: Calibration Curve of Tepotinib

Accuracy:

Accuracy assessment was performed employing the standard addition technique at three discrete concentration levels. For each accuracy tier, triplicate injections were administered, resulting in a mean percentage recovery of tepotinib of 99.59%, thereby corroborating the method's precision and reliability.

Table 4: Accuracy (%Recovery data)

% Level	Amount Spiked (µg/mL)	Amount Added (mg)	Amount Found(mg)	Mean %Recovery
50%	15	5	4.85	99.59%
	15			
	15			
100%	30	10	9.91	
	30			
	30			
150%	60	15	14.45	
	60			
	60			

Precision:

System Precision:

The % RSD 0.2 for the peak areas of Tepotinib obtained from six replicate injections of standard solution was within the limits are summarized in table no 5.

Method precision: The % RSD 0.7 for the peak areas of Tepotinib obtained from six replicate injections of standard solution was within the limits are summarized in table no 5.

Table 5: Robustness Results

System Precision		Method Precision
S. No	Area of Tepotinib	Area of Tepotinib
1.	192345	191345
2.	192432	191232
3.	192971	191671
4.	192899	191999
5.	192898	192898
6.	192333	194679
Mean	192646.3	192304.0
S.D	305.8	1308.1
%RSD	0.2	0.7

Robustness:

Robustness was appraised by intentionally varying critical chromatographic parameters, including the flow rate by ±0.1 mL/min, mobile phase composition by ±5%, and column temperature by ±3°C. The analytical method demonstrated resilience under these deliberate perturbations, with all system suitability criteria remaining within predefined acceptable thresholds and chromatographic conditions given in table no 6.

Table 6: Robustness Results

Parameters	Chromatographic condition	Tepotinib (SRN)
Flow rate	0.9ml/min	0.4
Flow rate	1.1ml/min	0.7
Mobile phase	75B:25A	0.4
Mobile phase	85B:15A	1.0
Temperature	25°C	0.4
Temperature	35°C	0.4

Stress Stability Studies: Stress studies were carried out as per ICH guidelines under different acid, base, oxidative, photolytic, and thermal conditions.[30]

Forced Degradation Studies:

Tepotinib demonstrated susceptibility to degradation across all applied stressors; however, no degradation products co-eluted with the principal analyte peak, thereby affirming the stability-indicating nature and specificity of the developed analytical method. From the results, degradation was evident upon exposing the samples to alkaline hydrolysis, thermal stress, photolytic conditions, and acidic environments. According to the stress degradation study, none of the degradation products co-eluted with the principal peaks of the active pharmaceutical ingredient, thereby confirming the method's specificity and stability-indicating potential and as you can confirm them values mentioned in table no.7.

Table 7: Forced Degradation Conditions for Tepotinib

S.NO	Degradation Condition	% Drug Un Degraded	% Drug Degraded
1	Acid	180528	7.07
2	Alkali	189212	2.60
3	Oxidation	183461	5.56
4	Thermal	190602	1.89
5	UV	188402	3.02

Conclusion

For the concurrent analysis of tepotinib, a precise, and robust reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and rigorously validated for the quantification of tepotinib in pharmaceutical dosage forms. The method exhibits high specificity, reproducibility, and accuracy, alongside stability-indicating capability, rendering it eminently suitable for routine quality control assessments and stability evaluations. Its demonstrated robustness and selectivity guarantee consistent and reliable analytical performance throughout drug development, manufacturing processes, and extend to applications in environmental monitoring.

Conflict of Interests

The authors declare no conflict of interest

Ethics Approval: Not applicable

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AI Tool Declaration
The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

Data Availability
Data will be available on request

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