International Journal of Research in Pharmacy and Pharmaceutical Sciences
www.pharmacyjournal.in
ISSN: 2455-698X
Received: 18-04-2026, Accepted: 19-05-2026, Published: 03-06-2026
Volume 11, Issue 2, 2026, Page No. 72-75

Rp-Hplc method development and validation for the estimation of Sevabertinib in bulk and pharmaceutical dosage form

Prasada Rao M[1], Narasimha Rao B V[1*], Udaya Krishna Veni A[2], Chaitanya C H[3], Chandrika D[3], Narasaraju G V[3], Sowmya K[3], Harshini K[3]

[1] Professor, Department of Pharmaceutical Analysis, MAM College of Pharmacy, Narasaraopet, Andhra Pradesh, India
[2]Assistant Professor, Department of Pharmaceutical Analysis, SS and N College of Pharmacy, Narasaraopet, Andhra Pradesh, India
[3] MAM College of Pharmacy, Narasaraopet, Andhra Pradesh, India
DOI: https://doi.org/10.66856/ijrpps.2026.11.2.11060

Abstract
This work developed and validated a reverse phase high performance liquid chromatography method for estimating sevabertinib in pharmaceutical formulations. In statistically planned studies, a number of method aspects were changed, such as mobile phase ratio and column type, to assess how these factors affected the chromatographic separation of sevabertinib. The separation was carried out on a Inertsil ODS C18 (150 × 4.6 mm, 5 µm) at room temperature under isocratic conditions at a flow rate of 1.0 mL/min using Acetonitrile: 0.1% OPA pH 2.3 in the ratio of 65:35 (v/v). A PDA detector operating at 252 nm for a total of 8 minutes made the detection. Calibration curves were linear between 2.5 and 15 µg/mL. The calculated LOQ of 2.952µg/mL and the observed LOD of 1.126µg/mL show how sensitive the developed technique is. The %RSD being less than 2 validated the robustness and ruggedness of the approach. The assay % for formulation analysis was 99.10. Consequently, this method was frequently used to analyse sevabertinib in bulk and pharmaceutical formulations.

Keywords: Sevabertinib, method development, method validation, robustness, linearity

Introduction
Adults with advanced non-squamous non-small cell lung cancer (NSCLC) with HER2 (ERBB2) mutations are treated with sevabertinib, an oral targeted tyrosine kinase inhibitor (TKI). It is taken once or twice daily with food and is especially recommended for people whose cancer has advanced following previous systemic therapy [1, 4]. As of right now, no analytical technique for estimating sevabertinib in pharmaceutical formulation and bulk has been published. Figure 01 depicts the chemical structure of sevabertinib.

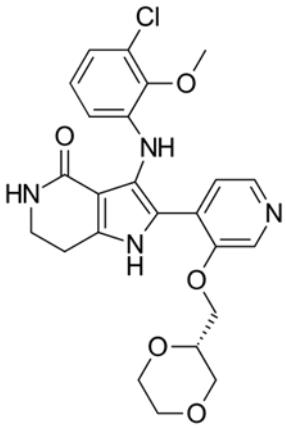

Fig 1: The Chemical structure of Sevabertinib

Mateials and Methods
Chemicals and Reagents
The working standard drug Sevabertinib (99.10% purity) & The formulation dosage form having brand name Hyrnuo containing 10 mg of Sevabertinib, were obtained from Zydus Life sciences Limited, Ahmedabad. HPLC grade Methanol, Water, Orthophosphoric acid and Acetonitrile were purchased form Merk chemicals private limited, Mumbai.

Preparation of Mobile Phase
Acetonitrile and 0.1% Orthophosphoric acid (P^H 2.3) were combined in a 65:35% v/v ratio. Before being used, the mobile phase was filtered through a 0.45 µm membrane filter after being sonicated for 15 minutes to eliminate dissolved gases.

Preparation of standard drug solution
After accurately weighing 10 mg of the standard sevabertinib in a 10 ml volumetric flask, it was completely dissolved in 5 mL of acetonitrile using an ultrasonicator. After using the same solvent to adjust the final volume in the volumetric flask, the solution was filtered using 0.45µm membrane filter paper. A 1000 µg/mL standard stock solution was obtained. The concentrations (10 µg/mL) required for method development and validation parameters were prepared from the stock (1000 µg/mL) solution of sevabertinib using mobile phase as a diluent.

Preparation of formulation solution
Twenty Sevabertinib tablets (label claim: 10 mg) were carefully weighed, ground into a fine powder, and added to a 10 mL volumetric flask together with around 7 mL of acetonitrile. The mixture was then sonicated for 15 minutes. The same diluent was used to adjust the volume once the solution had cooled to room temperature. The resultant solution was thoroughly mixed to create a sample stock solution with a concentration of 1000 µg/mL. The sample stock solution was filtered using a 0.45 µm membrane filter,

and the first few milliliters of the filtrate were thrown away. 1.0 mL of the filtered sample stock solution was carefully pipetted into a 10 mL volumetric flask and diluted to volume with the mobile phase to produce a working sample solution of 100 µg/mL. Additional dilutions were made from the working sample solution using the mobile phase in order to obtain a concentration of 10 µg/mL for assay and validation tests.

Method Development

Selection of Wavelength

The PDA detector was used to scan standard solutions of 10 µg/mL in order to choose an appropriate wavelength. A suitable wavelength for the detection was chosen based on the obtained wavelength maximum.

Table 1: Optimized Chromatographic Conditions

Parameter	Condition
Mobile Phase	Acetonitrile: 0.1% OPA pH 2.3 in the ratio of 65:35 (v/v)
Column	Inertsil ODS C18 (150 × 4.6 mm, 5 µm)
Flow Rate	1.0 ml/min
Wavelength	252nm
Injection Volume	20 µL
Temperature	Ambient
Run time	8min

Method Validation

The method was validated in terms of specificity, system suitability, LOD & LOQ, linearity, accuracy, precision, ruggedness, and robustness in compliance with the ICH requirements. Validation was carried out using duplicate injections of the sample and standard solutions into the column. [5]

Results and Discussion

Method Development

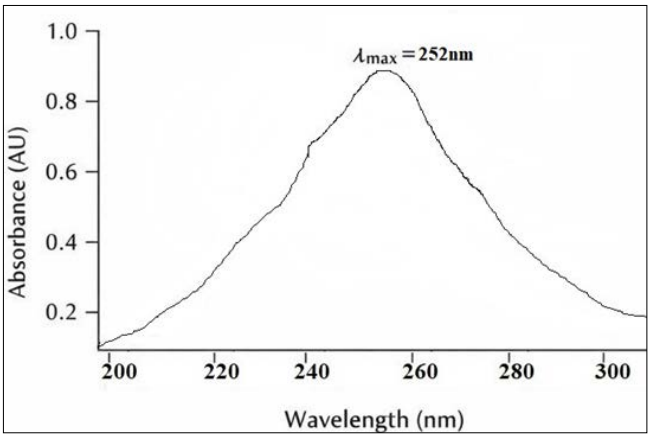

Fig 2: UV Spectra of Sevabertinib

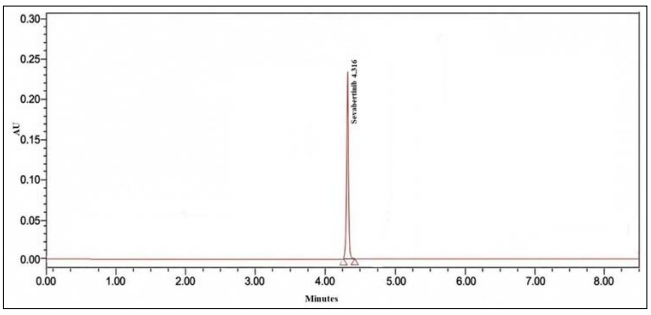

Fig 3: Optimized Chromatogram of Sevabertinib

Table 2: Results for Optimized Chromatogram

S.NO	Drug	Retention Time (min)	Theoretical Plates	Tailing Factor
1	Sevabertinib	4.316	4867	1.27

Method Validation

Specificity

No inference of diluent & Placebo

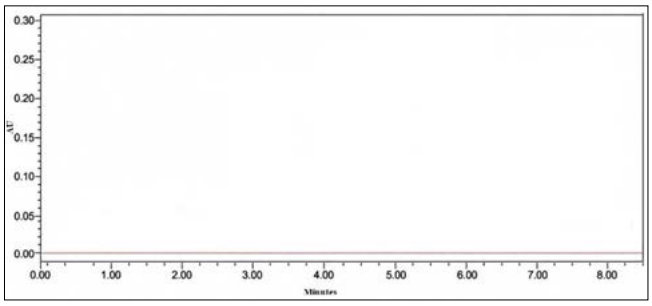

Fig 4: Chromatogram of Blank

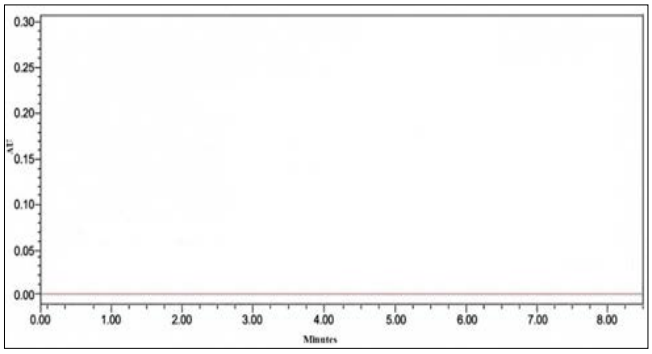

Fig 5: Chromatogram of Placebo

Linearity

Table 3: Results for Linearity

S. No	Level	Sevabertinib	
		Concentration in µg/mL	Peak Area
1	Level 1	2.5	76575
2	Level 2	5	154137
3	Level 3	7.5	230986
4	Level 4	10	309987
5	Level 5	12.5	389568
6	Level 6	15	468653

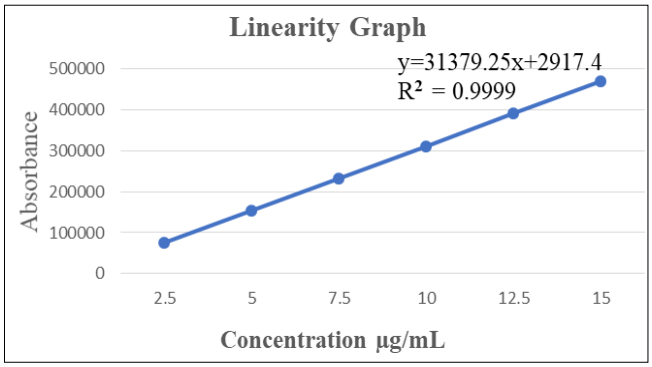

Fig 6: Linearity graph for Sevabertinib

LOD & LOQ

The LOD and LOQ of sevabertinib were reported to be 1.126µg/mL and 2.952µg/mL, respectively.

Precision

The system and method precision %RSD for sevabertinib were found to be 1.27 and 1.14, respectively. The %RSD was found to be within the permissible range of less than 2 for both system and technique precision.

Table 4: Results for Precision

S.NO	Injection	System Precision		Method Precision	
		Retention Time (Min)	Peak Area	Retention Time (Min)	Peak Area
1	Injection-1	4.322	314657	4.318	315674
2	Injection-2	4.317	307568	4.321	318752
3	Injection-3	4.316	311589	4.322	319267
4	Injection-4	4.323	314735	4.314	309746
5	Injection-5	4.315	305876	4.316	313742
6	Injection-6	4.324	316784	4.325	319976
Mean		4.3195	311868.2	4.319333	316192.8
STD		0.003594	3971.506	0.003727	3609.008
%RSD		0.083	1.27	0.08	1.14

Accuracy

It was demonstrated that the recovery percentage ranged from 98.80 to 99.33%. At 50%, 100%, and 150% spiking levels, the percentage RSD was found to be within the permissible limit for sevabertinib. The results showed that the suggested procedure was accurate, with an acceptance limit of 98–102% and a percentage RSD of less than two.

Table 5: Results for Accuracy

Recovery Level	Concentration in µg/ml			Amount Found	% Recovery	% RSD
	Target	Spiked	Total			
50%	5	2.5	7.5	7.44	99.20	0.16
	5	2.5	7.5	7.41	98.93	
	5	2.5	7.5	7.45	99.33	
100%	5	5	10	9.89	98.90	0.12
	5	5	10	9.10	99.10	
	5	5	10	9.88	98.80	
150%	5	7.5	12.5	12.39	99.12	0.13
	5	7.5	12.5	12.37	98.96	
	5	7.5	12.5	12.35	98.80	

Ruggedness (Intermediate Precision)

Sevabertinib's %RSD in the developed technique was 0.1. Ruggedness required to be expressed using a %RSD of less than 2. Results that are within the permitted range validate the ruggedness of the procedure.

Table 6: Results for Ruggedness

S.NO	Injection	Retention Time (Min)	Peak Area
1	Injection-1	4.317	314298
2	Injection-2	4.324	314876
3	Injection-3	4.318	314975
4	Injection-4	4.320	314574
5	Injection-5	4.316	313989
6	Injection-6	4.321	314759
Mean		4.319333	314578.5
STD		0.002687	342.6635
%RSD		0.06	0.1

Robustness

In the devised approach, the percentage change of sevabertinib was found to be within the acceptable range of less than 2. Consequently, it was demonstrated that when the analytical conditions were slightly altered, the suggested approach was appropriate for the analysis of sevabertinib. This shows that the results are unaffected by even minor changes to the analytical conditions.

Table 7: Results for Robustness

S. No	Condition	Sevabertinib		
		Retention Time	Peak Area	% Change
1	Standard	4.316	314275	--
2	+MP (70:30)	4.314	314168	0.03
3	-MP (60:40)	4.320	314099	0.05
4	Flow Rate 1.2ml/min	4.315	314289	0.01
5	Flow rate 0.8ml/min	4.323	314156	0.03
%RSD		0.08	0.03	

Assay

Sevabertinib's assay percentage in formulation analysis was 99.10%. As a result, the technique was demonstrated to be appropriate for the routine analysis of sevabertinib in formulations and bulk.

Table 8: Results for Formulation

S. No	Drug	Brand	Label Claim	Peak Area	Amount Found	% Assay
1	Sevabertinib	Hyrnuo	10 mg	307197	99.10mg	99.10

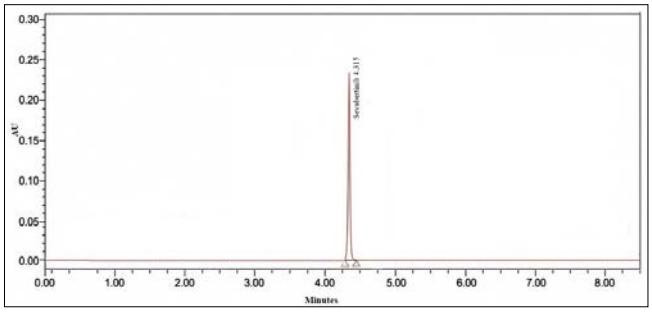

Fig 7: Chromatogram of Formulation

Conclusion

The LC determination of sevabertinib in pharmaceutical formulations was not previously documented in the literature. A sensitive, accurate, and precise RP-HPLC method for assessing sevabertinib in pharmaceutical formulations and bulk has been developed by the author. The recommended RP-HPLC method for the Sevabertinib test was shown to be suitable for routine quantitative analysis following validation. The HPLC method saved time while preparing the standard and sample and did away with the requirement for time-consuming extraction. The low standard deviation data show the exceptionally high precision of the new approach. The linearity, accuracy, specificity, and precision values were found to be within acceptable ranges. The chromatogram's lack of extra peaks showed that there was no conflict between the tablet's common excipients. Thus, it is shown that the devised RP-HPLC method is straightforward, linear, precise, sensitive, and reproducible. The new approach is therefore simple to use and offers a fast analytical time for routine quality

monitoring of sevabertinib in pharmaceutical formulations and bulk. The results indicate that the suggested approach has good precision and accuracy.

References

1. Hyrnuo- sevabertinib tablet, film coated". DailyMed, 2025. 3 December 2025. Retrieved 28 December 2025.
2. "FDA grants accelerated approval to sevabertinib for non-squamous non-small cell lung cancer". U.S. Food and Drug Administration (FDA), 2025. 19 November 2025. Retrieved 21 November 2025.
3. FDA Approved Drug Products: HYRNUO® (sevabertinib) tablets, for oral use, 2025.
4. US20250136599A1, "4H-pyrrolo [3,2-c] pyridin-4-one compounds", 2025. Published 1 May 2025.
5. ICH Q2 (R1). Validation of Analytical Procedures: Text and Methodology. Geneva: ICH, 2005.