RESEARCH PAPER

RP-HPLC Method Development and Validation for the Estimation of Eltrombopag Olamine in Pharmaceutical Dosage Forms

P. Sathya Sowmya[1*], Sidde Lahari[2], Kencha Swathi[3], K. Vijay Kumar[4], D. Venkatanarayana[5]

[1*] Assistant Professor, Department of Pharmaceutical Analysis, Sri Krishnadevaraya University College of Pharmaceutical Sciences, SK University, Anantapur, Andhra Pradesh, India.
Email: nicpsowmi@gmail.com (Corresponding Author)
[2] Assistant Professor, Department of Pharmaceutical Chemistry, JNTUA - Oil Technological and Pharmaceutical Research Institute, Anantapur, Andhra Pradesh, India.
[3] Assistant Professor, Department of Pharmaceutical Chemistry, Sri Raghavendra College of Pharmacy, Bangalore, Karnataka, India.
[4] Assistant Professor, Department of Pharmaceutical Chemistry, Aditya University, Surampalem, Kakinada District, Andhra Pradesh, India.
[5] Assistant Professor, Department of Pharmacognosy, Balaji College of Pharmacy, Anantapur, India.

Received: 2nd Mar, 2026 | **Revised:** 14th Mar, 2026 | **Accepted:** 4th Apr, 2026 | **Available Online:** 20th Apr, 2026

ABSTRACT

Purpose: Eltrombopag olamine is an orally active thrombopoietin receptor agonist used in the treatment of thrombocytopenia and aplastic anemia. Accurate analytical methods are required for quality control analysis of this drug in pharmaceutical formulations. The present study aimed to develop and validate a simple, rapid and precise reverse phase high performance liquid chromatographic method for the quantitative estimation of eltrombopag olamine in pharmaceutical dosage forms.

Methods: Chromatographic separation was achieved using DIKMA Spursil ODS C18 column (4.6 × 250 mm, 5 µm). The mobile phase consisted of 0.1% orthophosphoric acid buffer, acetonitrile and methanol in the ratio 30:60:10. The flow rate was maintained at 1.0 mL/min and detection was performed at 295 nm.

Results: The retention time of eltrombopag olamine was found to be 2.144 minutes. The developed method exhibited good linearity within the selected concentration range. The percentage recovery was 99.76% and %RSD was less than 1%, indicating good accuracy and precision. LOD and LOQ values were found to be 2.97 and 9.97 respectively.

Conclusion: The developed RP-HPLC method was found to be simple, accurate and reliable and can be successfully applied for routine quality control analysis of eltrombopag olamine in pharmaceutical dosage forms.

Keywords: Eltrombopag Olamine, RP-HPLC, Method Development, Method Validation, Pharmaceutical Dosage Forms.

How to cite this article: Sowmya PS, Lahari S, Swathi K, Vijay Kumar K, Venkatanarayana D. RP-HPLC Method Development and Validation for the Estimation of Eltrombopag Olamine in Pharmaceutical Dosage Forms. Int J Drug Deliv Technol. 2026;16(32s):951-956. DOI: 10.25258/ijddt.16.32s.105

Source of support: Nil.

Conflict of interest: The authors declare no conflict of interest.

RP-HPLC Method Development and Validation for the Estimation of Eltrombopag Olamine in Pharmaceutical Dosage Forms

INTRODUCTION

Eltrombopag olamine is a thrombopoietin receptor agonist used for the treatment of thrombocytopenia associated with chronic immune thrombocytopenic purpura and severe aplastic anemia. The drug stimulates platelet production by activating thrombopoietin receptors present on hematopoietic stem cells and megakaryocyte progenitors.

Quality control of pharmaceutical products is essential to ensure safety, efficacy and reliability. Analytical methods play a crucial role in the evaluation of pharmaceutical formulations. Reliable analytical procedures are necessary for determination of drug content, purity and stability.

High performance liquid chromatography (HPLC) is one of the most widely used analytical techniques in pharmaceutical analysis because of its high sensitivity, precision and reproducibility. In HPLC separation occurs based on the interaction between stationary phase and mobile phase which results in different retention times for compounds.

Reverse phase HPLC (RP-HPLC) is widely used in pharmaceutical industries because it provides better resolution, shorter analysis time and good reproducibility. In RP-HPLC a non- polar stationary phase such as C18 column is used with a polar mobile phase.

Method development involves optimization of chromatographic conditions including mobile phase composition, flow rate, detection wavelength and column selection. After development, the analytical method must be validated according to ICH guidelines.

Method validation confirms the reliability of analytical methods. Important validation parameters include linearity, accuracy, precision, limit of detection, limit of quantification and robustness.

The aim of the present study was to develop and validate a simple and accurate RP-HPLC method for the estimation of eltrombopag olamine in pharmaceutical dosage forms.

Materials and Methods Chemicals and Reagents

Eltrombopag olamine reference standard was obtained from MSN Laboratories. Orthophosphoric acid, methanol and acetonitrile of HPLC grade were used. All chemicals were of analytical grade and distilled water was used throughout the study.

Instruments

Chromatographic analysis was performed using a Waters HPLC system equipped with a 2695 separation module and UV detector with Empower software. UV-Visible spectrophotometer (Labindia UV-3000+), digital pH meter (Adwa AD-1020) and analytical balance (Afcoset ER- 200A) were used during the study.

Chromatographic Conditions

Separation was carried out using DIKMA Spursil ODS C18 column (4.6 × 250 mm, 5 µm). The mobile phase consisted of 0.1% orthophosphoric acid buffer, acetonitrile and methanol in the ratio 30:60:10. The flow rate was maintained at 1.0 mL/min and detection wavelength was set at 295 nm. Injection volume was 20 µL and run time was 10 minutes.

Method Validation

The developed RP-HPLC method was validated according to ICH guidelines for system suitability, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) and robustness.

Drug Profile

Parameter	Description
Drug Name	Eltrombopag Olamine
Molecular Formula	C29H36N6O6
Molecular Weight	564.6 g/mol
Category	Thrombopoietin receptor agonist
Solubility	Soluble in organic solvents
Half Life	21–32 hours

Table 1. Physicochemical properties of Eltrombopag Olamine

Drug Name: Eltrombopag Olamine

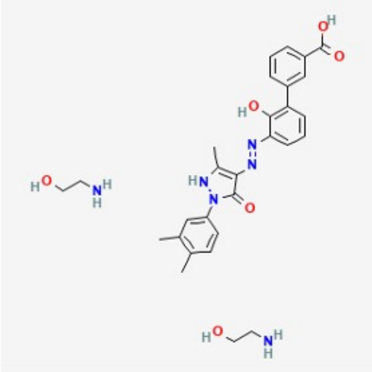

Figure 1. Chemical structure of Eltrombopag Olamine.

IUPAC Name: 2-aminoethanol; 3-[3-[[2-(3,4-dimethylphenyl)-5-methyl-3-oxo-1H-pyrazol- 4-yl] diazenyl]-2-hydroxyphenyl] benzoic acid

Molecular Formula: C29H36N6O6

Molecular Weight: 564.6 g/mol

Category: Thrombopoietin receptor agonist

Solubility: Soluble in organic solvents such as ethanol, dimethyl sulfoxide and dimethyl formamide.

Melting Point: 236–238 °C

pKa: −0.12

Protein Binding: Greater than 99%

Half Life: 21–32 hours

Mechanism

c ltrombopag olami

Experimental Method Instruments

The analysis was performed using a Waters High Performance Liquid Chromatography system equipped with a 2695 separation module and UV detector. UV-Visible spectrophotometer (Labindia UV-3000+), digital pH meter (Adwa AD-1020) and analytical balance (Afcoset ER- 200A) were used during the study.

Chemicals

Eltrombopag olamine was obtained from MSN Laboratories. Orthophosphoric acid, methanol, acetonitrile and distilled water were used for chromatographic analysis.

Chromatographic Conditions

Chromatographic separation was carried out using DIKMA Spursil ODS C18 column (4.6 × 250 mm, 5 µm). The mobile phase consisted of 0.1% orthophosphoric acid buffer, acetonitrile and methanol in the ratio of 30:60:10. The flow rate was maintained at 1.0 mL/min and detection wavelength was set at 295 nm. Injection volume was 20 µL and the run time was 10 minutes.

Parameter	Condition
Column	DIKMA Spursil ODS C18 (4.6 × 250 mm, 5 µm)
Mobile Phase	Buffer : Acetonitrile : Methanol (30:60:10)
Flow Rate	1.0 mL/min
Detection Wavelength	295 nm
Injection Volume	20 µL
Run Time	10 min
Retention Time	2.144 min

Table 2. Optimized chromatographic conditions for RP-HPLC analysis

Preparation of Mobile Phase

The mobile phase was prepared by mixing orthophosphoric acid buffer, acetonitrile and methanol in the ratio 30:60:10. The solution was filtered through a 0.45 µm membrane filter and degassed before use.

Method Validation

The developed RP-HPLC method was validated according to ICH guidelines for parameters such as linearity, accuracy, precision, limit of detection, limit of quantification and robustness.

Validation Parameters

The developed RP-HPLC method was validated according to **ICH guidelines** for the following parameters.

System Suitability

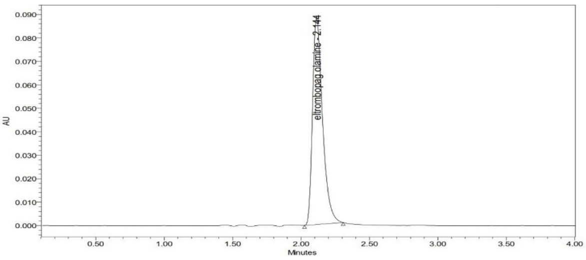

Figure 2:System suitability chromatogram of Eltrombopag Olamine

System suitability was evaluated by injecting the standard solution multiple times before analysis. Parameters such as retention time, theoretical plates, tailing factor and peak area were recorded to ensure proper functioning of the chromatographic system.

Parameter	Result
Retention Time	2.144 min
Theoretical Plates	>2000
Tailing Factor	<2
%RSD of Peak Area	0.7

Linearity: Linearity of the method was determined by preparing standard solutions of eltrombopag olamine at different concentration levels. Each solution was injected into the HPLC system and the calibration curve was constructed by plotting peak area versus concentration.

Precision: Precision of the method was evaluated by injecting the standard solution six times under the same chromatographic conditions. The results were expressed as percentage relative standard deviation (%RSD).

Accuracy: Accuracy of the method was determined by recovery studies using the standard addition method at different concentration levels. The percentage recovery was calculated to evaluate the accuracy of the method.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated based on the standard deviation of the response and slope of the calibration curve.

Parameter	Value
Limit of Detection (LOD)	2.97

Limit of Quantification (LOQ)	9.97

Robustness: Robustness of the method was studied by making small deliberate changes in chromatographic conditions such as flow rate and mobile phase composition and evaluating their effect on the results.

Sample Chromatogram

The developed RP-HPLC method was applied for the analysis of Eltrombopag Olamine in pharmaceutical dosage forms. The sample solution was prepared and injected into the chromatographic system. The chromatogram showed a well-defined peak at the same retention time as the standard drug, confirming the presence of Eltrombopag Olamine in the formulation.

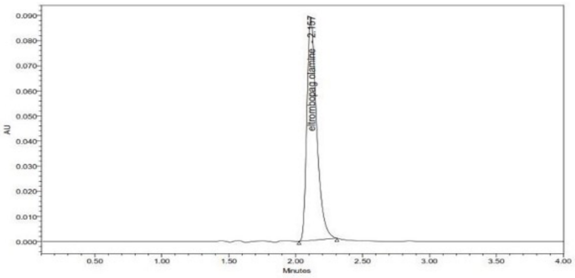

Fig 3: Sample chromatogram of Eltrombopag Olamine tablet formulation

Linearity Study

Linearity of the developed RP-HPLC method was evaluated by analyzing different concentrations of Eltrombopag Olamine ranging from **10–50 µg/mL**. The peak area was found to increase proportionally with concentration. The calibration curve was constructed by plotting concentration versus peak area. The correlation coefficient obtained was **0.999**, which indicates good linearity of the developed method.

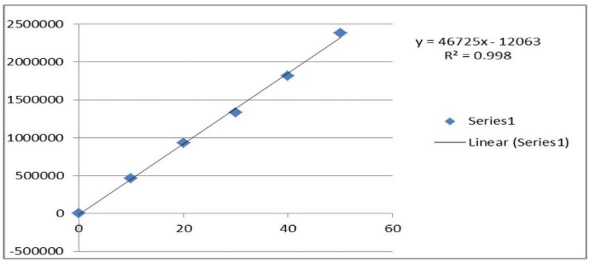

Fig 4: Calibration curve of Eltrombopag Olamine showing linear relationship between concentration and peak area

Precision

Precision of the method was evaluated by injecting the standard solution six times under identical chromatographic conditions. The peak areas obtained were consistent and the percentage relative standard deviation (%RSD) was found to be **0.7**, which is within the acceptable limit. These results indicate good repeatability and precision of the developed method.

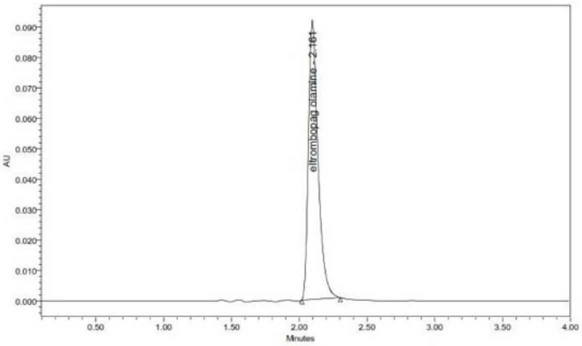

Fig 5: Chromatogram obtained during precision study

Limit of Detection

The limit of detection (LOD) was determined based on the signal-to-noise ratio obtained for the lowest detectable concentration of Eltrombopag Olamine. The signal-to-noise ratio was found to be **2.97**, indicating that the method is sensitive for detection of the drug at very low concentrations.

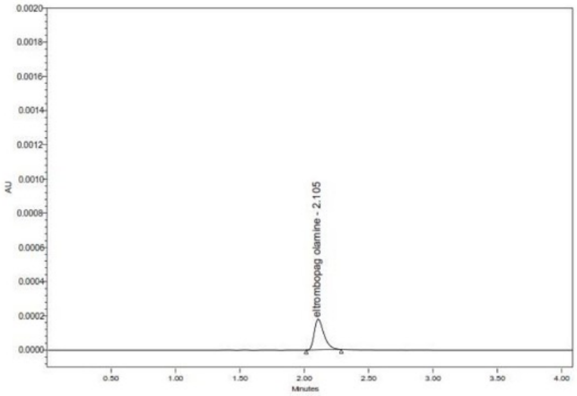

Fig 6 :Chromatogram showing limit of detection for Eltrombopag Olamine

Limit of Quantification

The limit of quantification (LOQ) was determined using the signal-to-noise ratio for the lowest quantifiable concentration of Eltrombopag Olamine. The signal-to-noise ratio obtained was **9.97**, indicating that the developed method is suitable for accurate quantification of the drug.

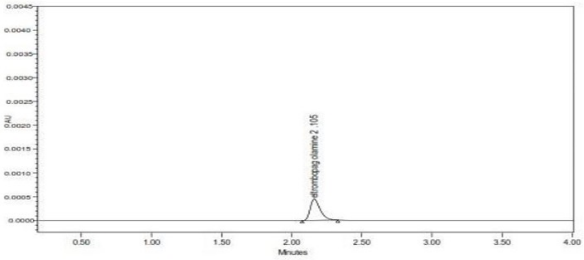

Fig 7 :Chromatogram showing limit of quantification for Eltrombopag Olamine

Robustness

Robustness of the developed RP-HPLC method was evaluated by making small deliberate changes in chromatographic conditions such as flow rate and mobile phase composition. The results showed no significant variation in retention time, peak shape or system suitability parameters. These observations confirm that the developed method is robust and reliable for routine analysis.

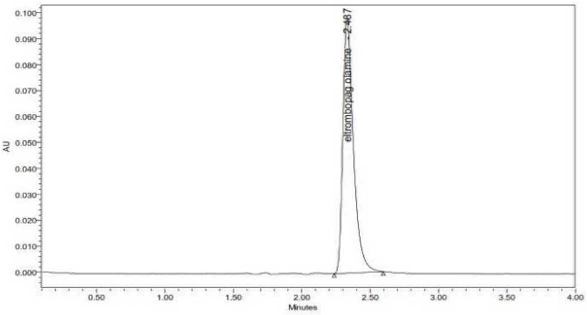

Fig 8: Chromatogram obtained at reduced flow rate during robustness study

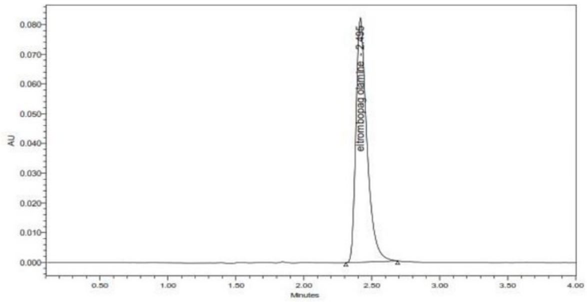

Fig 9: Chromatogram obtained after variation in mobile phase composition during robustness study

Conclusion

A simple and reliable RP-HPLC method was developed for the estimation of Eltrombopag Olamine in pharmaceutical dosage forms. The developed method showed good chromatographic separation with a retention time of about 2.144 min. The assay of tablet formulation was found to be **99.76%**, indicating suitability of the method for routine pharmaceutical analysis.

The method showed good linearity in the concentration range of **10–50 µg/mL** with a correlation coefficient of **0.999**. Precision studies showed %RSD values less than **1%**, confirming good repeatability of the method. Intermediate precision studies also showed

%RSD values within acceptable limits, demonstrating reproducibility under different conditions.

Accuracy studies showed a mean recovery of **100.50%**, indicating good accuracy of the developed method. The limit of detection and limit of quantification were found to be **2.97** and **9.97**, respectively, demonstrating adequate sensitivity. Robustness studies confirmed that small variations in chromatographic conditions such as flow rate and mobile phase composition did not significantly affect the results.

These results demonstrate that the developed RP-HPLC method is accurate, precise, sensitive and robust, and can be successfully applied for routine quality control analysis of Eltrombopag Olamine in pharmaceutical dosage forms.

Acknowledgement

The authors express their sincere gratitude to the Department of Pharmaceutical Analysis, Sri Krishnadevaraya University College of Pharmaceutical Sciences (SKUCOPS), Ananthapuramu, for providing the necessary laboratory facilities to carry out this research work. The authors are also thankful to the management and faculty members for their support and encouragement during the course of this study. Special thanks are extended to the guide and staff members of the analytical laboratory for their valuable guidance and assistance in completing this work successfully.

References

1. Snyder LR, Kirkland JJ, Dolan JW. Introduction to Modern Liquid Chromatography. 3rd ed. New York: Wiley; 2010.
2. Skoog DA, Holler FJ, Crouch SR. Principles of Instrumental Analysis. 6th ed. Belmont: Thomson Brooks/Cole; 2007.
3. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry. 4th ed. New Delhi: CBS Publishers; 2002.
4. Chatwal GR, Anand SK. Instrumental Methods of Chemical Analysis. Mumbai: Himalaya Publishing House; 2007.
5. Willard HH, Merritt LL, Dean JA, Settle FA. Instrumental Methods of Analysis. 7th ed. New York: CBS Publishers; 2008.
6. United States Pharmacopeia. USP 43–NF 38. Rockville: United States Pharmacopeial Convention; 2020.
7. British Pharmacopoeia. London: The Stationery Office; 2020.
8. Indian Pharmacopoeia Commission. Indian Pharmacopoeia. Ghaziabad; 2018.
9. ICH Q2(R1). Validation of Analytical Procedures: Text and Methodo
10. Meyer VR. Practical High-Performance Liquid Chromatography. 5th ed. Chichester: Wiley; 2010.
11. Kazakevich Y, Lobrutto R. HPLC for

Pharmaceutical Scientists. Hoboken: Wiley; 2007.

12. Dong MW. Modern HPLC for Practicing Scientists. Hoboken: Wiley; 2006.
13. Swartz ME, Krull IS. Analytical Method Development and Validation. New York: CRC Press; 2012.
14. Dandabattina R, et al. Stability indicating HPLC method for determination of impurities in Eltrombopag tablets. J Pharm Anal. 2017;7(3):190-196.
15. Manoharan A, et al. Development and validation of RP-HPLC method for estimation of Eltrombopag in pharmaceutical dosage form. Int J Pharm Sci Rev Res. 2018;52(2):114-118.
16. Ahir KB, et al. RP-HPLC method for estimation of Eltrombopag in tablet dosage form. Asian J Pharm Clin Res. 2019;12(5):201-205.
17. Maddela R, et al. LC-MS/MS method for quantification of Eltrombopag in human plasma. J Chromatogr B. 2018;1083:1-7.
18. Blessy M, et al. Development of forced degradation and stability indicating studies of drugs. J Pharm Anal. 2014;4(3):159-165.
19. Bakshi M, Singh S. Development of validated stability indicating assay methods. J Pharm Biomed Anal. 2002;28:1011-1040.
20. Dong MW. HPLC troubleshooting and maintenance. LCGC North America. 2003;21(10):1008-1014.
21. Kazakevich Y. HPLC method development for pharmaceutical analysis. J Chromatogr A. 2001;915:205-215.
22. Snyder LR, Kirkland JJ. Introduction to HPLC. J Chromatogr A. 2007;1126:3-10.
23. Kromidas S. HPLC Made to Measure. Weinheim: Wiley-VCH; 2006.
24. Bolton S, Bon C. Pharmaceutical Statistics. 5th ed. New York: Marcel Dekker; 2004.
25. Ermer J, Miller JH. Method Validation in Pharmaceutical Analysis. Wiley-VCH; 2005.
26. Miller JN, Miller JC. Statistics and Chemometrics for Analytical Chemistry. 6th ed. Pearson; 2010.
27. Shrivastava A, Gupta VB. Methods for determination of LOD and LOQ. Chron Young Sci. 2011;2(1):21-25.
28. Snyder LR. Classification of chromatographic methods. Anal Chem. 1978;50:1191- 1196.
29. Carr GP, Wahlich JC. Practical considerations in method validation. J Pharm Biomed Anal. 1990;8:613-618.
30. Ravisankar P, et al. Analytical method development and validation. Int J Pharm Sci Res. 2015;6(6):2320-2331.
31. Kumar S, et al. RP-HPLC method development and validation: A review. Int J Pharm Pharm Sci. 2012;4(3):13-20.
32. Snyder LR. Gradient elution in HPLC. J Chromatogr. 1980;189:3-18.
33. Poole CF. Chromatography Today. Amsterdam: Elsevier; 2013.
34. Dolan JW. HPLC method development strategies. LCGC North America. 2004;22:430- 436.
35. Niessen WMA. Liquid Chromatography-Mass Spectrometry. 3rd ed. CRC Press; 2006.
36. Dong MW. HPLC method validation for pharmaceutical analysis. Pharm Tech. 2006;30:82-92.
37. Meyer VR. Practical aspects of HPLC. J Chromatogr A. 2004;1023:1-8.
38. Ermer J. Validation in pharmaceutical analysis. J Pharm Biomed Anal. 2001;24:755- 767.
39. Snyder LR, Dolan JW. High-Performance Gradient Elution. Wiley; 2007.
40. Blessy M, et al. Stability indicating methods in pharmaceutical analysis. J Pharm Anal. 2014;4(3):159-165.