IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES
ISSN PRINT 2319 1775 Online 2320 7876
 Journal UGC CARE Listed (Group-I) Volume 15, Issue 01, 2026

# Time and Solvent-Saving RP-HPLC Method for Eltrombopag Olamine : Development, Validation, and Stability-Indicating Studies in API and Tablets

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## ABSTRACT

### Background:

Eltrombopag olamine is a thrombopoietin receptor agonist widely used in the management of platelet-related disorders, necessitating a reliable analytical method for routine quality control and stability assessment.

### Methods:

An RP-HPLC method with reduced run time and solvent usage was established and validated for the quantitative determination of eltrombopag olamine in active pharmaceutical ingredient and tablet dosage forms. Method validation was carried out in accordance with ICH Q2(R1), and forced degradation studies were conducted following ICH Q1A(R2) recommendations.

### Results:

Chromatographic separation was achieved on an Inert Sustain C18 column (4.6 × 250 mm, 5 µm) using a methanol–acetonitrile mobile phase (90:10, v/v). Detection was performed at 230 nm using a PDA detector. The analyte eluted at approximately 4.3 minutes. The method demonstrated linearity over the concentration range of 5–100 µg/mL with a correlation coefficient of 0.9936. The limits of detection and quantification confirmed adequate sensitivity. Stress degradation studies indicated effective separation of eltrombopag olamine from its degradation products.

### Conclusion:

The validated RP-HPLC method is rapid, economical, and stability-indicating, making it suitable for routine quality control and stability testing of eltrombopag olamine in pharmaceutical formulations.

**Keywords:** Eltrombopag olamine; RP-HPLC; Method validation; Stability-indicating method

 

## Introduction :

Eltrombopag olamine is a synthetic thrombopoietin receptor agonist used clinically to stimulate platelet production in conditions such as chronic immune thrombocytopenia, hepatitis C–associated thrombocytopenia, and severe aplastic anemia. Due to its therapeutic importance and widespread clinical use, accurate and reliable analytical methods are required to ensure the quality and stability of pharmaceutical formulations containing this drug.

From an analytical perspective, eltrombopag olamine presents challenges related to its limited aqueous solubility and susceptibility to degradation under stress conditions. These factors necessitate the development of a stability-indicating chromatographic method capable of distinguishing the intact drug from its degradation products while maintaining acceptable sensitivity and reproducibility.

Several reverse-phase high-performance liquid chromatography (RP-HPLC) methods have previously been reported for the estimation of eltrombopag in bulk drug and dosage forms. These methods utilize different stationary phases and mobile phase compositions, resulting in varying retention times and analytical performance. However, many reported procedures involve higher consumption of organic solvents or longer analysis times, which may limit their suitability for routine quality control environments.

Considering these limitations, the present study was undertaken to develop and validate a simple, rapid, and solvent-efficient RP-HPLC method for the determination of eltrombopag olamine in active pharmaceutical ingredient and tablet dosage forms. The method was further evaluated through forced degradation studies to confirm its stability-indicating capability in accordance with ICH guidelines.

## METHODS AND MATERIALS

### Chemicals and Reagents

Eltrombopag olamine was obtained as a reference standard of analytical grade. Methanol and acetonitrile of HPLC grade were procured from Merck (Mumbai, India). HPLC-grade water was used throughout the analysis. All reagents were used as received without further purification.

### Instrumentation

Chromatographic analysis was carried out using a Shimadzu LC-20AD high-performance liquid chromatography system (Shimadzu Corporation, Kyoto, Japan) equipped with a photodiode array (PDA) detector. UV–Visible spectroscopic measurements were performed using a Shimadzu UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). Sample weighing was conducted using an ATY224 analytical balance (Shimadzu, Kyoto, Japan). Sonication was performed using a SONICA 2200MH ultrasonicator (Soltec, Milan, Italy). A hot air oven (Vision Lab Equipment, Hyderabad, India) and a UV cabinet (Monoquartz, Chennai, India) were used for stress degradation studies.

### RP-HPLC Chromatographic conditions

### Chromatographic Conditions

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Separation was achieved on an Inert Sustain C18 column (4.6 mm × 250 mm, 5 µm particle size). The mobile phase consisted of methanol and acetonitrile in the ratio of 90:10 (v/v), delivered at a flow rate optimized to achieve adequate resolution and peak symmetry. Detection was performed at a wavelength of 230 nm using a PDA detector. The injection volume was set at 20 µL, and the total run time was maintained at 5 minutes.

### Preparation of Standard Solution

A standard stock solution was prepared by dissolving 10 mg of eltrombopag olamine in 10 mL of methanol to obtain a concentration of 1000 µg/mL. This solution was further diluted with the mobile phase to prepare working standard solutions within the required concentration range. All solutions were filtered through a 0.45 µm membrane filter prior to injection.

### Preparation of Sample Solution

Twenty tablets were accurately weighed and finely powdered. An amount of tablet powder equivalent to 10 mg of eltrombopag olamine was transferred to a 10 mL volumetric flask, dissolved in methanol, and sonicated for 10 minutes to ensure complete extraction. The solution was then diluted to volume with the mobile phase and filtered through a 0.45 µm nylon membrane filter. Further dilutions were prepared as required for analysis.

## RESULTS AND DISCUSSION

### SYSTEM SUITABILITY:

Under optimized chromatographic conditions, eltrombopag olamine produced a sharp and symmetrical peak with consistent retention behavior. System suitability parameters, including retention time, theoretical plate count, and tailing factor, were found to be within acceptable limits, confirming adequate system performance for analysis. The system suitability results are summarized in Table 1, and a representative chromatogram is shown in Figure 1.

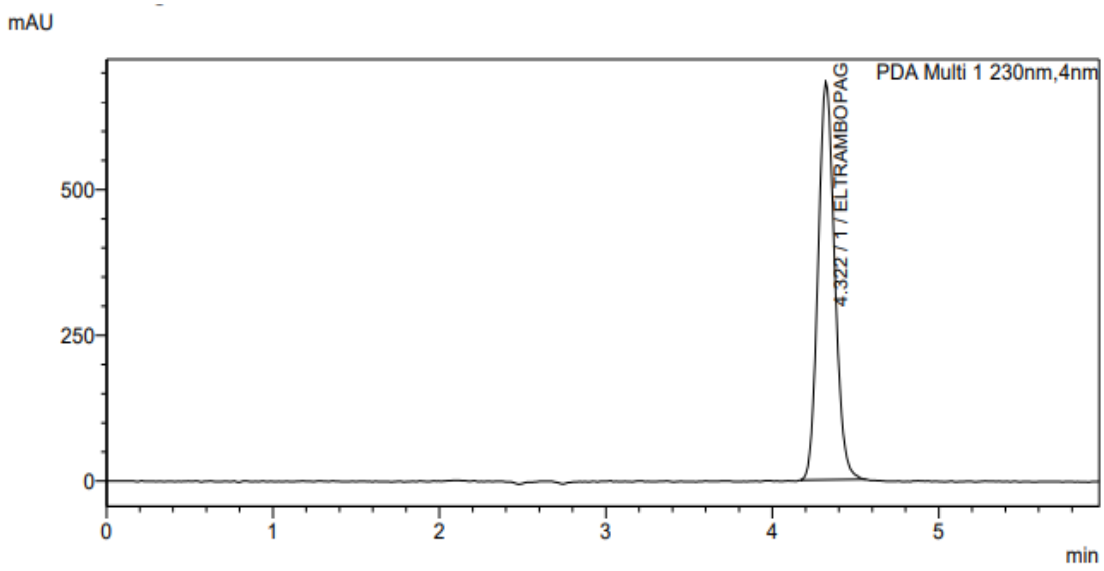


**Figure 1 : Chromatogram of system suitability**

 

**Table1: System requirements for the suggested HPLC technique**

| S.NO. | Peak area | Retention duration(min) | Theoretical plates | Tailing factor |
|---|---|---|---|---|
| 1. | 4798618 | 4.319 | 7562 | 1.127 |
| 2 | 4786895 | 4.323 | 7568 | 1.123 |
| 3 | 4702851 | 4.322 | 7561 | 1.123 |
| 4 | 4747667 | 4.324 | 7558 | 1.111 |
| 5 | 4793957 | 4.357 | 7576 | 1.122 |
| 6 | 4735967 | 4.353 | 7586 | 1.105 |
| Average | 4760992.50 | 4.33 | 7568 | 1.118 |
| STDEV | 38360.28 | 0.02 | 10.69 | 0.01 |
| %RSD | 0.81 | 0.40 | 0.14 | 0.68 |
| Limits | - | - | <2.0 | <2.0 |

**Linearity :**

The linearity of the method was evaluated over the concentration range of 5–100 µg/mL. A calibration curve was constructed by plotting peak area against corresponding drug concentration. The method demonstrated a linear response across the studied range, with a correlation coefficient ($R^2$) of 0.9936, indicating a strong linear relationship between concentration and detector response. Linearity data are presented in Table 2, and the calibration curve is illustrated in Figure 2.

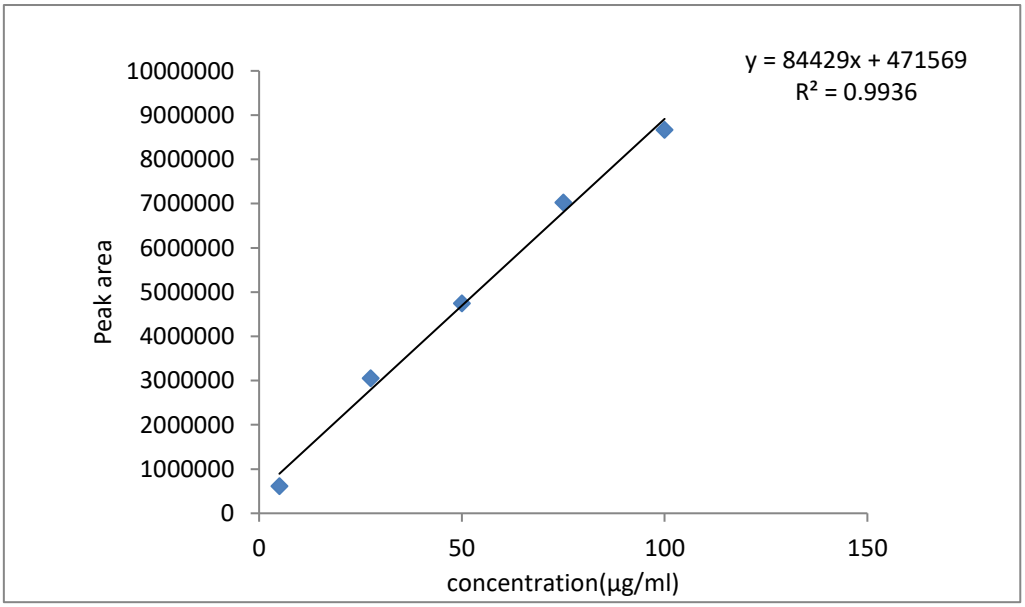


**Figure 2 : Eltrombopag olamine linearity graph**

**Table2: Linearity for Eltrombopag olamine**

| S.NO. | Level of linearity | Concentration | Peak area |
|---|---|---|---|
| 1. | 50% | 5 | 613256 |
| 2. | 75% | 27.5 | 3049708 |
| 3. | 100% | 50 | 4743155 |
| 4. | 125% | 75 | 7025735 |
| 5. | 150% | 100 | 8666377 |

 

**Precision:**

Method precision was assessed by repeated injection of a standard solution at 100% test concentration (500 µg/ml). The results showed low variability in peak area, with percentage relative standard deviation (%RSD) values below 2%, confirming the repeatability and reliability of the analytical method. Precision results are summarized in Table 3.

**Table 3: Precision for proposed HPLC method**

| S.NO | Day 1 | Day 2 | Day 3 |
|---|---|---|---|
| 1. | 4791434 | 4767675 | 4708342 |
| 2. | 4762070 | 4721759 | 4747234 |
| 3. | 4766399 | 4713217 | 4721691 |
| 4. | 4728642 | 4734535 | 4730050 |
| 5. | 4736661 | 4703630 | 4710065 |
| 6. | 4758539 | 4704102 | 4734171 |
| Average | 4757300.67 | 4724153.00 | 4725258.83 |
| STDEV | 22465.49 | 24292.03 | 14933.53 |
| RSD | 0.47 | 0.51 | 0.32 |
| Limits | %RSD:<2.0 | %RSD:<2.0 | %RSD:<2.0 |

**ACCURACY**:

The accuracy of the method was evaluated by recovery studies at three concentration levels (50%, 100%, and 150%) using tablet sample solutions. The percentage recovery values were found to be within the acceptable range of 98–102%, indicating that the method is accurate and free from interference by excipients present in the tablet formulation. The recovery results are presented in Table 4.

**Table 4:Accuracy displaying the recovery percentage**

| S.NO | Levels | Concentration taken(µg/ml) | Concentration added (µg/ml) | Peak area | Concentration recoverd (µg/ml) | % recovery | Average Recovery |
|---|---|---|---|---|---|---|---|
| 1 | 50% | 50 | 75 | 7073029 | 74.8546 | 99.8061 | 100.76 |
| 2 | | 50 | 75 | 7128140 | 75.8359 | 101.1145 | |
| 3 | | 50 | 75 | 7138692 | 76.0238 | 101.3650 | |
| 4 | 100% | 50 | 100 | 8592776 | 101.9146 | 101.9146 | 101.68 |
| 5 | | 50 | 100 | 8577538 | 101.6433 | 101.6433 | |
| 6 | | 50 | 100 | 8568296 | 101.4788 | 101.4788 | |
| 7 | 150% | 50 | 125 | 9967924 | 126.4000 | 101.1200 | 100.61 |
| 8 | | 50 | 125 | 9895839 | 125.1165 | 100.0932 | |
| 9 | | 50 | 125 | 9933248 | 125.7826 | 100.6261 | |

**LOD & LOQ:**

The sensitivity of the method was determined by calculating the limit of detection (LOD) and limit of quantification (LOQ). The LOD and LOQ values were found to be 1.50 µg/ml and 4.54 µg/ml respectively, demonstrating that the method is sufficiently sensitive for routine analysis,and the calculated values are summarized in Table 5.

 

**Table 5: LOD and LOQ for the HPLC technique**

| Parameters | Slope from Linearity | SD of peak from system Suitability |
|---|---|---|
| | 84429 | 38360.28 |
| LOD =3.3xSD/Slope | 1.50 µg/ml | |
| LOQ = 10xSD/Slope | 4.54 µg/ml | |

**Robustness:**

The robustness of the method was evaluated by making deliberate minor variations in chromatographic conditions. The observed results remained within acceptable limits, indicating that small changes in analytical parameters did not significantly affect method performance, thereby confirming the robustness of the proposed RP-HPLC method.

**DEGRADATION STUDIES**

**1.Acid Degradation:**

0.3 ml of the sample solution was transferred from the produced stock solution into a 10 ml volumetric flask.1 ml of 0.1N HCl was then added, and the mixture was heated to 50°C for 15 minutes before being cooled.One milli litre of 0.1N NaOH was added to this cooled solution to neutralise it and bring it up to the chromatogram is displayed in figure 5 after the mark with mobile phase was injected into HPLC and the peak responses were recorded

**2.Base degradation :**

From the prepared stock solution, 0.3 ml of the sample solution was transferred to a 10 ml volumetric flask. 1 ml of 0.1N NaOH was then added, and the mixture was heated to 50°C for 15 minutes before being cooled. After adding 1 millilitre of 0.1N HCl to this cooled solution to neutralise it and top it off with mobile phase, the mixture was put into an HPLC, the peak responses were noted.

**3. Peroxide Degradation:**

0.3 ml of the sample solution was taken from the prepared dummy solution and placed in a 10 ml volumetric flask. 0.1 ml of 3% H2O2 was added to the flask and brought up to the volumetric flask's mark using mobile phase. The solution was then allowed to sit at room temperature for 12 hours before being injected into an HPLC, where the peak responses were noted.

**4. Thermal Degradation:**

From the prepared stock solution, 0.3 ml of the sample solution was transferred to a 10 ml volumetric flask along with 1 milliliter of mobile phase. The mixture was then heated to 60°C for 10 minutes and allowed to cool. make up the difference with mobile phase, which was then injected into HPLC and the peak responses were noted.

**Table 6: Degradation studies**

| S. No | Condition | Peak area | %assay | %degradation |
|---|---|---|---|---|
| 1 | Acid | 4429464 | 93.037 | 6.963 |
| 2 | Base | 4404667 | 92.516 | 7.484 |
| 3 | Peroxide | 4281422 | 89.927 | 10.073 |
| 4 | Thermal | 4247839 | 89.222 | 10.778 |

 

## DISCUSSION :

The present study describes the development and validation of a rapid and solvent-efficient RP-HPLC method for the quantitative estimation of eltrombopag olamine in active pharmaceutical ingredient and tablet dosage forms. The method demonstrated satisfactory chromatographic performance, as evidenced by a well-resolved and symmetrical peak with consistent retention behavior. The observed retention time of approximately 4.3 minutes reflects efficient separation and contributes to reduced analysis time, which is advantageous for routine quality control laboratories.

Linearity of the method was established over the concentration range of 5–100 µg/mL with a strong correlation between analyte concentration and detector response ($R^2$ = 0.9936). This confirms that the method is capable of providing reliable quantitative results across a wide analytical range. Precision studies yielded %RSD values below 2%, indicating good repeatability and consistency of the chromatographic system. Accuracy assessment through recovery studies further demonstrated that excipients present in the tablet formulation did not interfere with analyte quantification, as recovery values remained within acceptable limits. Sensitivity of the proposed method was confirmed by the determined limits of detection and quantification. The obtained LOD and LOQ values indicate that the method is suitable for detecting and quantifying eltrombopag olamine at relatively low concentrations, which is essential for stability studies and quality assessment. Robustness testing showed that minor variations in chromatographic conditions did not significantly affect analytical performance, highlighting the reliability of the method under normal laboratory conditions.

Forced degradation studies under acidic, alkaline, oxidative, and thermal stress conditions confirmed the stability-indicating capability of the method. The drug peak remained well resolved from degradation products in all stress conditions, demonstrating the specificity of the developed RP-HPLC method. Approximately 10% degradation observed under certain stress conditions further supports the suitability of the method for stability testing, as recommended by ICH guidelines.

When compared with previously reported RP-HPLC methods for eltrombopag analysis, the present method offers notable advantages, including shorter run time and reduced organic solvent consumption. Many earlier methods required longer analysis times or higher proportions of organic solvents, which may increase operational costs and environmental burden. In contrast, the proposed method provides a balanced approach by achieving rapid separation without compromising sensitivity or accuracy, making it well suited for routine quality control and stability-indicating applications.

**Table: 7 Summary**

| S.No | Parameters | Results |
|---|---|---|
| 1 | Mobilephase | Methanol:Acetronitrile(90:10v/v) |
| 2 | Wavelength detection | 230nm |
| 3 | Calibration range(µg/ml) | 5-100µg/ml |
| 4 | Regression equation | y=84429x+471569 |
| 5 | Slope(m) | 84429 |
| 6 | Intercept(c) | 471569 |

 

| | | |
|---|---|---|
| 7 | Correlation coefficient ($r^2$) | 0.9936 |
| 8 | Retention time | 4.3min |
| 9 | Systems Suitability | 0.81% |
| 10 | LOD(µg/ml) | 1.50µg/ml |
| 11 | LOQ(µg/ml) | 4.54µg/ml |
| 12 | Accuracy | 100.82-102% |
| 13 | Inter-day Precision | 0.32-0.51% |
| 14 | Intraday Precision | 0.50-0.86% |
| 15 | Robustness Flow rate | 98.19-102.20% |
| 16 | Robustness Temperature | 98.90-102.07% |
| 17 | Robustness Wavelength | 98.88-102.10% |
| 18 | Assay | 99.98% |
| 19 | Acid degradation | 6.963 % |
| 20 | Base degradation | 7.844 % |
| 21 | H2O2 degradation | 10.073 % |
| 22 | Thermal degradation | 10.778% |

**Conclusion:**

**Acknowledgement**

This research was not funded by any one government or private organization self-finaced.

**Author contributions**

All authors made contributions design, acquisition of data and interpretation of data ,agreed to submit to the current journal, gave final approval of the version to be published.

**Ethical approvals**

In this study no experimental animals or human subjects were involved in this study.

**Data availability**

All the data generated and analysed are included in this research article

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