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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION AND FORCED DEGRADATION STABILITY-INDICATING STUDIES OF ELTROMBOPAG OLAMINE BY RP-HPLC AND UV IN BULK AND PHARMACEUTICAL DOSAGE FORM

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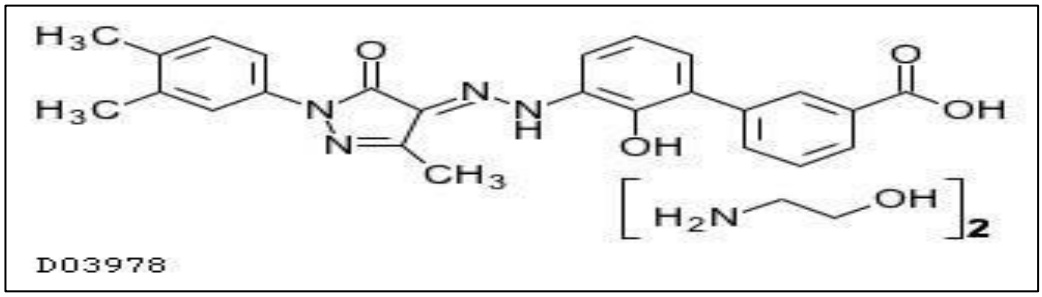
ABSTRACT:

In this proposed investigation, the aim was to develop and validate a rapid and accurate HPLC and UV spectrophotometric method for quantifying Eltrombopag Olamine in bulk and tablet formulations. The objectives involved creating an environmentally friendly method for assessing the substance using RP-HPLC and UV analysis, including forced degradation studies. Ethanol served as the optimal solvent, with an absorption wavelength of 423 nm, exhibiting linear response within a concentration range of 5-30 µg/ml and a regression coefficient of 0.996. The method underwent rigorous validation according to ICH criteria, demonstrating acceptable results. Overall, this novel and precise method was found to be suitable for Eltrombopag Olamine estimation in bulk and pharmaceutical formulations, utilizing an inertsil ODS-3V C18 150mm x 4.6mm x 5µ column with a mobile phase of buffer pH 3.5 and acetonitrile (90:10) at a flow rate of 1.0 ml/min, and detection at 280 nm via PDA.

Keywords: Eltrombopag Olamine, ICH guidelines, Method validation, Stability indicating, UV-Spectrophotometric

INTRODUCTION:

A brand-new therapeutic drug called Eltrombopag (ELT) has been approved for the treatment of persistent immunological thrombocytopenia. Eltrombopag Olamine is a small-molecule TPO-receptor agonist that binds with the transmembrane region of the human TPO-receptor and is orally bioavailable.When alternative medications or spleen removal surgery have not sufficiently improved low blood platelet counts in adults with persistent immune thrombocytopenia (idiopathic thrombocytopenia, or ITP), Eltrombopag Olamine is administered. ITP is a condition that might result in unexpected bleeding or swelling because the blood's platelet count is abnormally low. [1]
Eltrombopag Olamine, a biphenylhydrazone class member, increases the activation of the cytoplasmic tyrosine kinases Janus kinase (JAK) 2 and tyrosine kinase 2, as well as signal transducers and activators of transcription five (STAT) 5, via activating the thrombopoietin receptor, which leads to megakaryocyte proliferation and platelet differentiation. [2]

Fig. 

1.

Structure of Eltrombopag Olamine.

The IUPAC name for Eltrombopag Olamine is Bis(2-aminomethan-1-ol); 3-[(5E)-5-{2-[2(3,4-dimethylphenyl)-5-methyl-3-oxo-2,3-dihydro-1H-pyrazol-4-yl]hydrazin-1-ylidene}6-oxocyclohexa-1,3-dien-1-yl]benzoic acid.

Various analytical techniques have been reported in the literature for the estimation of ELT in both its formulation and bulk drugs, including spectrophotometric, HPLC, and HPTLC methods. However, these methods are associated with certain limitations such as prolonged analysis time and the requirement of expensive reagents. A UV spectrophotometric method for the determination of Eltrombopag Olamine in its pure form and formulation has not been identified in the literature review.

Forced degradation refers to the intentional degradation of both drug substance and drug product under conditions that are more severe than those employed in accelerated stability studies. This process serves multiple purposes, including the validation of stability-indicating methods to ensure specificity in addressing stability-related concerns. Furthermore, forced degradation offers valuable insights into the mechanisms and final products of degradation, thereby assisting in elucidating the structures of degradation products. Various stress conditions are typically applied during forced degradation experiments, encompassing oxidative, thermal, photolytic, acidic, and basic degradation pathways, with degradation rates typically ranging from 5% to 20%.

MATERIAL AND METHODS:

Instruments Used:

A double-beam UV-visible spectrophotometer manufactured by Jasco Companies in Tokyo, Japan was employed, along with an HPLC system from Shimadzu. The HPLC system included anSPD-20A PDA Detector, LC-20AD multi-solvent delivery system,and LC solution software. Reagent weighing was carried out using a high-precision electronic weighing balance provided by Mettler Toledo in Switzerland. The drug solubilization process involved ultrasonication. Mobile phase solvents underwent filtration using a vacuum pump filter.

Materials:

Pharmaceutical grade Eltrombopag Olamine was supplied by Hetero Drugs Ltd, Hyderabad, India. Analytical-grade ethanol is used as a solvent. From the research lab fine chem industry, we obtained orthophosphoric acid,acetonitrile, potassium dihydrogen phosphate,HPLC grade water, and triethylamine.

Method Development:

After deciding on the best solvent mixture and identifying the wavelengths in the literature, the development of a novel UV Spectrophotometric method was verified. A variety of solvents including ethanol, distilled water, sodium acetate (pH 6.2), ammonium acetate (6M), acetone, sodium citrate (1.25M), and methanol were employed to evaluate the solubility of the samples. After taking into account the solubility criteria, ethanol was chosen as the solvent. Using ethanol as the solvent, samples were scanned between 300 and 600 nm in the UV spectrum. At 423 nm, Eltrombopag Olamine demonstrated maximum absorption.

METHOD BY UV SPECTROSCOPY:

Preparation Of Standard Stock Solution:

Acquiring a 1000 µg/mL analyte concentration required precisely weighing 100 mg of ELT and transferring it into a 100 mL volumetric flask. Then, using ethanol as the solvent, the solution had been diluted to 100 mL. To reach an ELT concentration of 100 µg/mL, 10 mL of this ELT solution was pipetted from the volumetric flask and put into another 100 mL volumetric flask. Next, ethanol was used to adjust the volume to 100 mL.

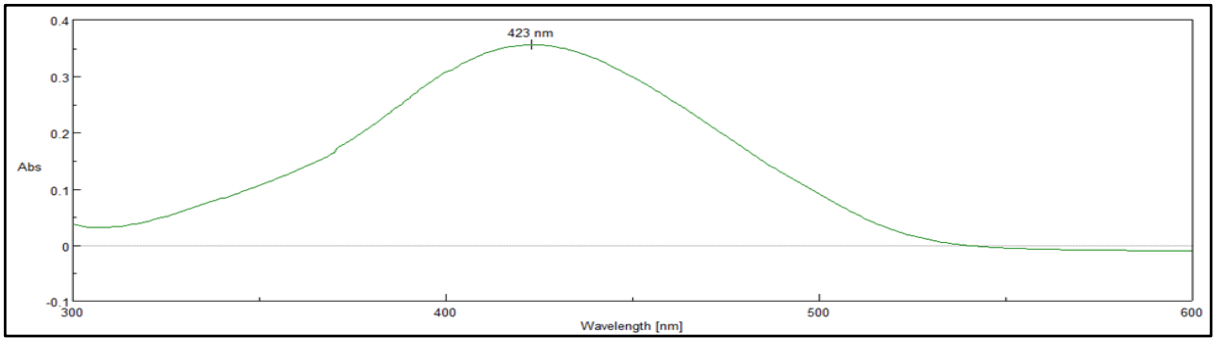


Fig. 2: UV Spectra of ELT for wavelength selection

Determination of Standard Calibration Curve:

Dilutions of the necessary quantities were prepared using ethanol from a stock solution, resulting in concentrations of ELT ranging from 5, 10, 15, 20, 25, and 30 µg/ml. Next, at 423 nm, the absorbance of these solutions was determined, and the results were entered into a

table. The observed absorbance values were plotted versus the solution concentrations on a graph. The findings demonstrated that the range of ELT concentrations that complied with Beer's law was 5–30 µg/ml.

METHOD BY RP-HPLC:

Chromatographic Condition:

A column of Inertsil ODS-3V C18 underwent chromatographic examination. Acetonitrile (90:10) and potassium dihydrogen phosphate (50 mM, pH 3.5) made up the mobile phase. Before use, the mobile phase was pumped at a flow rate of 1 ml/min after being filtered and degassed via a 0.45 mm membrane filter. Under these circumstances, the runtime was 6 minutes, as shown in Fig. 3.

Preparation of buffer, buffer, and diluents:

A 1000 ml beaker was filled with water, and into it, 1.36g of potassium dihydrogen phosphate and 2 ml of triethylamine were added. The mixture was subjected to sonication to ensure the complete dissolution of the components. The pH of the solution was then adjusted to 3.5 ± 0.05 using orthophosphoric acid. Subsequently, the solution was mixed and filtered using filter paper with a pore size of 0.45 micrometers.

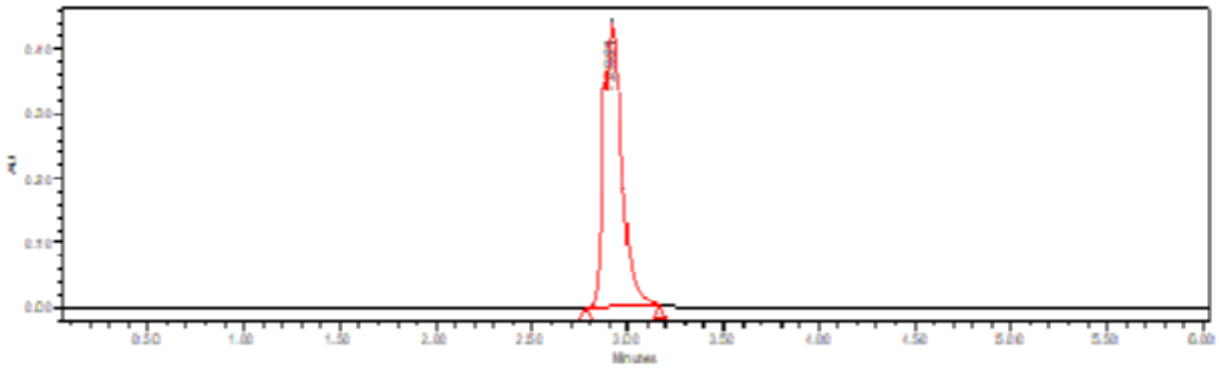


Fig. 3. Optimized chromatogram of ELT in buffer pH 3.5: Acetonitrile (90:10), flow rate=1 ml/min

Preparation of diluent:

A degassed mixture of buffer and acetonitrile was prepared in a 50:50% v/v ratio.

Mobile Phase:

In HPLC, the Binary technique was selected, with a pump B ratio set to 90.

Preparation of Standard stock solution:

Starting with the precise weighing of 100 mg of the pure drug, the obtained quantity was then mixed with 30 mL of the chosen diluent. This mixture was subsequently transferred into a 100 mL volumetric flask. An additional 100 mL of the diluent was introduced to reach the final volume, resulting in the creation of a stock solution possessing a concentration of 1000 mcg/mL.

Following the preparation steps, the stock solution underwent sonication to ensure thorough mixing. It was then subjected to filtration through a 0.45 mm filter to remove any particulate matter.

Before being introduced into the analytical system, the stock solution was subjected to further dilution using the diluent. This was done to attain the requisite concentration for the standard solutions within the range of 50-250 µg/mL.

STABILITY STUDY:

Preparation of stock solution for stability study:

To perform forced degradation, a quantity of 10 mg of the standard ELT drug was accurately weighed and subsequently placed into a volumetric flask. Subsequently, 70 ml of a suitable diluent was added to the flask, which was then subjected to sonication for a duration of 5 minutes. The final volume was adjusted as required using the diluent, to attain the necessary concentration.

ANALYTICAL METHOD VALIDATION

By UV Spectroscopy Method:

Linearity:

To investigate linearity, we prepared six ethanol solutions with different concentrations (5, 10, 15, 20, 25, and 30 µg/ml) using an ELT working standard solution. Subsequently, we recorded the absorbance at 423 nm for each of these solutions.

Precision:

To validate the method's precision, we conducted measurements for both repeatability (intra-day precision) and intermediate precision (inter-day precision). Intra-day precision was assessed by analyzing six replicates of the 10 µg/ml concentration (n=6) on the same day at various time intervals, confirming the method's repeatability. We calculated the % relative standard deviation across these six replicates.

Similarly, for intermediate precision (inter-day precision), we determined the percentage relative standard deviation after analyzing six replicates of the 10 µg/ml concentration for assay on three separate days.

Accuracy:

We assessed the accuracy of our ongoing investigation using the standard addition method. In this approach, known quantities of the standard were added at three distinct levels—50%, 100%, and 150%—to the tablet formulation of ELT. Subsequently, we conducted analyses using the established method, and this process was repeated three times. For ELT, we performed % recovery studies by spiking the tablet formulation with three different quantities of ELT standard (50%, 100%, and 150%) and calculated the % recovery for ELT at each level.

Repeatability:

The determination was made through the repetitive analysis of the same 10 µg/ml ELT standard solution.

Limit of detection (LOD) and limit of quantification (LOQ):

By ICH guidelines, we established the limit of detection (LOD) and limit of quantification (LOQ) concentrations for ELT using the residual standard deviation of response and slope method. To perform these calculations, we utilized the calibration curve developed during the linearity study. For LOD determination, we applied the equation $(3.3 \times \sigma) / S$, where σ represents the standard deviation of the response, and S signifies the slope of the calibration curve. Similarly, for LOQ calculation, we employed the equation $(10 \times \sigma) / S$.

Quantification of ELT in Formulation:

Ten ELT tablets were precisely weighed and then transferred to a clean and dry mortar. Subsequently, they were finely ground into a powder. The measured tablet powder, corresponding to 100 mg, was then carefully transferred to a 100 ml volumetric flask containing 30 ml of ethanol. This mixture was subjected to sonication for a duration of 15 minutes to facilitate drug dissolution. The volume was then adjusted to the flask's mark using ethanol, leading to a resultant concentration of 1000 µg/ml.

Following this, 10 ml of the prepared solution was moved to another 100 ml volumetric flask and subsequently diluted to the mark with ethanol, resulting in a concentration of 100 µg/ml.

In a sequential step, 2 ml of this solution was accurately transferred to a 10 ml volumetric flask to achieve a concentration of 20 µg/ml.

METHOD BY RP-HPLC:

Linearity:

A standard calibration was established using five distinct solutions spanning concentrations from 50 to 250 g/ml. Each of these standard solutions underwent chromatography three times for a duration of 10 minutes, adhering to optimal chromatographic conditions. To evaluate linearity, an average peak area-concentration correlation was analyzed via least squares linear regression. The range of 50-250 g/ml in the standard solution analysis was employed to assess the linearity of the method for ELT.

Precision:

Two main factors were considered while evaluating the precision of the method: intraday and interday changes. The same day was used to assess standard solutions in the intraday precision example. Three different days were used to analyze standard solutions for inter-day accuracy. To do precision analysis, the standard solution was injected six times at three distinct concentrations (50, 100, and 150 µg/ml), both on the same day and over a period of three days. The relative standard deviation (RSD) was used to quantify the results and express the degree of variation.

Accuracy:

To verify the accuracy, recovery tests were conducted by introducing predetermined amounts of pure drug substance into a sample solution previously analyzed with 50 µg/ml of ELT. The accuracy was assessed through the addition of known quantities of standard stock solution to the sample solution at concentrations of 50, 100, and 150%. The described procedure was employed to analyze these solutions, and subsequently, the mean recovery percentage was computed to evaluate the accuracy of the method.

Repeatability:

The determination was made through repetitive analysis of the standard solution containing 150 µg/ml of ELT.

Robustness:

The robustness study involved systematic variations of individual parameters and the subsequent observation of their impact on both system suitability tests and assay results. The following changes were introduced one at a time:

i. The mobile phase composition was altered by ± 1.0 mL of organic solvent.
ii. The flow rate was modified by ± 0.1 mL/min
iii. The wavelength was changed by ± 1 nm.

Change in mobile phase composition:

To evaluate the robustness of the developed method, the standard working solution underwent three injections while modifying the mobile phase composition by ±1.0 mL of organic solvent (phosphate buffer pH 3.5: acetonitrile) with v/v ratios of 89:11 and 91:09. This allowed us to assess the method's performance under variations in the mobile phase composition.

Change in pH:

To evaluate the impact of adjusting the sample pH, ranging from 3.4 to 3.6 with a ±1 variation, the standard working solution was injected three times using the newly developed method. The percent relative standard deviation (RSD) for the mean area was calculated to quantify the variation resulting from changes in this method parameter.

Change in flow rate:

To assess the impact of altering the flow rate by ±0.1 mL/min (0.9 mL/min and 1.1 mL/min), three standard injections of the working solution were performed using the developed method. The percent relative standard deviation (%RSD) of the mean area was calculated to quantify the variation resulting from this change in the method parameter.

Analysis of Marketed formulation using developed HPLC method:

Precisely weigh and transfer approximately 48 mg of ELT active pharmaceutical ingredient (API) into a 100 ml volumetric flask. Introduce around 80 ml of the designated diluent and subject it to sonication to facilitate dissolution. Subsequently, adjust the volume to the mark using the diluent and ensure thorough mixing. Further, transfer 5 ml of the prepared solution into a 50 ml volumetric flask, followed by dilution to the mark using the same diluent and thorough mixing.

Preparation of sample solution for assay:

Grind and precisely weigh 10 tablets of the blend to create a fine powder. Accurately measure and transfer an amount of the powder equivalent to 600 mg into a 200 ml volumetric flask. Add approximately 160 ml of the specified diluent and subject it to sonication for a duration of 15 minutes to aid dissolution. Adjust the solution's volume to the flask's mark using the diluent and ensure thorough mixing. Subsequently, transfer 4 ml of this solution into a 250 ml volumetric flask, followed by dilution with the same diluent and thorough mixing. Filter it through a 0.45 µ filter.

The theoretical average weight of ELT $[\frac{mg}{T}]$

$$\frac{AT}{AS} \times \frac{Ws}{100} \times \frac{5}{50} \times \frac{200}{WT} \times \frac{250}{4} \times \frac{P}{100} \times T - - - -[1]$$

Where,

AS = Average area of ELT peak obtained from standard solution

AT= Area of ELT peak in a sample solution

WT = Weight of sample taken in mg

Ws = weight of standard taken in mg

P= % Purity of standard

P =% Purity of ELT working standard used

T= Theoretical average weight of tablet sample

L= Label claimed of ELT in mg

% Label Amount= Content of ELT (mg/T)/Label claimed of ELT100

Solution stability of ELT:

The stability of the analytical solution was verified by analyzing both the standard and filtered sample solutions initially and at various predefined time intervals. These intervals were determined by storing the solutions within the sample compartment of the HPLC instrument under ambient conditions. To assess stability over the specified periods, the cumulative percentage relative standard deviation (% RSD) for the peak areas of ELT was calculated for both the sample and standard solutions. This analysis allowed for the evaluation of the solution's stability over time.

DEGRADATION STUDIES:

Acid degradation:

The acidic degradation was done against 1 N hydrochloric acid after 1 hour. For the preparation of stock solution 1. 10µg standard solution and 10 ml,methanol wasadded to make up the volume up to 1000 µg/ml from stock solution 1, 0.3 ml and 5 ml 1 N HCl were taken and make up the volume 10 ml with mobile phase and heated the solution at 70°. Further injected after 1 hour at 20 µg/ml and got 1.52% degradation.

Alkaline Degradation Study:

For the preparation of stock solution 1. 10 µg standard solution and 10 ml methanol were taken and added to make up the volume to 1000 µg/ml,
From stock solution 1, 0.3 ml and 5 ml 1 N NaOH were taken and make up the volume 10 ml with mobile phase and heated the solution at 70o. Further injected after 1 hr at 20 µg/ml and got 90.10 % degradation.

Oxidative Degradation Study:
The oxidative degradation was done against 10% Hydrogen peroxide After different time intervals:
For the preparation of stock solution 1. 10 µg standard solution and 10 ml methanol were taken and added to make up the volume to 1000 µg/ml,
From stock solution 1, 0.3 ml and 5 ml 10% H2O2 were taken and make up the volume 10 ml with mobile phase and heated the solution at 70o. Further injected after 1hr and then after 2 hr at 20 µg/ml and got 0 % degradation.

RESULT AND DISCUSSION:
Optimization by UV Spectrophotometric Method:
Linearity:
Linearity studies were conducted by scanning solutions of varying concentrations within the range of 5-30 µg/ml. Absorbance values were measured, and standard deviations (% RSD) were calculated, as detailed in **Table 1**. The linearity curve illustrated a consistent increase in absorbance as concentration rose, indicating a well-defined correlation. A calibration curve was generated, plotting the absorbance against concentration **[Figure 4].** The correlation coefficient value obtained was 0.9966, affirming the robustness of the relationship between absorbance and concentration.

Table 1: Linearity data of Eltrombopag Olamine	
Concentration (µg/ml)	Absorbance (nm)
5	0.185
10	0.340
15	0.543
20	0.755
25	0.996
30	1.182
R^2	0.9966
%RSD	0.003
Linear regression equation	y= 0.0399x -0.0266

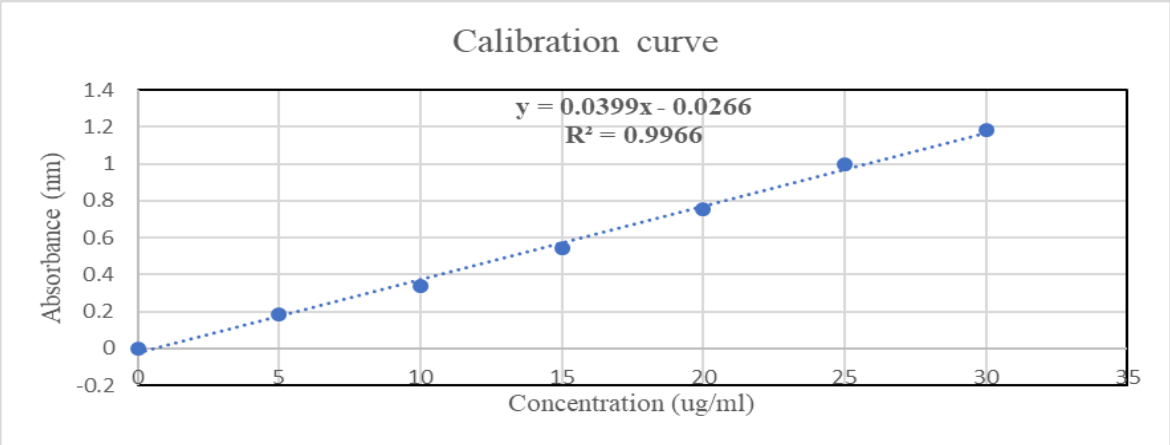
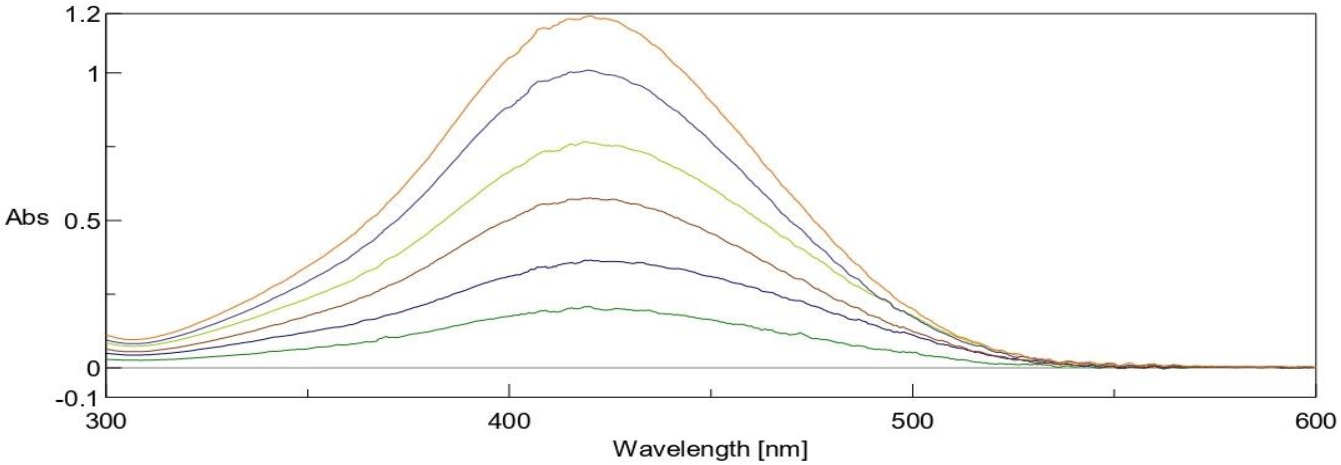


Fig. 4: Calibration curve plot of ELT at 423nm

Fig. 5: Overly spectrum of Eltrombopag Olamine (5 to 30 µg/ml) at 423 nm



Precision:

A precision study was conducted to assess the reproducibility of the outcomes obtained from the developed method. For the precision assessment, the ELT solution (10 µg/ml) was subjected to scanning at 423 nm. This procedure was carried out on the same day but at different time points, as well as on three distinct days. The recorded results have been organized in **Table 2**. The precision study verified the method's reproducibility and its adequate selectivity.

Table 2: Result of Intra-day precision (Repeatability)

Concentration	No.	**Absorbance at 423 nm**		
		Morning	**Afternoon**	**Evening**
10 µg/ml	1	0.3608	0.3622	0.3585
	2	0.3614	0.3625	0.3518
	3	0.3626	0.3609	0.3511
	4	0.3604	0.3601	0.3515
	5	0.3617	0.3635	0.3501
	6	0.3704	0.3657	0.3522
Average		0.3629	0.3625	0.3525
SD		0.004	0.002	0.003
%RSD		1.032	0.546	0.855

Table 3: Result of Intr-day precision (Intermediate precision)

Concentration	No.	**Absorbance at 423 nm**		
		Day 1	**Day 2**	**Day 3**
10 µg/ml	1	0.3338	0.3324	0.3301
	2	0.3319	0.3308	0.3283
	3	0.3329	0.3411	0.3271
	4	0.3405	0.3303	0.3291
	5	0.3319	0.3315	0.3231
	6	0.3305	0.3301	0.3206
Average		0.3336	0.3327	0.3264
SD		0.004	0.004	0.004
%RSD		1.067	1.263	1.144

Accuracy:

To evaluate the accuracy of the method, a recovery experiment was conducted across three varying levels: 50%, 100%, and 150%. The obtained % recovery fell within the range of 99.99% to 100.76%. The minimal % RSD values in Table 4 demonstrate both the accuracy and reproducibility of the method.

Table 4: Result of accuracy

Concentration taken (15pm) 50%		**Concentration taken (20ppm) 100%**		**Concentration taken (25ppm) 150%**	
Absorbance	**Conc. found**	**Absorbance**	**Conc. found**	**Absorbance**	**Conc. found**
0.5713	14.99	0.7699	19.97	0.9187	24.25
0.5877	14.74	0.7750	19.43	0.9243	24.42
0.5873	14.73	0.7719	19.89	0.8934	24.89
Mean of conc.	14.82		19.77		24.52
SD of conc.	0.15		0.29		0.33
RSD	1.02		1.47		1.35
%Recovery	98.79		98.83		98.08

Repeatability:

The method's accuracy and reproducibility are reflected in the low % RSD values. To assess repeatability, the same 10 µg/ml ELT standard solution was subjected to repeated analysis, as presented in **Table 5.**

Table 5: Result of repeatability

Conc. ug/ml	Absorbance
10	0.3849
10	0.3859
10	0.3853
10	0.3788
10	0.3791
10	0.3819
Mean	0.3827
Std. deviation	0.0032
%RSD	0.8316

LOD & LOQ:

The LODand LOQ of the developed method were investigated, and the results are summarized in **Table 5**. The determined values, i.e., LOD of 0.05 and LOQ of 0.16 µg/ml, fall within the acceptable range.

Table 6: Result of LOD & LOQ

LOD	**LOQ**
0.05	0.16

Quantification of ELT Olamine in formulation:

The λmax was precisely observed at 423 nm, and the % assay yielded a result of 98.85 ± 0.13% **[Fig. 5].** Through the developed method, the tablet assay of ELT was performed, revealing a content of 24 mg compared to the label claim of 25 mg, as detailed in **Table 7.**

Table 7: Result of ELT assay

The label claimed in mg	The average weight of the tablet (mg)	Absorbance at 423nm	The label claim found in (mg)	% Assay
25	20	0.37404	24	98%

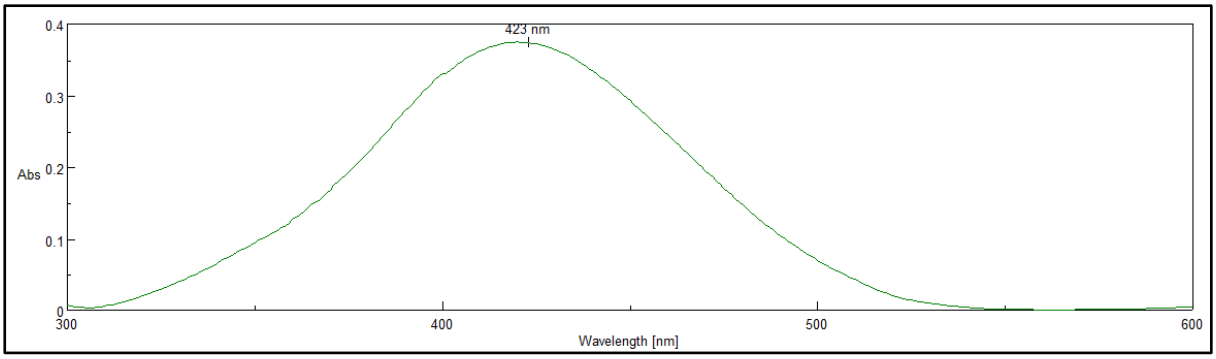


Fig. 6. UV graph of ELT from tablet assay

Optimization by HPLC Method:

Linearity:

Multiple solutions are created utilizing the Eltrombopag olamine working standard, with concentrations ranging from 20 µg/ml to 80 µg/ml of the desired level. The peak area response of the solution is assessed for Level 1 and Level 6 in six replicates each, while for Level 2 to Level 5, it is evaluated twice.

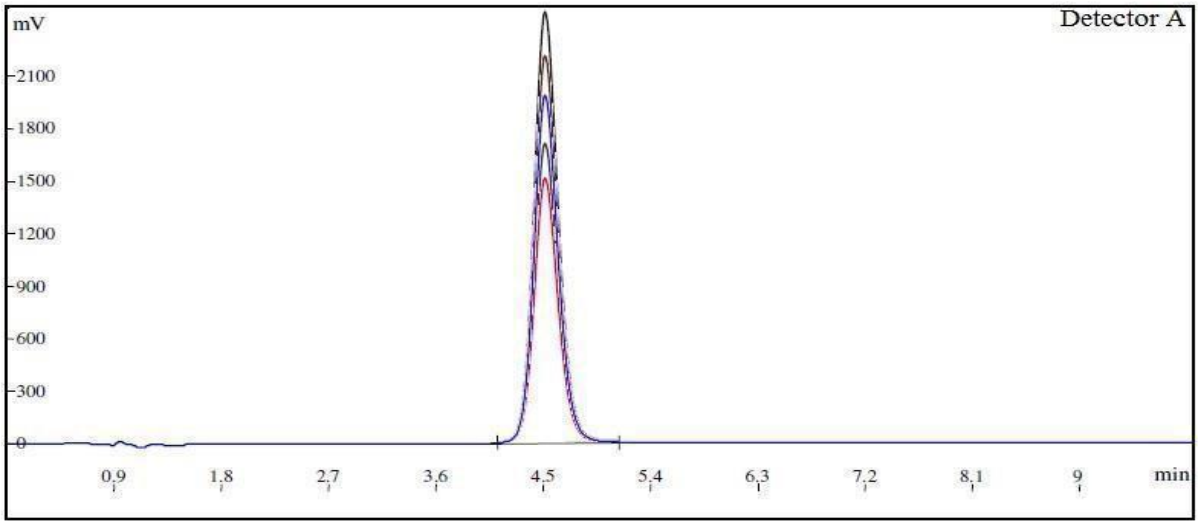


Fig.7: Overlay Chromatogram of different concentrations of Eltrombopag Olamine

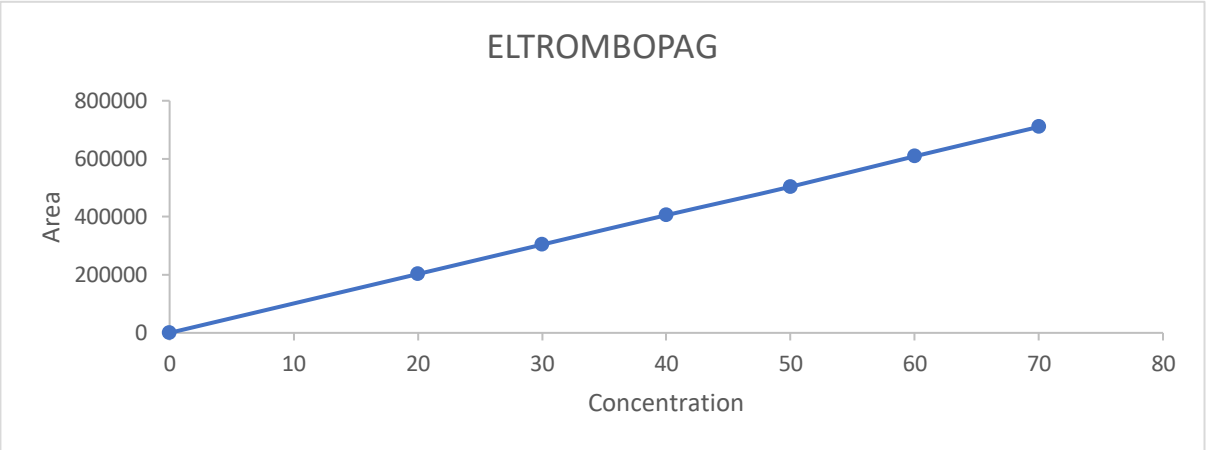


Fig. 8: Calibration curve of ELT by HPLC

Table 8: The linearity data of the ELT HPLC method			
Concentration (µg/ml)	Average Area	Statistical Analysis	
0	0	Slope	10432
20	202837	y-intercept	-134.3
30	304255	Correlation Coefficient	0.999
40	405674		
50	503566		
60	608511		
70	709929		

Precision:

Method precision was evaluated by computing the method's variations both intraday (repeatability, involving the analysis of standard solutions within the same day) and inter-day (repeatability, conducted by analyzing standard solutions on three distinct days). The precision investigation highlighted the remarkable consistency of outcomes as depicted in **Tables 9 and 10.**

Table 9: Data of Repeatability (System Precision)			
	Injection	**Peak Areas of ELT**	**% Assay**
Concentration 40ug/ml	1	405810	100.16
	2	405378	100.05
	3	405519	100.12
	4	405488	100.07
	5	405692	100.13
Statistical Analysis	**Mean**	405577	100.104
	SD	193.50	0.046
	%RSD	0.04%	0.04%

Table 10: Data of Repeatability (Method Precision)			
	Injection	**Peak Areas of ELT**	**% Assay**
Concentration 40ug/ml	1	405805	100.16
	2	405355	100.05
	3	405520	100.09
	4	405631	100.11
	5	405271	100.03
	6	405590	100.10
Statistical Analysis	**Mean**	405528	100.09
	SD	193.50	0.046
	%RSD	0.05%	0.05%

Accuracy:

The assessment of accuracy involved conducting recovery studies through the addition of defined concentrations of the pure drug to a previously analyzed sample solution containing 50µg/ml of ELT. For the initial analysis of the sample solution, a known quantity of standard stock solution was introduced, representing various levels at 50%, 100%, and 150%. The calculated % recovery ranged from 99.99% to 100.42% as illustrated in **Table 11**, which falls within the permissible limit according to the ICH guidelines.

Table 11: The accuracy data of the ELT HPLC method					
%Concentration	Area	Amount added (ug/ml)	Amount found (ug/ml)	% Recovery	Statistical Analysis of % Recovery
50	202788	20	20.02	100.16	Mean= 100.04 SD= 0.1153 %RSD= 0.12%
	202456	20	20.00	100.03	
	202289	20	19.96	99.92	
100	405612	40	40.03	100.05	Mean= 100.05 SD= 0.020 %RSD= 0.02%
	405578	40	40.02	100.06	
	405565	40	40.03	100.09	
150	608256	60	60.05	100.11	Mean= 100.11 SD= 0.030 %RSD= 0.03%
	608142	60	60.05	100.08	
	608593	60	60.08	100.14	

Table 12: Data of repeatability					
Sr. No.	Conc. (ug/ml)	Area	Mean	S.D	%RSD
1		405805			
3	40	405520	405528	193.50	0.05%
4		405631			
5		405590			

Table 13: The LOD and LOQ data of the ELT HPLC method	
LOD	LOQ
0.038	0.115

Degradation Studies:

Stability-indicating methods were systematically examined for the established method via forced degradation studies. The extent of degradation is outlined in **Table 15**. Among the various degradation conditions, the least degradation of 0.00% was witnessed in oxidative degradation, while acid hydrolysis induced a degradation of 3.02%. Graphical representations of all degradation processes are depicted in **Figures 9 to 11.**

To formulate the UV spectroscopic method for Eltrombopag, ethanol was chosen as the solvent. A solution of appropriate concentration underwent UV testing within the range of 300 to 600 nm. The peak absorbance was notably observed at 423 nm, subsequently chosen for further analysis. Refer to **Figure 6** for the UV spectra depiction. The Eltrombopag calibration curve was established across concentrations spanning 5-30 µg / mL. Notably, the absorbance increased proportionally with concentration, yielding a consistent standard slope.

This newly developed UV spectroscopic method was rigorously validated in line with ICH guidelines. The validation encompassed aspects such as linearity, precision, accuracy, repeatability, and stability studies, ensuring its robustness and reliability.

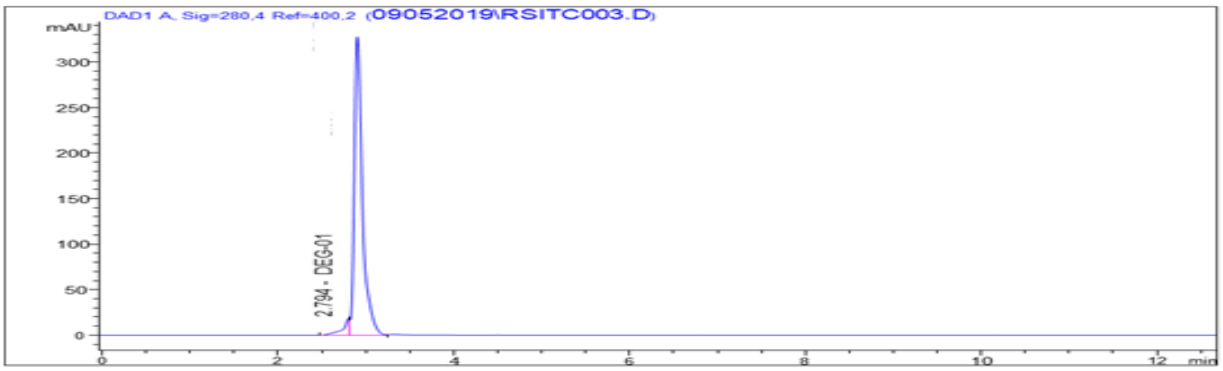


Fig. 9. The chromatogram of ELTunder acid degradation

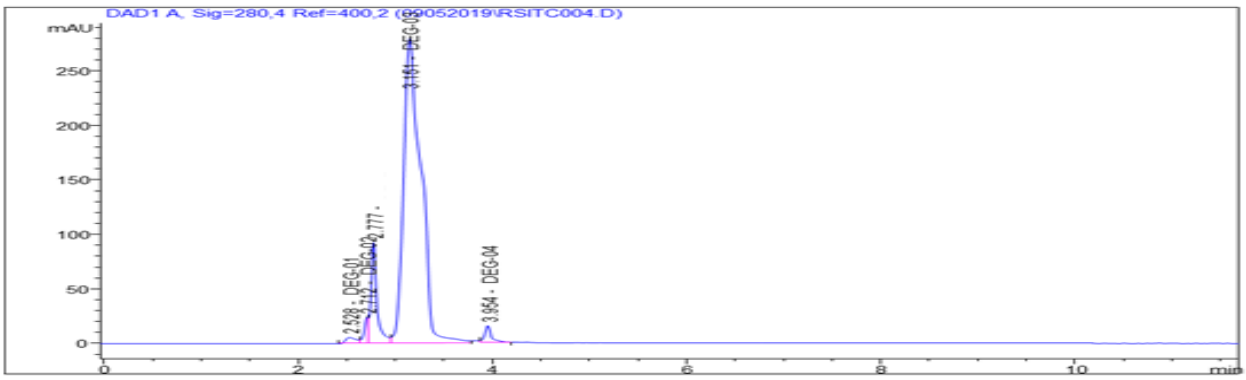


Fig. 10. The chromatogram of ELT from alkali degradation

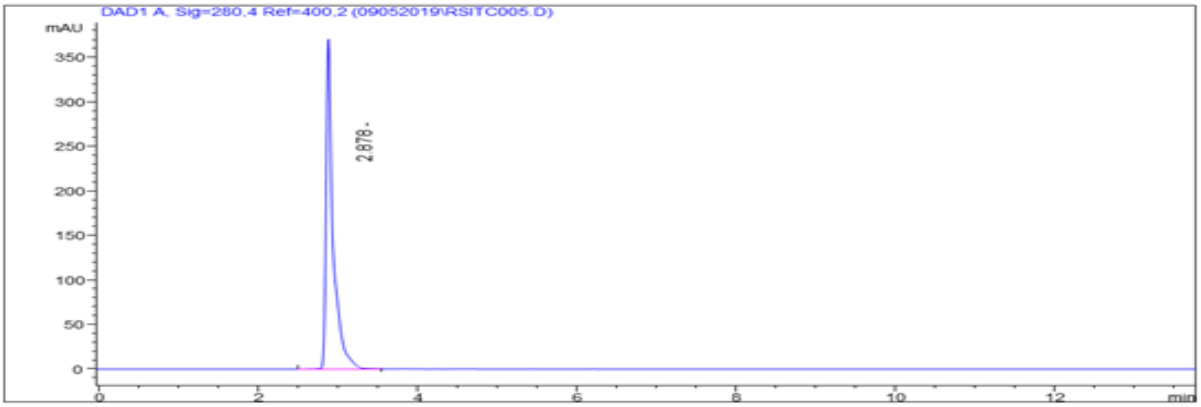


Fig. 11. The chromatogram of ELT in oxidative degradation

Parameters	Degradation %
Standard	00
Acid	3.02
Base	90.10
Oxidative	00

Conclusion:

Linearity studies were conducted by subjecting solutions to varying concentrations ranging from 5 to 30 µg/ml. Absorbance, along with standard deviation and % RSD, were calculated. The linearity curve depicted a consistent increase in absorbance as the concentration rose, indicating ideal variation. Precision analysis was undertaken to verify the replicability of the method's outcomes. Specifically, the ELT solution (10 µg/mL) was subjected to scanning at 423 nm on the same day but at different times and on three distinct days. This precision assessment established the method's reproducibility and selectivity.

Method accuracy was confirmed through a recovery experiment encompassing three levels: 50%, 100%, and 150%. The recovery percentages ranged from 98.08% to 98.83%. The presence of low % RSD values underscored the method's accuracy and reproducibility. Repeatability was determined by iteratively testing the same 10 µg/ml ELT standard solution. Furthermore, the method's limit of detection (LOD) and limit of quantification (LOQ) were investigated and found to be within acceptable bounds, specifically LOD at 0.05 µg/ml and LOQ at 0.16 µg/ml. The developed method was effectively employed for estimating ELT in selected commercial formulations.

The UV spectrum graph, shown in Figure 6, exhibited a λmax precisely at 423 nm, with a calculated % dosage of 102%. This was in comparison to the labelled indication of 25 mg for ELT, revealing 24 mg through the application of this developed method in the ELT tablet study.

A stability-indicating RP-HPLC method has been successfully developed and validated for the precise and accurate estimation of both bulk and tablet forms of ELT, adhering to ICH guidelines. The method is characterized by its simplicity, rapidity, specificity, sensitivity, precision, and selectivity.In this RP-HPLC method, a wavelength of 280 nm was carefully selected. The mobile phase consisted of a buffer with a pH of 3.5 in combination with acetonitrile, maintaining a ratio of 90:10, and a flow rate of 1.0 ml/min was employed. The optimized ELT peak is clearly depicted in Figure 12. Detection was carried out using a PDA detector at the wavelength of 280 nm.he method's validation encompassed various aspects including linearity, precision, accuracy, limit of detection (LOD), limit of quantification

(LOQ), and robustness. Through these validation steps, the method's reliability and effectiveness were confirmed.

Linearity was established through the creation of a standard calibration curve spanning concentrations from 20 µg/mL to 70 µg/mL. Precision was assessed by examining method variability in both intraday (repeatability, conducted on the same day using the standard solution) and inter-day (repeatability, performed on three separate days using the standard solution) settings. The precision investigation demonstrated close alignment with the results obtained. Accuracy was ascertained via recovery studies, involving the addition of a precise drug concentration to a previously examined 50 µg/mL ELT sample solution. Various levels of a standard stock solution, namely 50%, 100%, and 150%, were introduced to the previously analyzed sample solution. The recovery rate fell within the range of 99.92% to 100.16%, which adheres to the acceptable limits outlined in the ICH guidelines. The limits of detection (LOD) and quantification (LOQ) were determined as 0.038 and 0.115, respectively. The robustness of the developed method was assessed by altering the mobile phase composition, adjusting the solvent system's pH, and modifying the flow rate.

To evaluate the stability indicators for the developed method, forced degradation studies were conducted. Acid hydrolysis was initiated using 1N HCl, followed by neutralization with a 1N NaOH solution. Alkaline hydrolysis was induced using 1N NaOH, subsequently balanced with a 1N HCl solution. The percentage of degradation was computed for these forced degradation investigations. In a parallel manner, oxidative degradation was executed. The lowest degradation, at 0%, was observed in water oxidative experiments, whereas alkaline hydrolysis resulted in a substantial 90.10% degradation.

The RP-HPLC method developed was employed for the analysis of the commercial formulation of ELT. The degradation observed in this study amounted to 100.1%, a value that falls within the acceptable range as defined by ICH guidelines. Consequently, we can affirm that the developed method is precise, reliable, sensitive, and consistent for the quantitative determination of ELT in both its bulk form and its formulated product.

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