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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF ELTROMBOPAG OLAMINE IN PHARMACEUTICAL DOSAGE FORM

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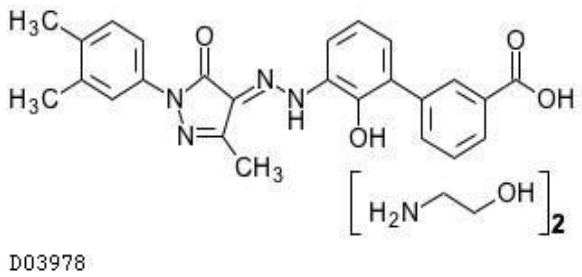
ABSTRACT
A reversed-phase liquid chromatographic method has been developed and validated for estimation of Eltrombopag Olamine in Pharmaceutical Dosage Form. RP-HPLC method, Column used was 150 x 4.6mm 5µ C18, Hypersil BDS with mobile phase containing 10mM Potassium dihydrogen Phosphate + 1% TEA (pH 4.5 Adjusted using Ortho phosphoric acid): Acetonitrile (50:50). The flow rate (1.0 ml/min) and wavelength (226 nm). The retention time of Eltrombopag Olamine was found to be 4.509 min, respectively. Correlation co-efficient for Eltrombopag Olamine was found to be 0.999. Assay result of marketed formulation was found to be in 99.7% for Eltrombopag Olamine. The proposed method was validated with respect to linearity, accuracy, precision and robustness. Percentage recovery for Eltrombopag Olamine was found to be 99.7 – 100.0%. Analysis proves that the developed method was successfully applied for the analysis of pharmaceutical formulations and can be used for routine analysis of drugs in Quality Control laboratories.

KEYWORDS: Eltrombopag Olamine, HPLC, ICH, Analytical method development, validation.

INTRODUCTION
The IUPAC name of Eltrombopag Olamine is Bis(2-aminoethan-1-ol); 3-[(5E)-5-{2-[2(3,4dimethylphenyl)-5-methyl-3-oxo-2,3-dihydro-1H-pyrazol-4-yl]hydrazin-1-ylidene}6oxocyclohexa-1,3-dien-1-yl]benzoic acid. With molecular formula and molecular weight $C_{29}H_{36}N_6O_6$ and 564.64 g/mol respectively.

The molecular structure of the drug is given in **Fig.1**



- The Eltrombopag Olamine is used as a Thrombopoeitin receptor agonist.
- The Eltrombopag Olamine increasing cells in bone marrow. It also helps to increase the number of platelets, a type of blood cells that helps to reduce or prevent bleeding.

However no HPLC method has been reported till date for the estimation of Eltrombopag Olamine using the RP-HPLC method. The present paper describes the analytical method development and validation of estimation of Eltrombopag Olamine in Pharmaceutical dosage form using RP-HPLC. The proposed method are optimized and validated as per ICH guidelines.

MATERIALS AND METHODS
Materials
a) Instruments
- Analytical Weighing Balance
- Sonicator
- FT-IR spectrophotometer
- HPLC system
- Millipore Filter Unit
- pH Meter
- UV Spectrophotometer

b) Glasswares
- Beaker
- Conical flask
- Measuring cylinder
- Petri dish

- Pipette
- Volumetric flask.

c) Chemicals

- Standard Eltrombopag Olamine Gifted by Amneal Pharmaceautical Pvt Limited.
- The commercial fixed dose **Revolade** 25mg Tablets 7's manufactured by Glexo smithkline Pharmaceutical Ltd was procured from local market. All solvents (HPLC grade) were obtained from S.D. fine chemical.

d) Method

• Chromatographic method.

Methods Working Standard preparation

Solution Preparation of Eltrombopag Olamine: (25 µg/ml)

About 2.5mg of Eltrombopag was dissoved in 100mL of water with 15minutes sonication to achieve 25µg/mL concentration of Eltrombopag.

• Sample Preparation for marketed formulation

Transferred 1 tablet in to 100mL volumetric flask and added 70-75 mL of diluent, sonicated for 45 minutes and then it was shaked for 30 minutes by mechanical means, tablet was checked visually if it got dispersed and then volume was made up to mark with diluent and mixed well. The solution was filtered through 0.45 µ PVDF filter. Further 5mL of filtrate was transferred to 50mL of volumetric flask and volume was made up to mark with diluent.

Method Validation

Chromatographic conditions and System Suitability Parameters

Mode of chromatography: Reversed Phase Chromatography **Mode of Elution:** Isocratic **Flow Rate:** 1.0 ml/min

Oven: Oven Temperature: 25° ± 2°C

Detector: Type: UV detector

Wavelength: 226 nm

Column: 150 x 4.6mm 5µ C18, Hypersil BDS

Sample Volume: 20 µl

Run time: 10 min

Mobile Phase: 10mM Potessium dihydrogen Phosphate + 1% TEA (pH 4.5, adjust with 1% orthophosphoric acid): Acetonitrile (50:50).

System Suitability Parameters

Table 1: System Suitability Test Parameters for Eltrombopag Olamine.

Sr. No.	System suitability Parameter	Eltrombopag Olamine
1	Retention time (min)	4.509
2	Theoretical plate number (N)	12457
3	Tailing factor (T)	1.0

Linearity and Range (n=3)

- The range of analytical method is the interval between the upper and lower levels of analytes that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity.
- The linearity was determined at five levels over the range of 12.5-37.5 µg/ml for Eltrombopag Olamine. Peak area of above linearity solution preparations were taken at each concentration three times. Mean Peak Area at each concentration was calculated and Graph of Mean Peak Area (y axis) versus Concentration (x-axis) was plotted.

Precision Repeatability

Six replicate of 25µg/ml concentration of Eltrombopag Olamine were prepared and chromatographic were recorded at the optimized condition. SD and RSD were calculated.

Intraday Precision and Interday Precision

Variations of results within the same day (intra-day), variation of results between days (interday) were analyzed. Intra-day precision was determined by analyzing both standard solutions for three times in the same day. Interday precision was determined by analyzing the drugs daily for three days. %RSD was calculated.

Accuracy (% Recovery)

Accuracy is the closeness of the test results obtained by the method to the true value. To study the accuracy 5 tablet powder were weighed and analysis was carried out as per assay. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels (80%, 100% and 120%) taking into consideration percentage purity of added bulk drug samples. These solutions were subjected to re-analysis by the proposed method and Results are calculated.

Robustness

The robustness of the method was established by making deliberate minor variations in the following method parameter.

a) Flow rate: ±0.2 ml/min
b) Change in the ratio of component in the mobile phase: ± 2%.
c) pH of mobile phase: ±0.2

RESULT

Validation Parameter

Linearity and Range

Linear correlation was obtained between peak area and concentration of Eltrombopag Olamine in the range of 12.5-37.5 µg/ml. The linearity of the calibration curves was validated by the value of correlation coefficients of the regression (r).

Table 2: Linearity data for Eltrombopag Olamine.

% Linearity Level	Concentration (µg/ml)	Mean area	Correlation Coefficient
50	12.5	3783448	0.999
80	20.0	6063026	
100	25.0	7582549	
120	30.0	9098784	
150	37.5	11423543	

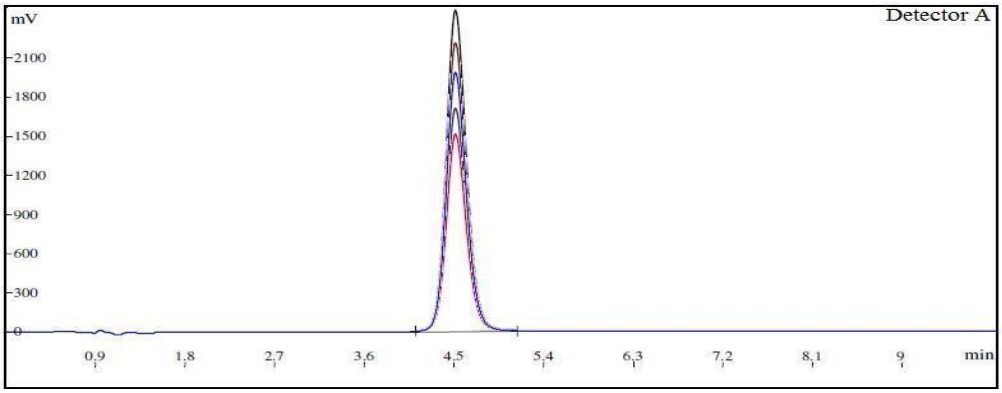


Figure 2: Overlay chromatogram of different concentration of Eltrombopag Olamine.

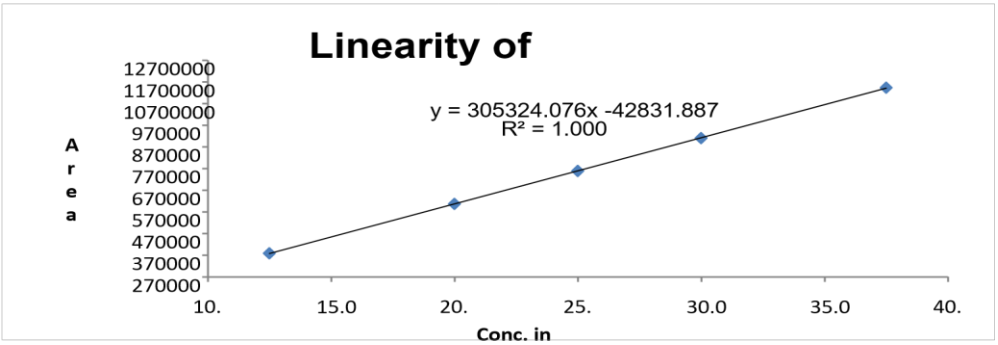


Figure 3- Calibration Curve of Eltrombopag Olamine.

Accuracy

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. Percentage recovery for Eltrombopag Olamine was found to be 99.8 – 100.0%.

Table 3: Recovery Data of Eltrombopag Olamine.

Accuracy Level %	Set no.	Amount Added(mg)	Amount Recovery(mg)	% Recovery	Mean	% RSD
80	1	20	19.84	99.2	99.8	0.8
	2	20.25	20.40	100.1		
	3	19.75	19.66	99.5		
100	1	25.25	25.43	100.7	100.5	0.6
	2	25.24	25.00	101.0		
	3	24.88	24.82	99.8		
120	1	30.13	30.10	99.9	100	0.7
	2	30.25	30.05	99.3		
	3	29.95	30.16	100.7		

Precision

Repeatability (Method precision, n=6)

Table 4: Repeatability of Eltrombopag Olamine.

Sr. no	Area	Mean	SD	%RSD
1	7551263	7553265	7741.2	0.1
2	7548965			
3	7551282			
4	7550236			
5	7568923			
6	7548923			

Repeatability

The data for repeatability of Eltrombopag Olamine is shown in Table 4 The % RSD for Repeatability data was found to be 0.1 respectively.

Intraday precision

The data for intraday precision for Eltrombopag is shown in Table-5. The % RSD For intraday precision was found to be 0.23%.

Table 5: Intraday precision for Eltrombopag Olamine (n=3).

Sr. No.	Concentration (µg/ml)	Mean Area ± SD	% RSD
1	12.5	3775791± 8697.0	0.23
2	25.0	7553593± 15697.9	0.21
3	37.5	11379918± 30064.0	0.26
Mean			0.23

Interday precision

Table 6: Interday precision for Eltrombopag Olamine (n=3).

Sr. No.	Concentration (µg/ml)	Mean Area ± SD	% RSD
1	12.5	373775± 30525.0	0.82
2	25.0	7482058± 65006.3	0.87
3	37.5	11270505± 115482.6	1.02
Mean			0.90

The data for interday precision for Eltrombopag Olamine is shown in Table-10. The % RSD For intraday precision was found to be 0.90%.

Limit of Detection and Limit of Quantification

The Limit of detection (LOD) and Limit of quantitation (LOQ) of Eltrombopag Olamine as mention below table Results of LOD and LOQ.

Drug	Eltrombopag Olamine
LOD	0.17
LOQ	0.52

Robustness

Robustness

The method is found to be robust as the results were not significantly affected by slight variation in composition of mobile phase, Mobile phase pH and flow rate of the mobile phase.

Table 7: Change the flow rate.

Standard repetitions (n=6)	0.9ml/min	1.1ml/min
	Eltrombopag Olamine	Eltrombopag Olamine
Mean Area	8289684	8660888
% RSD	0.3	0.5

Table 8: Change the mobile phase composition.

Standard repetitions (n=6)	48:52	52:48
	Eltrombopag Olamine	Eltrombopag Olamine
Mean Area	7584756	7586610
% RSD	0.4	0.6

Table 9: Change the mobile phase pH.

Standard repetitions (n=6)	4.3	4.7
	Eltrombopag Olamine	Eltrombopag Olamine
Mean Area	7562706	7560944
% RSD	0.1	0.1

System Suitability tests

Table 10: System Suitability Test Parameters for Eltrombopag Olamine.

Sr. No.	System suitability Parameter	Eltrombopag Olamine
1	Retention time (min)	4.509
2	Resolution (R)	-
3	Theoretical plate number (N)	12457
4	Tailing factor (T)	1.0

Assay preparation (Marketed formulation)

Label claim: Eltrombopag Olamine-25mg.

Sample preparation of marketed formulation

Transferred 1 tablet in to 100mL volumetric flask and added 70-75 mL of diluent, sonicated for 45 minutes and then it was shaked for 30 minutes by mechanical means, tablet was checked visually if it got dispersed and then volume was made up to mark with diluent and mixed well. The solution was filtered through 0.45 µ PVDF filter. Further 5mL of filtrate was transferred to 50mL of volumetric flask and volume was made up to mark with diluent and it was injected.

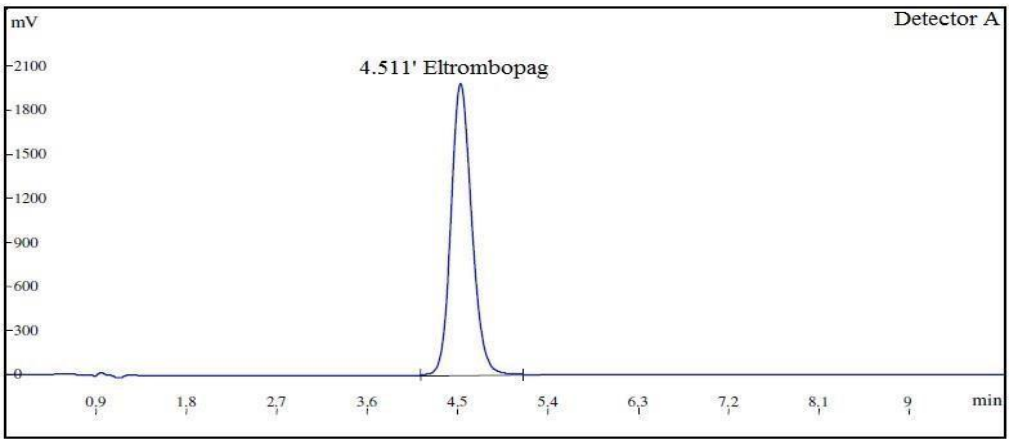


Figure 4: Injection of marketed formulation.

Peak Table

Table 11: Injection of marketed formulation.

Sr. No	Peak	Retention time	Area	Tailing factor	Theoretical Plates	Resolution time
1	Eltrombopag Olamine	4.511	7528769	1.0	12785	-

OBSERVATIONS

In formulation sample preparation, peaks are found symmetrical with good peak shape.

% Assay Results from Formulation

Table 12

Sr. No.	Sample name	% Assay of Eltrombopag Olamine
1	Revolade (Eltrombopag Olamine) 25mg Tablet	99.7 %

Summary of Regression Parameters

Table 13: Summary of Regression Parameters for Eltrombopag Olamine.

Sr. No.	Parameters	Eltrombopag Olamine	REMARK
1	Linearity (µg/ml)	12.5-37.5 µg/ml	Linear
2	%Recovery	99.8-100	Accurate
3	Precision (%RSD)	0.1%.	Precise (%RSD < 2)
	Repeatability (n=6)		
	Intra-day (n=3)	0.23%	
	Inter-day (n=3)	0.90%	
4	Specificity	Specific	Specific (No interference)
7	Robustness	Robust	(No difference in result)

DISCUSSION

A simple, accurate and precise RP-HPLC method for the estimation of Eltrombopag Olamine in Pharmaceutical Dosage form has been developed and validated. 10mM Potassium dihydrogen Phosphate (pH 4.5, adjust with 1% Orthophosphoric acid): Acetonitrile (50:50% v/v) Separation of drugs was carried out using mobile phase at 10 min. run time and 226 nm. The Rt value for Eltrombopag Olamine was found to be 4.509 ± 0.01 min. respectively.

The drug response with respect to peak area was linear over the concentration range 12.5-37.5µg/ml Eltrombopag Olamine. The percentage recovery of Eltrombopag Olamine was found to be 99.8-100% respectively.

The %RSD values for intra-day precision study and inter-day study were ≤ 2.0%, confirming that the method was sufficiently precise.

The %RSD values of Robustness study were ≤ 2.0%, confirming that the proposed method was found to be robust enough to withstand such deliberate changes and allow routine analysis of the sample. Interference studies reveals that the common excipients and other additives usually present in the dosage form did not interfere in the proposed method.

So it is concluded that the developed method is specific. The system test parameters were also performed and were found to be within acceptable criteria. The method can be successfully employed for the estimation of Eltrombopag Olamine in pharmaceutical dosage form.

CONCLUSION

A simple, economic, specific and robust RP-HPLC method has been developed and validated for the estimation of Eltrombopag Olamine in pharmaceutical dosage form. There was no interference from any excipients in the determination of drugs in tablets which indicates the method is specific. All method validation parameters lie within its acceptance criteria as per ICH Q2(R1) guideline so we can conclude that method is Specific, Linear, Accurate and Precise. Hence it can be successfully used for the routine analysis of Eltrombopag Olamine in pharmaceutical dosage form.

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