Journal of Advanced Scientific Research
Available online through https://sciensage.info
ISSN 0976-9595
Research Article

DEVELOPMENT AND VALIDATION OF VISIBLE SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF ELTROMBOPAG OLAMINE IN BULK AND TABLET DOSAGE FORM

Gangu Sreelatha*, Sai Chaitanya RG, T. Harshana, Y. Divya Spandana, Y. Abhishek Reddy
Department of Pharmaceutical Analysis, CMR College of Pharmacy, Kandlakoya, Hyderabad, Telangana, India
**Corresponding author: sreelatha1801@gmail.com*

Received: 24-07-2023; Accepted: 03-09-2023; Published: 30-09-2023

 https://doi.org/10.55218/JASR.202314808

ABSTRACT

A simple and sensitive visible spectrophotometric method has been developed for the quantitative estimation of Eltrombopag Olamine in its bulk and tablet dosage form. It is an orally active ethanolamine salt of Eltrombopag, a small-molecule, nonpeptide thrombopoietin receptor agonist with megakaryopoiesis-stimulating activity. The maximum absorbance of drug was found at 425nm. Eltrombopag Olamine was found to be dissolved in Methanol so it was used as solvent for the conduction of work. Linearity range for the method was finalized as 3-18 µg/ml with R^2 value of 0.9995. % RSD less than 2 which is well within indicates the method is precise and robust. The % recovery rate was found to be 100-101% indicating the method's accuracy. The method was validated as per ICH guidelines provided and the results were found to be satisfactory. LOD & LOQ values were obtained as 0.150 & 0.456 respectively. Stability studies shows that the drug is unstable in various stress conditions like acid, base, water and oxidation.

Keywords: Olamine, Eltrombopag, Ethanolamine, Nonpeptide thrombopoietin, Agonist, Megakaryopoiesis, Visible Spectrophotometric, Methanol, ICH guidelines, Stress condition.

1. INTRODUCTION

Spectroscopy is a branch of science that studies the interaction between EMR and matter. The basic law that relates absorbance and concentration is the Beer-Lambert law. Beer-Lambert's law is an essential relationship in spectroscopy that describes the connection between the concentration of a solution and the amount of light absorbed by that solution. The law states that the absorbance (A) of a sample is directly proportional to the concentration (c) of the absorbing species in the sample and the path length of the light through the sample [1-10].

Eltrombopag Olamine, a small molecule, belongs to the class of Antihemorrhagics, nonpeptide thrombopoietin receptor agonist with megakaryopoiesis-stimulating activity, is the orally active ethanolamine salt of Eltrombopag [11, 12].

A search through the literature revealed that the drug has been examined with a variety of analytical approaches, notably HPLC, RP-UPLC, UPLC-MS-MS, and LC-tandem mass spectrometry. The development of a basic, precise, reliable, and repetitive Visible Spectrophotometric method for the quantification of Eltrombopag Olamine in bulk and in tablet dosage form is described in the current work. According to ICH Guidelines, the developed approach was validated [13-27].

Table 1: Drug profile of Eltrombopag Olamine

Criteria	Features
IUPAC name	2-aminoethanol;3-[3-[[2-(3,4-dimethylphenyl)-5-methyl-3-oxo-1H-pyrazol-4-yl] diazenyl]-2-hydroxyphenyl] benzoic acid
MF	$C_{29}H_{36}N_6O_6$
MW	564.6 g/mol
λ_{max}	425nm
Solubility	Soluble in Methanol
Brand name	Revolade (75mg)

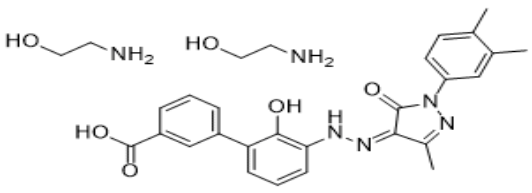

Fig. 1: Structure of Eltrombopag Olamine

2. MATERIAL AND METHODS

2.1. Instrumentation

The proposed work was carried out on a TG Ultraviolet visible spectrophotometer, T-60 Model, which is a double beam double detector configuration with a 1 cm glass matched cell. All weighing was done on PGB-200 model weighing balance.

2.2. Selection of Solvents

On the basis of solubility study Methanol was selected as the solvent for dissolving Eltrombopag Olamine.

2.3. Method development

2.3.1. Preparation of Standard Stock Solutions of Eltrombopag olamine

2.3.1.1. Stock Solution A (1mg/ml)

Accurately weighed 10mg of Eltrombopag Olamine into a 10ml volumetric flask, dissolved in 1ml methanol and made up the volume to 10ml.

2.3.1.2. Stock Solution B (100µg/ml)

Five ml of stock solution A was taken and diluted up to 50 ml with Methanol**.**

2.3.1.3. Dilutions

Further serial dilutions were done by taking 0.3, 0.6, 0.9, 1.2, 1.5,1.8 ml of stock B and madeup the volume with Methanol up to 10ml to give concentrations 3,6,9,12,15,18µg/ml.

2.3.2. Selection of Analytical Wavelength

An appropriate aliquot portion of 0.9ml from stock solution B was transferred to 10mL volumetric flasks; the volume was made up to the mark using Methanol (9µg/ml - working standard). Drug solution was scanned against a Methanol blank between 400 nm to 800 nm range. The drug showed λmax at 425nm.

2.4. Method validation

The suggested approach has undergone rigorous validation in terms of linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness, ruggedness and assay.

2.4.1. Determination of Linearity

A working standard solution of the drug was divided into six separate 10mL volumetric flasks using an appropriately measured proportionate fraction. Methanol was used to make up the volume to the required level in order to produce concentrations (3-18 g/mL). The absorbance of these solutions was measured at 425nm. The calibration curve was displayed in a concentration versus absorbance graph.

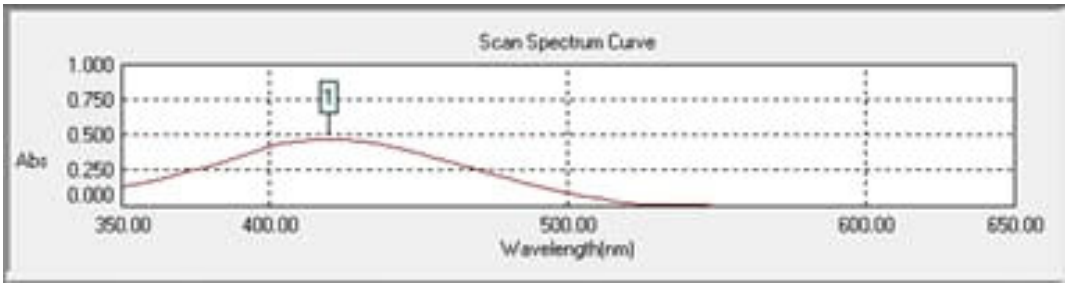

Fig. 2: UV spectrum of Eltrombopag Olamine (425nm)

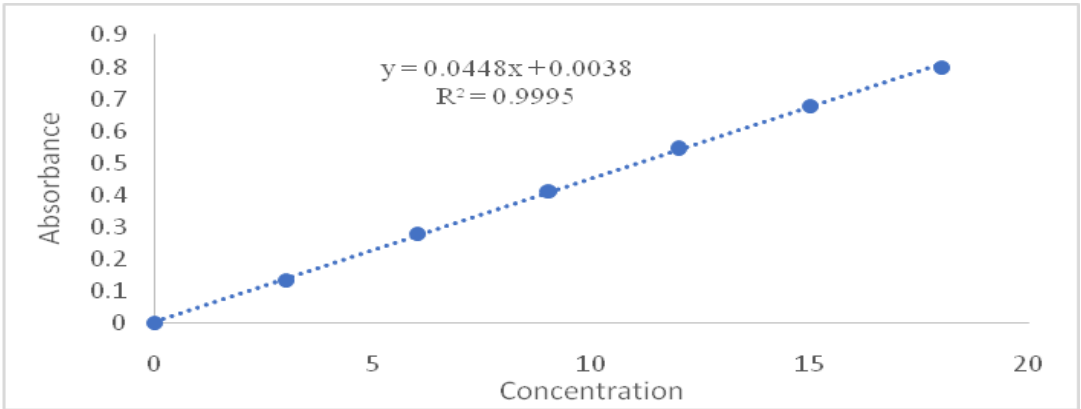

Fig. 3: Calibration Curve of Eltrombopag Olamine

2.4.2. Accuracy

On the basis of a recovery, research carried out using the conventional addition approach, the proposed method's accuracy was determined. The tablet powder was re-analysed using the recommended method after being mixed with a known quantity of standard medication solutions to make final concentrations of 50%, 100%, and 150%. The absorbance recorded and the % recoveries were calculated.

2.4.3. Precision

Precision was determined as intra-day and inter-day variations. The results of the intra-day and inter-day precision research were determined by calculating absorbance at the same drug concentrations (9 ppm) three times on the same day and three different days over the course of a week at a wavelength of 425nm. The results were reported.

2.4.4. Ruggedness

The suggested method's durability was assessed through the analysis of portions from uniform slots by two different analysts under similar operational and environmental circumstances. The results were reported accordingly.

2.4.5. Robustness

Robustness was obtained by performing the analysis at two different wavelengths (±2 nm). The results were reported.

2.4.6. LOD & LOQ

LOD & LOQ gives information about the sensitivity of the method. The values reported indicate that the method is highly sensitive.

2.4.7. Assay

Table 2: Assay results

Formulation	Labelclaim mg/tab	Amountfound Mean ±S.D	Assay	%RSD
Tablet	75	99.89 ± 0.0064	99.89	0.0064

3. RESULTS AND DISCUSSION

3.1. Method Validation

3.1.1. Linearity

The calibration curve has a regression coefficient of 0.9995 and displayed linearity in the 3-18 µg/ml range.

Table 3: Linearity of Eltrombopag Olamine

S. No	Eltrombopag Olamine	
	CONC	ABS
1	3	0.132
2	6	0.278
3	9	0.411
4	12	0.548
5	15	0.678
6	18	0.799
Regression equation	y=0.448x + 0.0038	
R^2	0.9995	

3.1.2. Accuracy

The % RSD of the present work was found to be within and less than 2. The % recovery was between 100-102%.

3.1.3. Precision

The % RSD of Eltrombopag Olamine was found to be 0.614 for intraday precision and 0.66759 for intraday precision, respectively.

3.1.4. Robustness

A small variation of the wavelength (±2nm) was applied to the presented method and it was found that the %RSD was within the limits and the values were 0.243309 and 0.140247.

Table 4: Accuracy data of the UV method

% Sample Spiked	Sample (Tablet)	Standard (9 ppm)	Mean	SD	%RSD	%Recovery
50%	0.9	0.45	0.612	0.001	0.163399	101.6865
	0.9	0.45				
	0.9	0.45				
100%	0.9	0.9	0.813	0.001	0.123001	100.6944
	0.9	0.9				
	0.9	0.9				
150%	0.9	1.35	1.012	0.00058	0.057032	100.0882
	0.9	1.35				
	0.9	1.35				

Table 5: Intraday Precision Results

	10:00 AM Mean± SD	%RSD	1:00 PM Mean ±SD	%RSD	4:00 PM Mean ±SD	%RSD
LQC (3PPM)	0.132167± 0.000735	0.569563	0.132333± 0.000516	0.390225	0.132333± 0.000816	0.617
MQC (9PPM)	0.410833± 0.000753	0.183231	0.411667± 0.000816	0.198339	0.4115± 0.001049	0.254875
HQC (18PPM)	0.798833± 0.000753	0.094234	0.798167± 0.000983	0.123181	O.798333± 0.000816	0.102275

Table 6: Interday precision Values

	10:00 AM Mean± SD	%RSD	1:00 PM Mean ±SD	%RSD	4:00 PM Mean ±SD	%RSD
LQC (3PPM)	0.1325± 0.000548	0.413376	0.131833± 0.001169	0.88676	0.132± 0.000894	0.677596
MQC (9PPM)	0.411167± 0.000753	0.183082	0.411833± 0.001169	0.283864	0.4115 0.000548	0.133104
HQC (18PPM)	0.798± 0.000894	0.112084	0.798± 0.001095	0.137274	0.797833± 0.000983	0.123233

Table 7: Robustness Values

Conc(µg/ml)		425nm	
		Analyst 1	Analyst 2
9	Mean	0.412	0.411667
9	SD	0.001	0.001155
9	%RSD	0.242718	0.280494

3.1.5. Ruggedness

Two different analysts performed the ruggedness studies under same conditions and it was found that the % RSD was within the limits.

Table 7: Ruggedness Result

Conc(µg/ml)		425nm	
		423nm	427nm
9	Mean	0.411	0.411667
9	SD	0.001	0.000577
9	%RSD	0.243309	0.140247

3.1.6. LOD & LOQ

The study for LOD & LOQ was carried out and the obtained results are described in the table below.

Table 8: LOD & LOQ Values

Drug	LOQ	LOD
Eltrombopag Olamine	0.150	0.456

3.1.7. Stability Studies

A 9µg/ml solution of Eltrombopag Olamine was obtained and its stability was tested (48 hours) in 0.1N HCl, 0.1N NaOH, Hydrogen Peroxide, and Hydrolysis. The results are shown in below table.

Table 9: Stabilities Studies of Eltrombopag Olamine

Solution	Absorbance		% Degradation	
	Day 1	Day2	Day 1	Day 2
HCl	0.074	0.041	82%	90%
NaOH	0.023	0.014	94%	97%
H2O2	0.025	0.011	94%	97%
Water (H_2O)	0.011	0.008	97%	98%

4. DISCUSSION

Using visible spectrophotometry, we created a method in this work for estimating Eltrombopag Olamine in both bulk and pharmaceutical dosage forms. With the use of various techniques like HPLC, RP-UPLC, and others, there have been numerous methods. We used visible spectrophotometry to carry out the method's development and validation.

5. CONCLUSION

Based on the aforementioned findings, it can be said that the suggested method is straightforward, sensitive, exact, repeatable, and affordable to determine Eltrombopag Olamine in bulk and tablet dosage form. The drug's R^2 value is 0.9995, and an %RSD of less than 2 suggests the method is robust and precise. The lowest LOD value can be used to determine the method's sensitivity. % Degradation indicates high level of degradation that means the drug is highly unstable in different stress conditions.

Conflict of interest
None declared

Source of funding
None declared

6. REFERENCES

1. Chatwal GR, Anand SK. Instrumental method of Chemical Analysis, 5th ed, Himalaya Publishing House, New Delhi, 2011, 2.108-2.110p.
2. Skoog DA, Holler FJ, Nieman TA. Principles of Instrumental Analysis, 5th ed, Thomson (Brook/ Cole), 2004, 378p.
3. Davidson AG (2002) Ultraviolet-visible absorption spectrophotometry. In BeckettAH, Stenlake JB, (4thedn), Practical Pharmaceutical chemistry. CBS Publishers and distributors, New Delhi, 275-278.
4. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry, 4th ed, Part 2, CBS Publisher and Distributor New Delhi, 2004, 275-278p.
5. Patil KM, Bodhankar SL. *J Chromatogr B Analyt Technol Biomed Life Sci.,* 2005; **823:**152-157.
6. Connors KA. A textbook of pharmaceutical analysis, 3rd ed, John Wiley and Sons, 2007, 179-185p.
7. Watson DG. A textbook for pharmacy students and pharmaceutical chemists, 2nd ed, Elsevier Churchill Livingstone, 2005, 89-91p.
8. Willard HH, Merritt LL, Dean JA, Settle FA. Instrumental methods of Analysis, 7th edn, CBS Publishers New Delhi 1986.159-161p.
9. Sharma BK. Instrumental Method of Chemical Analysis, 28th ed, Goel Publishing house, 2012, S-72-S-74p.
10. Chatwal GR, Sham K Anand. Instrumental Methods of Chemical Analysis. 6th edition. Mumbai: Mrs. Meena Pandey; 2011. Chapter 5, Visible Spectrophotometry and Colorimetry; 2.108-2.109
11. Eltrombopag olamine | CAS#496775-61-2 | 496775-62-3 | thrombopoietin receptor agonist | MedKoo Biosciences [Internet]. www.medkoo.com. [cited 2023 Jul 21].Available from: https://www.medkoo.com/products/10323
12. PubChem. Eltrombopag olamine [Internet]. pubchem.ncbi.nlm.nih.gov. [cited 2023 Jul 21]. Available from: https:// pubchem.ncbi.nlm.nih.gov/compound/135449331
13. Antypenko LM, Solodovnyk VA. *Dhaka University Journal of Pharmaceutical Sciences*, 2017 Jul 30; **16(1):**37-42.
14. Li J, Jiang Y, Sun T, Ren S. *Journal of pharmaceutical and biomedical analysis.* 2008 Aug 5; **47(4-5):**929-33.
15. Dandabattina R, Subramanian VB, Merugu KS, SharathBabu H, Katari NK, Kowtharapu LP. *Journal of AOAC International.* 2023 Mar 1; **106(2):**267-275.
16. Soto C, Saavedra R, Toral MI, Nacaratte F, Poza C. *Microchemical Journal,* 2016 Nov 1; **129:**36-40.
17. Satani BH, Patel JV, Gami RB, Patel CN. *Journal of Pharmacy Research,* 2013; **6(1):**102.
18. Patel AI, Gosai S, Jadav K, Patel AB, Patel NK. *Journal of the Turkish Chemical Society Section A: Chemistry,* 2020 Oct 10; **7(3):**845-850.
19. Jayagopal B, Murugesh S. *Journal of Pharmaceutical and Biomedical Analysis*, 2021 Sep 5; **203:**114231.
20. Chouhan P, Saini TR. *Indian drugs,* 2016 Mar; **53:**32-39.
21. Ali OI, Abd Allah FI, Fahmy EA. *Egypt. J. of Appl. Sci.,* 2022; **37(11-12):**34-38.
22. BASHA D, Reddy GV, Rani TS, Susithra E, Chekkara R. *Asian Journal of Chemistry,* 2015 Nov 1; 27(12).
23. Mohan TS, Mukkanti K, Jogia HA. *Oriental journal of chemistry*, 2018; **34(2):**1098.
24. Nacaratte F, Toral M, Soto C. *Journal of the Chilean Chemical Society,* 2016 Sep; **61(3):**3102-3107.
25. Deng Y, Madatian A, Wire MB, Bowen C, Park JW, Williams D, Peng B, Schubert E, Gorycki F, Levy M, Gorycki PD. *Drug metabolism and disposition*, 2011 Sep 1; **39(9):**1734-1746.
26. Ahir K, Singh S, Dharti P, Patel M. *Int. J. Pharm. Biol. Arch.* 2020 Dec 15; **11:**182-189.
27. Maddela R, Gajula R, Pilli NR, Siddiraju S, Maddela S, Makula A. *Journal of Pharmaceutical and Biomedical Analysis,* 2014 Sep 1; **98:**68-73.