Advances in Bioresearch
Adv. Biores., Vol 15 (5) September 2024: 333-339

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.15.5.333339

Advances in Bioresearch

ORIGINAL ARTICLE

Development and Validation of Stability Indicating UV-Spectrophotometric Method for the Estimation of Eltrombopag Olamine in Bulk and pharmaceutical formulation

Mohammed Aabid, Rashid Akhtar, Kaleem Ahmed, Faizan Ahmed, Arbaz Khan
Royal College of Pharmaceutical Education and Research, Abdul Muttalib Campus, Sayne Khurd, Malegaon Dist. Nashik (M.S), India-423203
Corresponding Author: Mohammed Aabid Nisar Ahmed
E-Mail: mohammedaabid02@gmail.com

ABSTRACT
In the proposed investigation, an effort was made to design and validate a quick and accurate UV spectrophotometric method for estimating Eltrombopag Olamine in bulk drugs.To propose a UV-Spectrophotometric approach that is easy to use, precise, reliable, sensitive and accurate for estimating Eltrombopag Olamine in bulk medications. The optimum conditions for drug analysis were found using Ethanol as the solvent. 423 nm was discovered to be the greatest absorption wavelength. It responded linearly between concentration levels of 5-30 µg/ml. The coefficient of linear regression was found to be 0.996. The method was evaluated for linearity, precision, accuracy, and robustness using ICH criteria, and all validation values were found to be acceptable. As a result, it is possible to infer that the method was novel, easy, selective, specific, and precise for estimating Eltrombopag Olamine in bulk and pharmaceutical formulation.
Keywords: *Eltrombopag Olamine, ICH guidelines, Method validation, Stability indicating, UV-Spectrophotometric*

Received 24.05.2024 Revised 01.06.2024 Accepted 11.07.2024

How to cite this article:
Mohammed A, Rashid A, Kaleem A, Faizan A, Arbaz K. Development and Validation of Stability Indicating UV-Spectrophotometric Method for the Estimation of Eltrombopag Olamine in Bulk and pharmaceutical formulation. Adv. Biores., Vol 15 (5) September 2024: 333-339.

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]

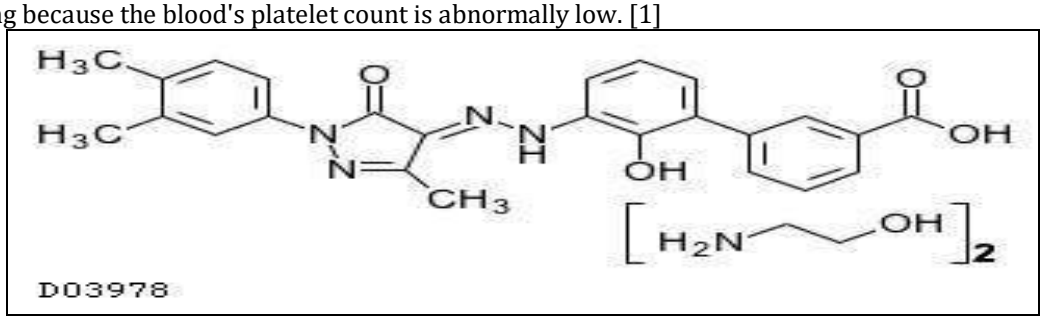

Figure 1. Structure of Eltrombopag Olamine.

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]

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

The literature has published analytical techniques for estimating ELT in the formulation and bulk drugs, such as spectrophotometric, HPLC, and HPTLC methods. The aforementioned techniques have some drawbacks, such as the fact that they take more time and need the use of pricey, sophisticated chemicals. There is no UV spectrophotometric method for assessing Eltrombopag Olamine in its pure form and formulation found, according to the review of the literature.

Forced degradation is the degradation of drug substance and drug product at conditions harsher than accelerated conditions. It must demonstrate the specificity of stability indicating techniques to address stability-related issues and it also offers insight into the processes and end products of degradation, aiding in the clarification of the structure of degradation products. The medicine is subjected to a variety of stress conditions, including oxidative, thermal, photolytic, acidic, and basic degradation, with a degradation rate of 5-20%.

MATERIAL AND METHODS

INSTRUMENTS USED:

A double-beam UV -visible spectrophotometer (Jasco Companies, Tokyo, Japan), and a high-precision electronic weighing balance (Mettler Toledo, Switzerland) are used for weighing the reagents. Ultrasonication was used for the solubilization of the drug.

MATERIALS:

Pharmaceutical grade Eltrombopag Olamine was supplied by Hetero drugs Ltd, Hyderabad, India. Analytical-grade ethanol is used as a solvent.

Method Development:

After deciding on the best solvent combination 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.

Preparation of standard stock solution:

To achieve a concentration of 1000 g/ml of the analyte, 10 mg of ELT was precisely weighed and transferred to a volumetric flask of 10 ml. The solution was then made up to 10 ml using solvent ethanol. 1 ml of the ELT solution was pipette out of the volumetric flask and transferred to a 10 ml volumetric flask in order to get an ELT concentration of 100 g/ml. Then, ethanol was used to raise the volume to 10 ml.

Forced degradation studies: [3- 4]

To assess the stability of the well-established UV-Spectroscopic method, samples were subjected to acid, base, oxidation, and photolytic degradation. The percentage of degradation was estimated for each study. The limit established by the study on forced degradation is acceptable and within limitations.

Acid degradation study: [5]

Acid degradation was seen when 10 mg of ELT was weighed in 10 ml of a volumetric flask. 1000 g/ml concentration was attained by dissolving in ethanol. For ELT, secondary stock solutions with a 100 g/ml concentration were prepared. This solution of 10 g/ml ELT was subjected to two hours of stress in 0.1 N HCl on a water bath at 80 °C. After scanning the samples in the 300–600 nm UV region, spectra were observed.

Base degradation study: [6- 7]

ELT solution (10 g/ml), which was made from primary and secondary stock solutions and diluted with 0.1 NaOH, was created for the Base degradation investigation. The spectra of both solutions were scanned after being stressed for two hours at 80°C.

Oxidation degradation study: [8]

From primary and secondary stock solutions, ELT solution (10 g/ml) was produced and diluted with 30% hydrogen peroxide. The spectra of both solutions were scanned after being strained at 80°C for two hours.

Photodegradation study: [9]
In the photodegradation investigation, 10 mg of the ELT drug was exposed to UV light at 423 nm for 24 hours. After that, ethanol was used to create solutions with a final ELT concentration of 10 mg/ml, which were subsequently scanned to get the appropriate spectra.

Formula to calculate % degradation

$$\% \text{ Degradation} = \frac{(\text{Initial degradation} - \text{Final degradation})}{\text{Initial degradation}} \times 100$$

Method Validation:
The method was developed and validated by ICH guidelines to determine Linearity, accuracy and system suitability, Precision, Robustness, Stability, LOD, and LOQ. ELT has a linearity range of 5-30 µg/ml. The calibration curve was created by plotting the area versus the concentration. The precision study was performed in the system, intraday, and interday precision, and the results were expressed in percent RSD.

Table No. 1: Parameters of method development	
Parameters	**Specifications**
Analytes	Eltrombopag Olamine
Solvent	Ethanol
λmax of Eltrombopag	423nm

Robustness was measured by changing the laboratories and system recording the instrument's percent RSD. The solution was stabilized in bench and freeze conditions for 72 hr. The linearity slope was used to calculate the LOD and LOQ.

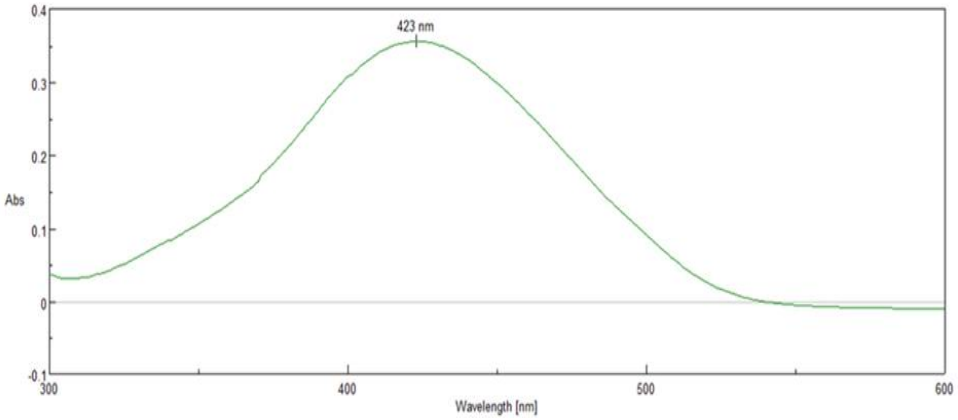

Fig 2: UV spectrum of Eltrombopag Olamine

RESULTS AND DISCUSSION
The solvent development method utilizes ethanol, and Eltrombopag Olamine demonstrated a spectrum with maximum absorbance at 423 nm. The parameters for method development and validation were presented in **(Table 1).**

Linearity:
As per the ICH Q2 (R1) guidelines, the linearity of an analytical procedure verifies that the test results have a direct relationship with the concentration (amount) of the analyte within the sample. For the linearity study, six solutions of various concentrations (5, 10, 15, 20, 25, and 30 µg/ml) were ready in ethanol from a working standard solution of ELT, and therefore the absorbance of each solution was noted at 423 nm.

Table 3: 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

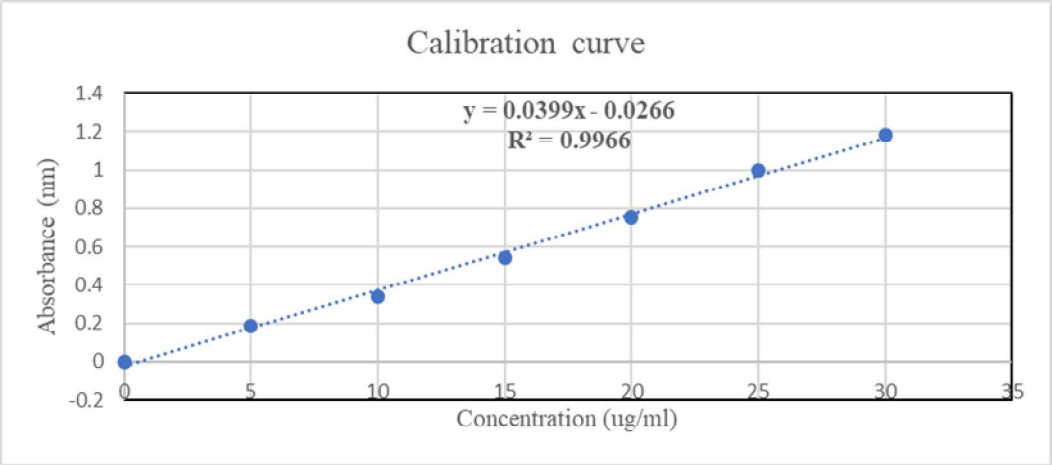

Fig 3: Calibration curve plot of Eltrombopag Olamine at 423nm

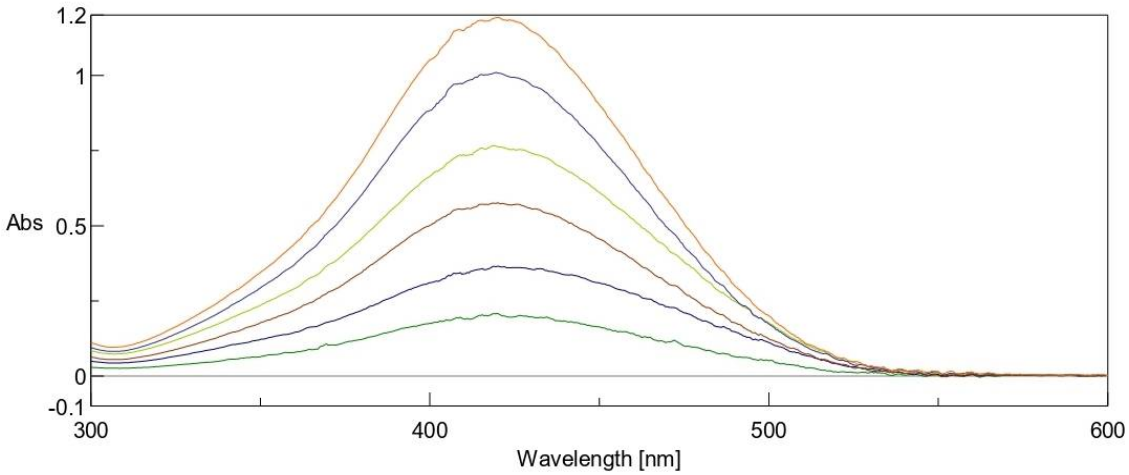

Fig 4: Overly spectrum of Eltrombopag Olamine (5 to 30 µg/ml) at 423 nm

Precision:

The ICH guidelines state that an analytical procedure's precision refers to how closely the outcomes of repeated measurements of the same homogenized sample match. To demonstrate the accuracy of the method, repeatability (intra-day precision) and intermediate precision (inter-day precision) measurements were done. Six replicates of the 10 g/ml concentration (n=6) were analyzed on the same day to confirm the test methodology's repeatability (intra-day precision). % relative standard deviation across six replicates was calculated. Similar to this, for intermediate precision (inter-day precision), the percentage relative standard was determined after six replicates of the 10 g/ml concentrations were examined for assay on three separate days.

Table 4: 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 5: 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:

The accuracy of a commenced investigation was appraised by standard addition methods, where a known amount of the standard was added in three different levels, i.e., 50, 100, and 150% to the in-house tablet formulation of ELT and analyzed by the commenced method in a set of three. The % recovery studies for ELT were carried out by spiking three different amounts of ELT standard (50, 100, and 150%) to the in-house tablet formulation. The % recovery of ELT was estimated for each level.

Table 6: Result of accuracy					
Concentration took (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

LOD & LOQ:

The limit of detection and limit of quantification concentrations for ELT were determined based on the residual standard deviation of response and slope method as per ICH guidelines. A calibration curve prepared in the linearity study was used for this purpose. For LOD calculation equation (3.3 x σ)/S and LOQ equation (10 x σ)/S were used. Where σ indicates the standard deviation of the response and S is the slope of the calibration curve.

Table 5: Result of LOD & LOQ	
LOD	**LOQ**
0.05	0.16

Robustness:

Robustness is often interpreted as the capability to reproduce the (analytical) method in diverse laboratories or under different conditions without the occurrence of unexpected differences within the obtained results, and a robustness test as an experimental set-up to assess the robustness of a method. to check the capability of the proposed method, different UV spectrophotometers were used present within the different laboratories for the determination of absorbance.

Table 6: Result of Robustness		
Conc. ug/ml	**Absorbance on system 1**	**Absorbance on system 2**
10	0.3849	0.3740
10	0.3859	0.3787
10	0.3853	0.3719
10	0.3788	0.3811
10	0.3791	0.3767
10	0.3819	0.3745
Mean	0.3827	0.3762
Std. deviation	0.0032	0.0034
%RSD	0.8316	0.8954

Forced Degradation:
The results of the forced degradation study using ethanol as a solvent were summarized in Figure 5 (A-D).
Acid degradation study:
There was extreme acid degradation of the drug found. More than 10% deterioration was seen in 0.1 N HCl after two hours of heating at 80°C, as shown in Figure 5 A.
Base degradation study:
Figure 5B demonstrates that heating at 80°C with 0.1 N NaOH causes higher degradation of ELT than other methods.
Oxidation Degradation study:
When compared to the other degradation methods, oxidation degradation using 30% hydrogen peroxide revealed very little degradation (Figure 5 C).
Photolytic degradation study:
Photolytic degradation showed very slight changes in the spectra and the level of the degradation was very less compared to other degradations Figure 5 D.

Table 7: Data of forced degradation study

Con	Eltrombopag at 423nm		
	Initial	Final	%Degradation
Acid	0.152	0.128	12.05
Base	0.325	0.271	13.25
Oxidation	0.578	0.503	11.06
Thermal	0.323	0.269	15.23
Photolytic	0.302	0.289	7.11

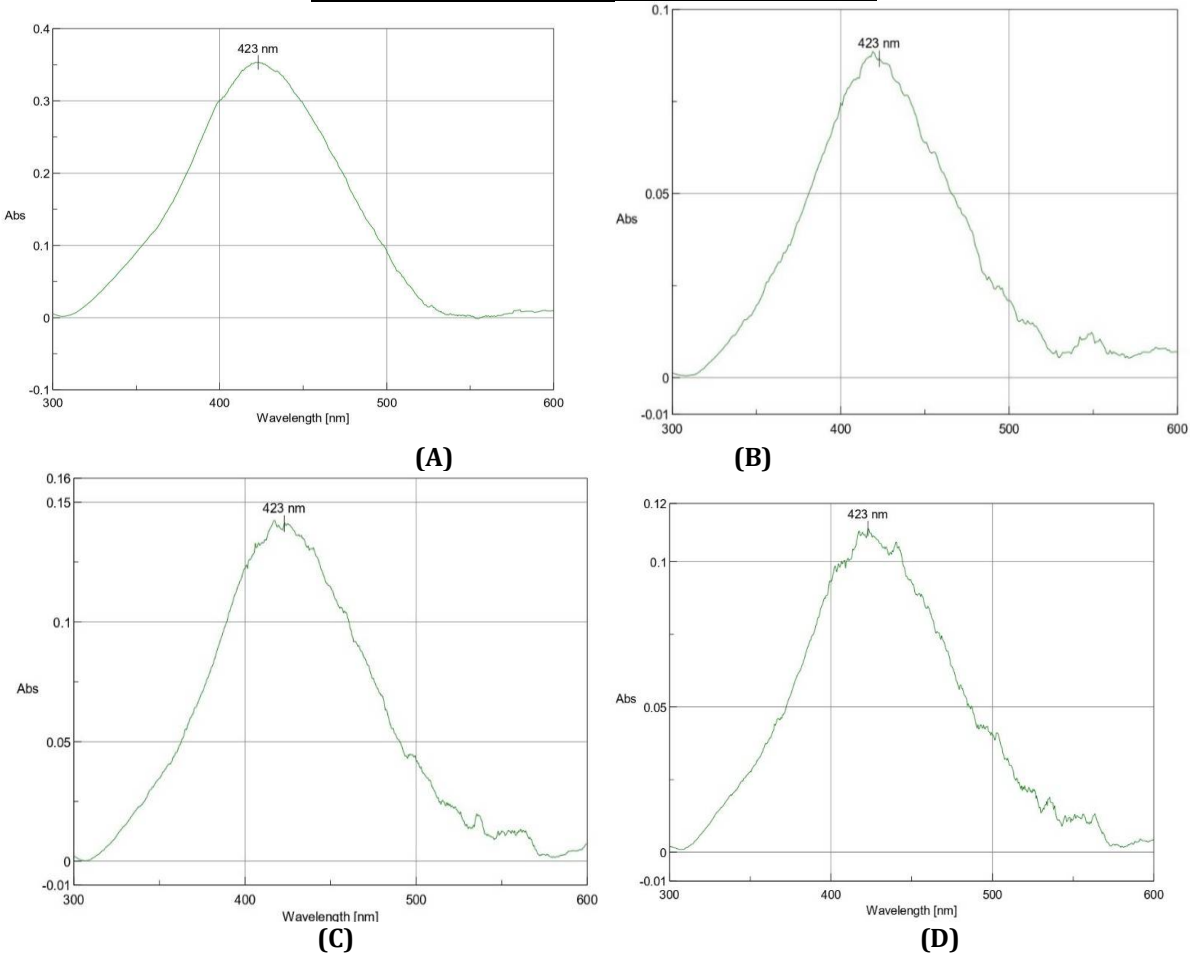

Fig 4: UV spectra of Eltrombopag Olamine (10 µg/ml) obtained in the stress degradation assays using Acidic (A), Basic (B), Oxidative(C), Photolytic (D)

CONCLUSION
A precise, accurate, cost-effective, and robust UV Spectroscopic method has been produced for the assessment of Eltrombopag Olamine in bulk drugs. The developed UV Spectroscopic method for the

estimation of Eltrombopag Olamine is simple and rapid. Data obtained from precision shows result in terms of RSD less than 2, which conclude that the method is reproducible and precise. The accuracy range is between 98-99% recovery ensures good accuracy and specificity. The excellent % recovery of a drug shows that the excipients present in the tablet formulation have no obstruction in the determination of Eltrombopag Olamine indicating that the method is specific with a good response for the estimation of Eltrombopag Olamine. Therefore, the developed method can be used for the regular analysis of Eltrombopag Olamine in bulk drugs.

ACKNOWLEDGMENTS

The authors show sincere thanks to Novartis, Hyderabad for providing the drug samples. Special and sincere thanks to the staff of the Royal College of Pharmaceutical Education and Research (RCPER) for permitting us to carry out the research work. The authors also acknowledge the Principal Dr. Masood Siddiqui for their constant support during the work.

CONFLICT OF INTEREST:

The authors state that there is no conflict of interest in publishing this paper.

REFERENCES

1. Jose Ramon, G., and Jose, M. (2018). Eltrombopag in immune thrombocytopenia: efficacy review and update on drug safety; Therapeutic Advances in Drug Safety., 9(6): 263-285.
2. Amolkumar, K., Ashok, T., Kiran, J. (2015). Development and validation of UV spectrophotometric method to study stress degradation behavior of rizatriptan benzoate. Guang Pu Xue Yu Guang Pu Fen Xi. 35(1): 137-40.
3. Supriya, S., Sunil, S., Akshay, K., Bhaskar, K. (2022). Development and Validation of Stability Indicating UV-Spectrophotometric Method for the Estimation of Hesperidin in Bulk Drugs, Plant Extract, Ayurveda Formulation, and Nanoformulation. Indian Journal of Pharmaceutical Education and Research 56(3): 865-872.
4. Kiran, V., Dinesh, L., Geet, P., Pranali, R, Mrityunjaya, B. (2013). Stability-indicating stress degradation studies of lafutidine using UV spectrophotometric method. Pharmaceutical Methods., 4(1): 21-25.
5. Tharlapu, S., Khagga, M., and Hitesh, A. (2018). Stability Indicating UHPLC Method Development and Validation for Estimation of Eltrombopag and its Related Impurities in Tablet Dosage form. Oriental Journal of Chemistry 34(2): 1098-1104.
6. R., Gandhimathi, S., Vijayaraj, M.P., Jyothirmaie. (2012). Analytical Process of Drugs by Ultraviolet (UV) Spectroscopy- A review. International Journal of Pharmaceutical Research and Analysis., 2(2): 72-78.
7. G. Manoharan. (2018). Development and Validation of a Stability-indicating RP-HPLC Method for the Estimation of Eltrombopag in Bulk and Tablet Formulation. Asian Journal of Chemical Sciences., 4(1): 1-9.
8. Tharlapu, S., Khagga, M., and Hitesh A. (2018). Stability Indicating UHPLC Method Development and Validation for Estimation of Eltrombopag and Related Impurities in Tablet Dosage Form. Oriental Journal of Chemistry., 34: 1098-1104.
9. "https://pubchem.ncbi.nlm.nih.gov/compound/Eltrombopag-olamine".
10. Panchumarthy, R., MD. Shaheem, P. Srinivasa. (2017). Development and validation of stability-indicating UV spectrophotometric method for determination of Apremilast in bulk and pharmaceutical dosage form. Indian Journal of Research in Pharmacy and Biotechnology. 5(1): 47-53.
11. Mayank S, Geeta V., Priyanka S, and Suleman S. (2018). Development and Validation of Stability Indicating Rp-Hplc Method for Estimation of Eltrombopag Olamine in Pharmaceutical Dosage Form. European Journal of Biomedical AND Pharmaceutical sciences. 5(11): 454-460.
12. Maddela R, Gajula R, Pilli NR, Siddiraju S, Maddela S, Makula A. (2014). Liquid chromatography-tandem mass spectrometric assay for eltrombopag in 50μL of human plasma: a pharmacokinetic study. J Pharm Biomed Anal. 98: 68-73.
13. ICH – Harmonized Tripartite Guideline. Stability testing of new drug substance and products Q1A (R2). International Conference on Harmonization. IFPMA, Geneva, Switzerland, 2003.
14. Blessy M, Patel RD, Prajapati PN and Agrawal YK. (2013). Development of forced degradation and stability indicating studies of drugs – A review. J. Pharm. Anal. 4: 159- 65.
15. Pal N, Pravalika P, Bhavani DS, Geetha R, Afreen A and Rao AS. (2018). New method development and validation for the determination of Eltrombopag in bulk and tablet dosage form by HPLC. World journal of Pharmacy and Pharmaceutical science. 7: 1176-84.
16. Madasu RK, Isreal S and Rao NV. (2018). Development and validation for detection of DIC and IHC in Eltrombopag olamine tablet by RP-HPLC method. World journal of Pharmacy and Pharmaceutical science. 7: 744-62.
17. Basha DM, Reddy GV, Rani TS, Susithra E and Chekkara R. (2015). RP-UPLC-MS assay method for Eltrombopag: application in pharmaceuticals, human plasma and urine samples. Asian J. Chem. 27: 4615-9.