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NEW METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF ELTROMBOPAG IN BULK AND TABLET DOSAGE FORM BY HPLC

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ABSTRACT

The present analytical work is a unique method development and validation for the determination of Eltrombopag by using reverse phase High performance liquid chromatography (HPLC) with isocratic elution technique. Here the stationary phase used was Xterra C18 (150mm x 4.6mm x 5µm), mobile phase was 40% potassium di-hydrogen phosphate (0.01N) and 60% Acetonitrile. pH of the mobile phase was maintained at 3.0, flow rate 1 ml/minute. Eluted material underwent for monitoring at the detector wavelength of 282 nm. Retention time for Eltrombopag was found to be 2.24 minutes; linearity range was 25 µg/ml to 150 µg/ml. The new method was evaluated according to ICH guideline and as far as validation results are concern correlation coefficient value was 0.998 for the very compound, LOD 0.062 µg/ml, LOQ 0.188 µg/ml, percentage recovery 99.83%, repeatability results relative standard deviation (%RSD) 0.40 for Eltrombopag. The developed HPLC method was found to be a simple and rapid one for regular analysis in professional laboratory.

KEY WORDS: Eltrombopag, HPLC, method development, validation.

INTRODUCTION

Eltrombopag (ELT) is a drug of choice for those patients who suffer from abnormally low platelet counts. It works by reinforcing the physiological actions of receptor c-mpl (TpoR). This receptor is the physiological target of a hormone called thrombopoietin.[1,2]

Eltrombopag is an approved drug by U.S. Food and Drug Administration.

Its recommended for the treatment of thrombocytopenia cases in patients with a chronic immune (idiopathic) thrombocytopenic purpura and who have had an insufficient or improper response to drugs like corticosteroids or procedure like immunoglobulin therapy, or splenectomy. This drug is available in tablet form, 25 mg and 50 mg and in the form of powder for suspension. For the evaluation of such a delicate drug in bulk and formulations its very essential to have a good analytical procedure. We therefore, planed for finding out a new simple technique for the evaluation of ELT in bulk and tablet dosage form.

Chemically ELT is 3'-{(2Z)-2-[1-(3,4-dimethylphenyl)-3-methyl-5-oxo-1,5-dihydro-4H-pyrazol-4-ylidene]hydrazino}-2'-hydroxy-3-biphenylcarboxylic acid

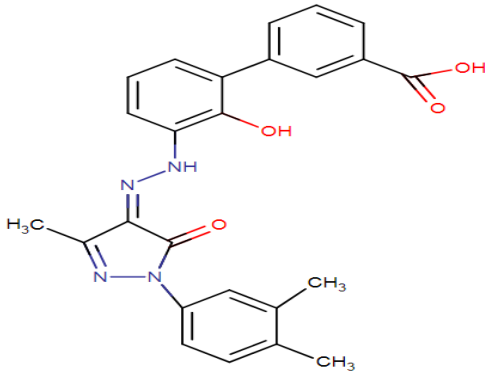

Figure 1: Struture of Eltrombopag.

As per literature survey[3-7] it is learned that there are very few methods available for the determination of Eltrombopag. S.Marakatham, R.V. Vallikumari and Meruva Sathish Kumar developed Spectrophotometric method for determination of Eltrombopag in bulk and pharmaceutical formulation. Rambabu Maddela, Ramakrishna Gajula, Nageswara Rao Pilli, Sridhar Siddiraju, Srinubabu Maddela and Ajitha Makula developed a Liquid chromatography-tandem mass spectrometric method for the assay for Eltrombopag in 50uL of human plasma. Gunasekar Manoharan developed a RP-HPLC method for the estimation of same in bulk and tablet formulation. Madasu Raja Kumar, Samson Isreal and V.V.Nageswara Rao, developed a method for detection of DIC and IHC in Eltrombopag olamine tablet by RP-HPLC. Mohan T.S.S.J, Mukkanti K and Jogia H.A developed a stability indicating UHPLC method for Eltrombopag and its related impurities in tablet dosage form.

We observed in all these cases that the compound needs a more simple method for its estimation. Therefore, in our present project we have attempted to use a very simple

chromatographic condition as well as ensured that the time consumption must be as low it was possible and in fact it is the lowest one as on date.

MATERIALS AND METHODS

Materials: Eltrombopag pure drug was procured from Hetero drugs Ltd Hyderabad. Eltrombopag tabtets (Revalde) was supplied by Medindia Pharma network. HPLC grade water, Distilled water, acetonitrile, methanol, potassium di-hydrogen phosphate, ortho-phosphoric acid were from Rankem.

Instruments: WATERS 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software.

UV-VIS spectrophotometer: PG Instruments T60, bandwidth of 2 nm, matched quartz cells integrated with UV win 6 Software was used to measure the absorbance.

Experimental: The new method development work[8,9] we started after adequate amount of literature survey and getting adequate idea about the different chromatographic factors which ultimately could deliver a very good method for the determination.

Diluent: Based upon the solubility of the drugs, diluent was selected. It was observed that water and acetonitrile at 50:50 ratios was suitable.

Preparation of stock solutions (Standard): Accurately weighed 25mg of Eltrombopag and transferred to 25 ml volumetric flask. 3/4th of diluent (as mentioned) was added to the flask and subjected for sonication for 10 minutes. Flask volume till the mark was made up with diluents and labeled as stock solution standard (1000µg/ml of Eltrombopag).

Preparation of Standard working solutions (100% solution): 1ml solution from stock preparation was pipetted out and collected into a volumetric flask (10ml) and made up the required volume with diluent. (100µg/ml Eltrombopag).

Preparation of stock solutions (Sample): 10 tablets were weighed, average weight of single tablet was calculated, then the weight (equivalent) of 1 tablet was collected into a volumetric flask (50 ml), 25ml of diluents was mixed and sonicated for around 25 minutes. It was then subjected for making the volume with diluent and filtered by membrane filters (1000µg/ml of Eltrombopag).

Preparation of working sample solutions (100% solution): 1ml volume of filtered sample solution was transferred to volumetric flask (10ml) and the volume was made up with same diluent. (100µg/ml of Eltrombopag).

Preparation of buffer

0.01% potassium di-hydrogen phosphate: 3.4gm was dissolved in 1000ml with HPLC grade water.

Validation Parameters

The method was evaluated as per protocol of ICH.[10] The evaluation parameters took into consideration were system suitability parameters, precision accuracy, intermediate precision, linearity, limit of quantification, limit of detection, robustness studies etc.

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of Eltrombopag (100ppm) and the solutions were injected six times and the parameters like peak tailing, theoretical plate count, retention time etc were determined.

Specificity: Checking of interference if any in the optimized method. We should not find any interfering peak in blank in this method so that the method can be considered as specific.

Accuracy: The accuracy for the present HPLC methods was examined by calculating the extant of recoveries of Eltrombopag by the method called standard addition. Correct amount of solutions (standard) of Eltrombopag (each1 50%, 100%, and 150%) were added and injected to pre-quantified solution of sample. The quantity of each substances recovered were determined.

Precision: The experimental repeatability as well as intermediate precision was examined by repeatedly applying six injections containing Eltrombopag (100 ppm) at two subsequent days. Number of theoretical plates, retention time, peaks resolution, peak symmetry etc was the subject of observation.

Linearity: Following concentration for both the compound were designed to conduct linearity test.

Eltrombopag: 25 ppm, 50 ppm, 75 ppm, 100 ppm, 125 ppm, 150 ppm. To build up calibration curve, concentration and area were considered at X and Y axis respectively.

LOD and LOQ: Calculation for Limit of detection as well as Limit of quantification had been done by using standard Equations. LOD = 3.3×σ/S, LOQ = 10×σ/S. Here σ denotes for standard deviation of intercepts of regression lines, S denotes for slope.

Robustness: Evaluation for robustness had been conducted by making alteration in different chromatographic parameters. These parameters included flow rate, temperature, mobile phase composition etc.

Assay of marketed formulation: Assay of marketed product was carried by injecting sample corresponding to equivalent weight into HPLC system, percentage purity was found out by the following formula

$$\text{Conc.}_{\text{unknown}} = \left(\frac{\text{Area}_{\text{InternalStd.in known}}}{\text{Area}_{\text{InternalStd.in unknown}}}\right) \times \left(\frac{\text{Area}_{\text{unknown}}}{\text{Area}_{\text{known}}}\right) \times (\text{Conc.}_{\text{known}})$$

RESULTS AND DISCUSSION

Method development: A unique method was innovated by using different columns, mobile phases with various compositions were tried by taking standard and sample in individual. Column temperature, flow rate, different buffers with slightly varying pH value and solvents were applied. The different mobile phases which were prepared were subjected for filtration through membrane filters prior to their use. The mobile phase containing 40% potassium di-hydrogen phosphate (0.01N) and 60% Acetonitrile pH around 3.0 was identified to be best to obtain peaks of Eltrombopag at 2.163 minutes.

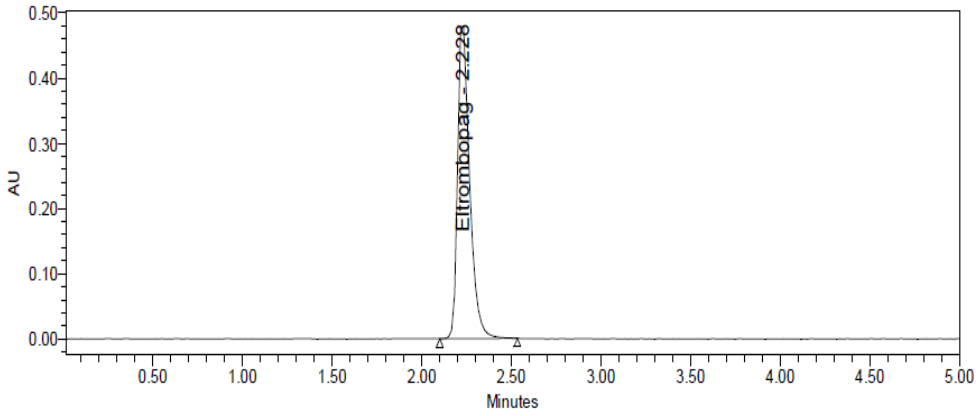

Figure 3: Optimized chromatogram of Eltrombopag.

Validation Results

System suitability parameter: The optimized chromatographic method as developed resulted in the elution of Eltrombopag at 2.163 minutes. Figure3 is the representative chromatogram of standard Eltrombopag. System suitability results were evaluated taking six replicates of standard at 100µg/ml for the compound Eltrombopag. Table 1 narrates about the results of system suitability parameters.

Table 1: System suitability.

Compound	Rt(minutes)	Area	USP plate count	Tailing factor
Eltrombopag	2.163	2235301	5580	1.41

Accuracy results: Recovery of Eltrombopag standard 50%, 100% and 150% was 99.84% as a mean. Table 2 contains all the results of accuracy studies.

Table 2: Accuracy results.

% Level	Amount spiked	Amount recovered	% Recovery	Mean recovery
50%	50	50.32	100.65	99.84
	50	49.59	99.17	
	50	50.13	100.27	
100%	100	100.48	100.48	
	100	99.66	99.66	
	100	99.33	99.33	
150%	150	150.52	100.35	
	150	148.70	99.14	
	150	149.16	99.45	

Precision results: Result of intraday precision as mean area of peak and %RSD for ELT standard injections were –2244693 and 0.41. For ELT sample injection results were 2244871 and 0.40. Results of inter day precision study in terms of average area of peak and %RSD for ELT standard injections were 2255338 and 0.42. Table 3 narrates precision results in details.

Table 3: Precision results.

S.No	Peak area of ELT standard (intra day)	Peak area of ELT standard (inter day)
1	2235376	2281087
2	2246311	2251589
3	2258557	2255898
4	2250606	2260261
5	2242928	2248059
6	2234380	2235106
Mean	2244693	2255333
SD	9231.16	15257.35
%RSD	0.41	0.67

Regression Analysis: Results of linearity test revealed that mean Y intercept value, slope value, and value of correlation coefficient for ELT was 21876, 17974, 0.998 at the concentration range of 25 µg/ml to 150 µg/ml. Table 4 explains about sensitivity and results of regression analysis.

Table 4: Regression analysis and sensitivity test results.

Parameters	Eltrombopag
Linearity (µgm/ml)	25µg/ml to 150 µg/ml
Correlation Coefficient.(r)	0.998
Regression slope	17974
%RSD of Slope	0.144
Regression Intercept (mean)	17974
%RSD of Intercept	0.322
LOD	0.061
LOQ	0.187

Robustness results: This evaluation had been done by bringing variation in certain chromatographic parameters such as increasing and reducing flow rate, variation in the ratio of aqueous phase and organic phase, temperature of column etc. Retention time, plate counts, asymmetric or tailing factor etc was observed with very negligible variation. All the observed values are given in table 5 as tabular form.

Table 5: Robustness results.

	Chromatographic condition	Retention time	USP plate count	Asymmetric factor	%Assay
ELTROMBOPAG	Flow rate (1.2ml/min)	2.098	5133	1.30	99.98%
	Flow rate (0.8ml/min)	2.361	5536	1.38	100.02%
	Buffer : ACN(35:65)	2.203	4137	1.30	100.00%
	Buffer : ACN(45:55)	2.267	4695	1.33	99.16%
	Temperature(32ºc)	2.215	4067	1.34	100.25%
	Temperature(28ºc)	2.265	4770	1.35	100.22%
	Mean	2.234	4723	1.33	99.94

Assay of marketed formulation: The formulation (Tablet- Revalde) was procured from Medindia Pharma network. Ten tablets had been taken, weighed and collected in a mortar. Tablets were triturated to powder form and collected an equivalent quantity of ELT 50 mg in a volumetric flask (50 ml). Powders were treated with diluent and subjected for sonication. The volume was made with diluent. 1 ml of the solution was pipetted out into a volumetric

flask (10 ml) and the volume was made with diluent. 10 µl of resultant solution was injected to the Chromatographic system and result was studied as compared to standard. Peak area response was taken into consideration.

CONCLUSION

The present HPLC method for the determination of Eltrombopag was found to be the least time consuming, simple, highly accurate technique as results of all the validation parameters were with low value of %RSD. It also proved that the innovated technique is precise and robust. Therefore the above mentioned novel analytical technique is suitable for the evaluation of bulk and tablet formulation of Eltrombopag in laboratory.

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