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RESEARCH ARTICLE

Dissolution Method Validation with Reverse Phase Chromatographic Method for Determination of Eltrombopag Drug Release in Dissolution Samples of Tablets

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ABSTRACT

The present analytical work is a unique method development and validation for the determination of dissolution of Eltrombopag using reverse phase high-performance liquid chromatography (HPLC) with isocratic elution technique. HPLC method for quantification of drug in dissolution samples of Eltrombopag tablet is developed and validated. About 0.5% polysorbate 80 in phosphate buffer of pH −6.8 is used as dissolution medium and paddle (USP-II) as apparatus at 50 rpm. The sample was withdrawn after 45 min. The developed HPLC method was used for quantitative estimation of drug release in dissolution samples of Eltrombopag tablet. Here, the stationary phase used was Xbridge C18 (50 mm × 4.6 mm × 5 µm), mobile phase was 25% ammonium formate and 75% acetonitrile. pH of the buffer solution was maintained at 3.0, flow rate 1.0 ml/min. Eluted material underwent for monitoring at the detector wavelength of 230 nm. Retention time for Eltrombopag was found to be 2.16 min; and linearity range was 3.516 µg/mL–131.862 µg/mL. The new method was evaluated according to the ICH guideline and as far as validation results are concern correlation coefficient value that was 1.0000 for the compound, percentage recovery 99.4%, and repeatability results relative standard deviation 0.6 for Eltrombopag. The developed HPLC method was found to be a simple and rapid one for regular analysis in professional laboratory.

Keywords: Eltrombopag, high-performance liquid chromatography, method development, validation

DRUG PROFILE

Eltrombopag olamine

Description: Eltrombopag used in Severe Aplastic Anemia and hemostasis

Structure

Description: Orange to red Crystalline Solid.
IUPAC name: 3-(3-{2-[(4Z)-1-(3,4-dimethylphenyl)-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-4-ylidene]hydrazin-1-yl}-2-hydroxyphenyl)benzoic acid.
Molecular Weight: 564.643 g/mol.
Molecular Formula: $C_{25}H_{22}N_4O_4$.
Wave length: 230 nm.
Melting Point: 242-244°C.
Solubility: Sparingly Soluble in Water and Freely Soluble in dimethylformamide.
pKa: 3.99.
log P: 6.3.

Mode of action

Eltrombopag is an orally bioavailable, small-molecule thrombopoietin (TPO)-receptor agonist that interacts with the transmembrane domain of the human TPO-receptor. Eltrombopag is a stimulator of STAT and JAK phosphorylation. Unlike recombinant TPO or romiplostim, Eltrombopag does not activate the AKT pathway in any way. It should be noted that when given to patients with aplastic anemia, other lineages besides platelet

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count were increased, suggesting that either Eltrombopag enhanced the effect of TPO *in vivo*; or there is a yet uncovered mechanism of action at work.

Absorption

Peak absorption of Eltrombopag occurs around 2–6 h following oral administration, and the total oral absorption of drug-related material following a 75 mg dose was estimated to be at least 52%. Eltrombopag tablets 25 mg, 50 mg, and 75 mg are approved drug product by USFDA.

OBJECTIVE AND PLAN OF STUDY

As per literature survey, it is learned that there are very few methods available for the determination of Eltrombopag. Dharti and Miral developed precipice and accurate Spectroscopic high-performance liquid chromatography (HPLC) method for determination of dissolution of Eltrombopag in bulk and pharmaceutical formulation.

- To develop HPLC method for dissolution of simultaneous estimation of Eltrombopag Tablet and validated method according to the ICH guidelines.
- To apply validated method for the estimation of Eltrombopag in pharmaceutical formulation in QC laboratory and R&D lab Scale.[1-5]

MATERIALS AND METHODS

Materials

Eltrombopag tablets supplied by Medindia Pharma network., DI Water, HPLC Grade water, acetonitrile, methanol, potassium dihydrogen phosphate, ammonium formate, glacial acetic acid, sodium hydroxide, dimethyl formamide, and polysorbate 80 [Figure 1-3].

Instruments

HPLC instruments used a Shimadzu's HPLC (LC-1020C HT) with PDA detector and autosampler (Shimadzu Corporation, Kyoto, Japan) with Empower-3 Software.
SHIMADZU 1800 double-beam Ultraviolet (UV) - Visible spectrophotometer with software LC Solution (Shimadzu Corporation, Kyoto, Japan), Dissolution Apparatus of Electro lab.

Methods

Dissolution medium preparation

Weight about 68 g of potassium dihydrogen phosphate and transfer into a 8000 ml of water. Sonicate to dissolve it and mixed well. Adjust pH 6.80 ± 0.05 using NaoH solution and mixed well Dilute to volume up to 10,000 ml with water and mixed well degas it. Weigh and transfer 50.0 g of polysorbate 80 into same beaker and dissolve.[6-10]

Dissolution parameters

Apparatus: USP Type-II Paddle.
Medium Volume: 900 mL.
Speed: 50 RPM.
Temperature: 37 ± 0.5°C.

Diluent

Based on solubility data of drug, dissolution medium was selected. It was observed 0.5% polysorbate 80 in phosphate buffer pH 6.8.

Preparation of buffer solution

Weight and transfer about 0.63 g of ammonium formate and transfer into suitable container containing 1000 ml of water. Sonicate to dissolve it and mixed well. Adjust the pH 3.00 ± 0.05 using glacial acetic acid solution and mixed well.

Mobile phase preparation

Prepare a mixture of buffer solution and acetonitrile in ratio of 25:75 %v/v, respectively. Mixed well and degas it by sonication.

Chromatographic parameters

HPLC column: Xbridge C18 (50 mm × 4.6 mm × 5 µm).
Pump Flow: 1.0 ml/min.
Injection volume: 10 µL.

Wavelength: UV detector at 230 nm.
Column oven temperature: 25°.
Sample oven temperature: 25°.
Run time: 5 min.

Preparation standard stock solution of eltrombopag

Weigh accurately 112.0 mg of Eltrombopag reference standard and transfer into a 50 ml volumetric flask. Add about 20 mL of dimethyl formamide and sonicate to dissolve. Make up the volume up to the mark with methanol and mix well.

Preparation standard solutions of eltrombopag

Pippete 8.0 mL of standard stock solution and transfer in a 250 mL volumetric flask and
Diluted up to mark with Diluent and mix well.

Preparation sample solutions of eltrombopag

Placed one tablet in each individual jar (six tablets in six individual jars) which was contained 900 ml of dissolution medium maintained at 37.0. The paddle was rotated at speed of 75 rpm. Aliquot was withdrawn after 45 min. Filtered through 0.45 µm PVDF filter and injected in chromatographic system.

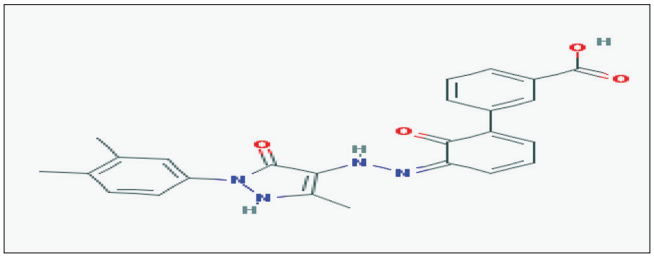

Figure 1: Structure of Eltrombopag olamine

Validation parameter

Method was evaluated as per ICH. The evaluation parameter took into Consideration was system suitability, precision, accuracy, intermediate precision, linearity, robustness study, etc.[11-13]

Specificity

The specificity was determine by the comparison of diluent, standard solution, and sample solution. There no interference is observed at the peak of Main Peak (Eltromopag) in Blank, hence, this method considered as specific.

System suitability parameter

This parameter is determine by preparing standard solution of Eltrombopag and solution was injected 5 times and parameters such as tailing, plate count, and retention time were determined.

Accuracy

The accuracy for the present HPLC methods was determined by calculating the extant of recoveries of Eltrombopag by the method called standard addition. Correct amount of solutions (standard) of Eltrombopag (each 25%, 100%, and 200%) was added and injected to pre-quantified solution of sample. The quantity of each substances recovered was determined.

Precision

Precision is usually measured as the coefficient of variation or relative standard deviation (RSD)

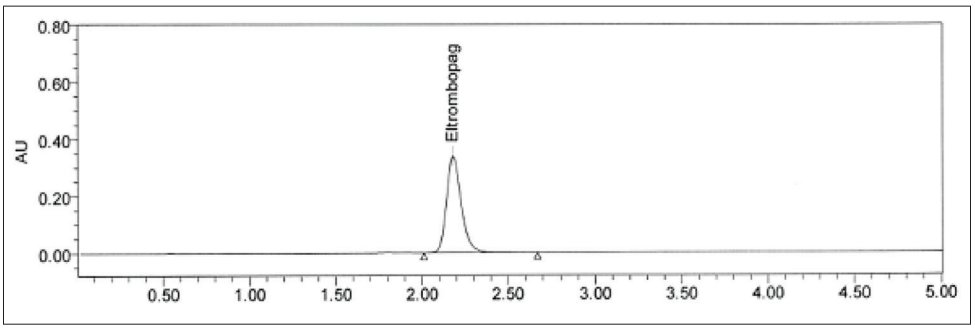

Figure 2: Standard solution chromatogram of Eltrombopag olamine

Table 1: Contains all the results of accuracy studies

% of Eltrombopag standard added (µg/ml)	Amount of standard eltrombopag added (µg/ml)	Amount of eltrombopag standard added (µg/ml)	Amount of eltrombopag standard recovered (Mg/ml)	Amount of eltrombopag standard recovered %	Average % recovery	% RSD
25%	11	11.564	11.703	98.8	99.3	1.1
		11.550	11.490	100.4		
		11.523	11.705	98.4		
100%	43	43.044	43.417	99.1	99.3	0.4
		43.041	43.447	99.1		
		43.036	43.121	99.8		
150%	65	65.013	65.205	99.7	99.5	0.3
		64.987	65.143	99.8		
		65.006	65.626	99.1		
Overall % recovery					99.4	
Overall % RSD					0.6	

Table 2: Explains about results of linearity analysis

Linearity level	Concentration (µg/mL)	Peak area
4%	3.516	123,521
50%	43.954	1,564,481
80%	70.326	2,524,287
90%	79.117	2,833,655
100%	87.908	3,143,224
110%	96.699	3,478,806
120%	105.489	3,799,830
150%	131.862	4,740,748
Correlation coefficient: 1.0000	Y-intercept: −10965.7971	
Slope: 36027.3439	Y-intercept bias at 100% level: −0.3%	

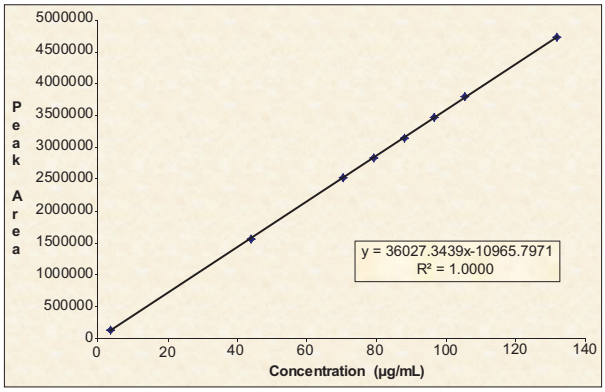

Figure 3: Linearity plot of Eltrombopag olamine/calibration curved of Eltrombopag

of analytical results acquired from independently prepared samples (six tablets in case of dissolution). Method precision was evaluated by performing the dissolution using proposed method (dissolution parameters and chromatographic method) on six tablets of Eltrombopag tablets and calculated % release of Eltrombopag in each sample. The %RSD for set of six tablets was calculated. The intermediate precision of the method was also evaluated using different day and a different instrument in the same laboratory by carrying out dissolution on six more tablets using proposed method and calculated % release of Eltrombopag in each sample.

Linearity

The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in sample. The linearity of Eltrombopag olamine is established by analyzing linearity solutions of different concentrations from 4% to 150% of working concentration of method for dissolution. The linearity curve is plotted for peak area versus concentration.

Robustness

Robustness study is performed by analyzing the standard and sample at different conditions.

The results obtained with altered conditions are compared against results obtained under normal chromatographic conditions. The parameter included changed flow rate, temperature, pH of buffer, mobile phase ratio, dissolution medium, and RPM.

Table 3: Robustness results comparison with precision result – variation in flow rate (Sample Solution) (±0.2 mL/min)

Injection #	% drug release			
	Flow rate: 0.8 mL/min	Actual flow rate: 1.0 mL/min	Flow rate: 1.2 mL/min	Actual flow rate: 1.0 mL/min
1	94	97	94	97
2	99	97	99	97
3	101	96	101	96
4	99	97	99	97
5	102	96	102	96
6	102	98	102	98
Mean	100	97	100	97
% RSD	3.0	0.8	3.0	0.8
Absolute difference	3		3	

Table 4: Robustness results comparison with precision result – Variation in column oven Temperature (Sample solution) (±5°C)

Injection #	% drug release			
	Column oven temperature: 20°C	Actual column oven temperature: 25°C	Column oven temperature: 30°C	Actual column oven temperature: 25°C
1	94	97	94	97
2	99	97	99	97
3	101	96	102	96
4	99	97	99	97
5	102	96	102	96
6	102	98	102	98
Mean	100	97	100	97
% RSD	3.0	0.8	3.1	0.8
Absolute difference	3		3	

Table 5: Robustness results comparison with precision result – variation of pH in Buffer solution (sample solution) (±0.20 pH)

Injection #	% drug release			
	pH of buffer solution: 2.80 pH	Actual pH of buffer solution: 3.00 pH	pH of buffer solution: 3.20 pH	Actual pH of buffer solution: 3.00 pH
1	94	97	94	97
2	99	97	99	97
3	102	96	102	96
4	99	97	100	97
5	102	96	103	96
6	103	98	103	98
Mean	100	97	100	97
% RSD	3.3	0.8	3.4	0.8
Absolute difference	3		3	

RESULTS AND DISCUSSION

System suitability parameter [Tables 1-8]

The optimized chromatographic method as developed resulted in the elution of Eltrombopag at 2.16 min. Figure 2 is the representative chromatogram of standard Eltrombopag. System suitability results were evaluated taking six replicates of standard at 50 mg for the compound Eltrombopag. Table 9 narrates about the results of system suitability parameters.

Table 6: Robustness results comparison with Precision result – variation of organic solvent (acetonitrile) in mobile phase composition

Injection#	% drug release			
	−5% Variation of Acetonitrile in Mobile Phase composition: (Buffer solution: Acetonitrile [288:712])	**Actual composition of Mobile Phase composition : (Buffer solution: Acetonitrile [250:750])**	**+5% Variation of Acetonitrile in Mobile Phase composition: (Buffer solution: Acetonitrile [212:788])**	**Actual composition of Mobile Phase composition: (Buffer solution: Acetonitrile [250:750])**
1	100	97	100	97
2	102	97	102	97
3	99	96	100	96
4	103	97	103	97
5	97	96	97	96
6	101	98	101	98
Mean	100	97	101	97
% RSD	2.2	0.8	2.1	0.8
Absolute difference	3		4	

Table 7: Results of robustness-variation in media volume for sample (±5%)

Injection #	% drug release			
	Media volume: 855 mL	**Actual media volume: 900 mL**	**Media volume: 945 mL**	**Actual media volume: 900 mL**
1	101	97	100	97
2	98	97	101	97
3	98	96	100	96
4	99	97	100	97
5	93	96	100	96
6	101	98	100	98
Mean	98	97	100	97
% RSD	3.0	0.8	0.4	0.8
Absolute difference	1		3	

Table 8: Results of robustness-variation in RPM for sample (±2 RPM)

Injection #	% drug release			
	RPM: 48	**Actual RPM: 50**	**RPM: 52**	**Actual RPM: 50**
1	95	97	99	97
2	91	97	95	97
3	98	96	99	96
4	102	97	100	97
5	97	96	96	96
6	99	98	100	98
Mean	97	97	98	97
% RSD	3.9	0.8	2.2	0.8
Absolute difference	0		1	

Accuracy

% Recovery of Eltrombopag 25%, 100%, and 150% was 99.4 as mean.

PRECISION RESULTS

Result of precision as mean area of peak and %RSD for Eltrombopag standard injections was 1,683,698

Table 9: System suitability

Compound	RT (min)	Area	USP plate count	Tailing factor
Eltrombopag	2.18	2000644	3259	1.2

Table 10: Results of precision and intermediate precision standard solution

Sr. no.	Peak area of Eltrombopag standard (Precision)	Peak area of Eltrombopag standard (Intermediate precision)
1	2,000,644	2,064,525
2	2,002,355	2,061,243
3	2,003,582	2,067,171
4	2,001,160	2,068,153
5	2,001,551	2,065,803
Mean	2,001,858	2,065,379
%RSD	0.1	0.1

Table 11: Results of precision and intermediate precision sample solution

Sr. no	Peak area of Eltrombopag sample (Precision)	Peak area of Eltrombopag sample (Intermediate precision)
1	1,912,536	2,005,515
2	1,912,365	2,012,255
3	1,892,565	1,992,556
4	1,912,244	2,001,565
5	1,912,222	1,992,565
6	1,925,465	2,012,555
Mean	2,002,184	2,002,835
% RSD	1.07	0.4

and 0.3%. For Eltrombopag sample injection results were 1,674,925 and 0.7%. Results of intermediate precision study in terms of average area of peak and %RSD for Eltrombopag standard injections were 1,668,277 and 0.6%. For Eltrombopag sample injection results were 1674,058 and 0.7%
Tables 10 and 11 narrate precision and intermediate results in details.

Linearity

Results of linearity test revealed that mean Y intercept value, slope value, and value of correlation coefficient for Eltrombopag were −9493.3632, 33463.9802x, 1.0000 at the concentration range of 3.516 μg/mL–131.862 μg/mL

ROBUSTNESS RESULTS

Robustness study is performed by analyzing the standard and sample at different conditions. The results obtained with altered conditions are compared against results obtained under normal chromatographic conditions. Altered condition includes flow rate variation in column temperature, organic ratio, and change in buffer Ph.

CONCLUSION

Newly, developed method is cost effective, precise, accurate, linear, robust, selective, and specific. Therefore, above newly developed analytical method is suitable for the evaluation of bulk and tablet formulation of Eltrombopag in laboratory analysis.

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