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DEVELOPMENT AND VALIDATION OF STABILTY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ELTROMBOPAG AND ROMIPLOSTIM IN BULK AND TABLET DOSAGE FORM

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Abstract:

A simple, economical, sensitive and reproducible stability indicating RP-HPLC method for the simultaneous estimation of Eltrombopag and Romiplostim in bulk and tablet dosage form has been developed and validated. Chromatographic isocratic separation was carried out on Symmetry ODS C18 (4.6 x 150 mm, 5µ particle size) column using a mobile phase composed of phosphate buffer: acetonitrile (adjusted to pH 2.5 with 0.1 % OPA) in the ratio of 20:80 % v/v at a flow rate of 0.8 ml/min. The analyte was monitored using UV detector wavelength at 274 nm at ambient column temperature. The retention time was found to be 2.003 min and 5.067 min for Eltrombopag and Romiplostim respectively. The proposed method was found to be linear in the concentration range of 10-50 µg/ml for Eltrombopag and 25-125 µg/ml for Romiplostim with correlation coefficient value of 0.999 respectively. The mean % recoveries obtained were found to be 99.6–99.7% for Eltrombopag and 99.7–99.8% for Romiplostim respectively. Stress testing which covered acid, alkali, peroxide, photolytic and thermal degradation was performed on under test to prove the specificity of the method and the degradation was achieved. The developed method has been statistically validated according to ICH guide lines and found to be linear, precise, accurate and robust. The proposed method can be successfully applied for the stability indicating RP-HPLC simultaneous estimation of Eltrombopag and Romiplostim in bulk and tablet dosage form and in routine quality control analysis.

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INTRODUCTION:

Introduction to Analytical Chemistry

Analytical chemistry is often described as the area of chemistry responsible for characterizing the composition of matter, both qualitatively (what is present) and quantitatively (how much is present). Analytical chemistry is not a separate branch of chemistry, but simply the application of chemical knowledge.

Pharmaceutical Analysis

Pharmaceutical Analysis is the branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter. It is mainly involved in the qualitative identification or detection of compounds and quantitative measurements of the substances present in bulk and pharmaceutical preparation.

The technique employed in quantitative analysis is based upon the quantitative performance of suitable chemical reactions and either measuring the amount of reagent needed to complete the reaction, or ascertaining the amount of reaction product obtained.

Introduction to Chromatography

Chromatography was originally developed by the Russian botanist Michael Tswett in 1903 for the separation of colored plant pigments by percolating a petroleum ether extract through a glass column packed with powdered calcium carbonate. It is now, in general, the most widely used separation technique in analytical chemistry having developed into a number of related but quite different forms that enable the components of complex mixtures of organic or inorganic components to be separated and quantified. A chromatographic separation involves the placing of a sample onto a liquid or solid stationary phase and passing a liquid or gaseous mobile phase through or over it, a process known as elution. Sample components, or solutes, whose distribution ratios between the two phases differ will migrate (be eluted) at different rates, and this differential rate of migration will lead to their separation over a period of time and distance.

Eltrombopag (rINN, codenamed SB-497115-GR) is a medication that has been developed for certain conditions that lead to thrombocytopenia (abnormally low platelet counts). It is a small molecule agonist of the c-mpl (TpoR) receptor, which is the physiological target of the hormone thrombopoietin Romiplostim is a fusion protein analog of thrombopoietin, a hormone that regulates platelet production

MATERIALS AND METHOD AND INSTRUMENTATION:

HPLC-auto sampler –UV detector, Separation module2695, UV.detector2487, Empower-software version-2, Waters, U.V double beam spectrometer, UV 3000+, U.V win soft ware, Lab India.

Eltrombopag and Romiplostim, KH_2PO_4, Water and Methanol for HPLC, Acetonitrile for HPLC, HCL, H_2O_2, NaOH.

CHROMATOGRAPHIC CONDITIONS

Trial-5

Optimized chromatogram is obtained by following conditions

Mode of operation	:	Isocratic
Column	:	Symmetry C18 (4.6 x 150mm, 5µm, Make: XTerra) or equivalent
Buffer pH	:	2.5
Mobile phase	:	20% buffer 80% acetonitrile
Flow rate	:	0.8 ml per min
Wavelength	:	274 nm
Temperature	:	ambient.
Run time	:	7min.

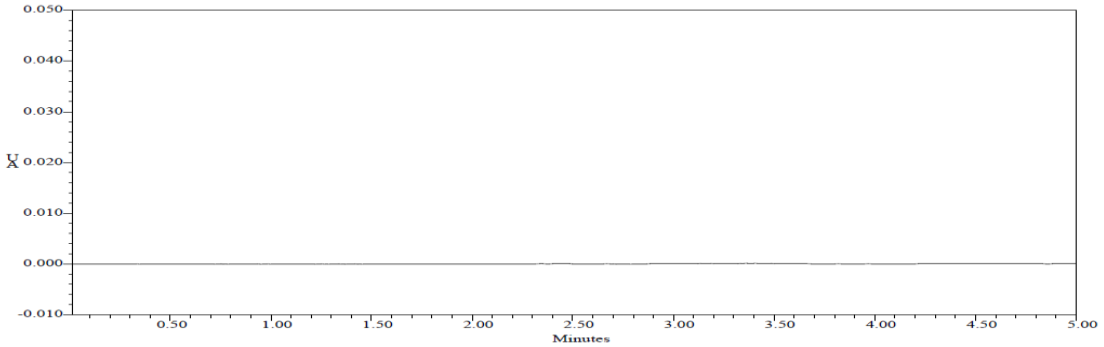

Figure 1 Chromatogram for blank

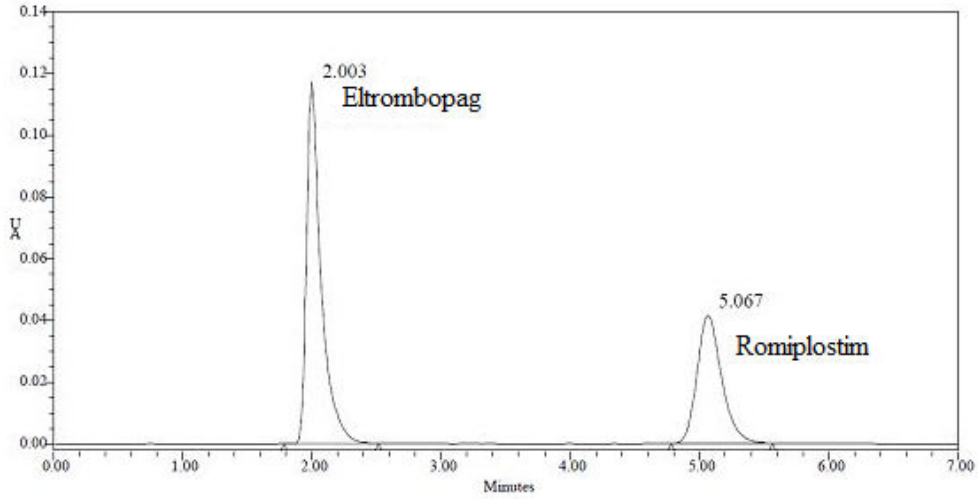

Figure no 2 Chromatogram for Romiplostim and Eltrombopag

Table 1 DetailsofTrial-5

S.No	Peakname	R_t	Area	Height	USPPlate count	USP Tailing	USP Resolution
1	Eltrombopag	2.003	4757682	785743	2342	1.13	
2	Romiplostim	5.067	6331252	126622	2413	1.14	1.41

Observation: It was observed that the Romiplostim and Eltrombopag peaks are well separated with less retention time, less tailing and more plate count with good peak shape.

VALIDATION REPORT

SYSTEM SUITABILITY

Table 2 Results of system suitability parameters for Romiplostim and Eltrombopag

S. No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Eltrombopag	2.003	920101	116666	11.0	1.6	2711.8
2	Eltrombopag	2.653	952058	41531	11.0	1.3	3428.2
3	Eltrombopag	2.655	954824	41257	12.0	1.2	5426.3
4	Eltrombopag	2.657	930202	21543	11.1	1.1	2451.6
5	Eltrombopag	2.665	901021	22451	11.3	1.2	2411.7
Mean		2.526	931641.2				
Std. Dev		0.0292	12511.84				
% RSD		1.155	1.3429				

S. No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Romiplostim	5.067	920101	116666		1.5	2311.8
2	Romiplostim	5.075	950538	61531	11.0	1.3	3448.2
3	Romiplostim	5.427	955824	41287	12.0	1.1	4426.3
4	Romiplostim	5.245	930802	71543	12.1	1.1	4451.6
5	Romiplostim	5.263	991021	23451	11.3	1.2	2411.6
Mean		5.215	949657.2				
Std. Dev		0.01497	11289.451				
% RSD		0.287	1.1887				

Specificity

Table 3 Details of Standard Injection

	Peak Name	RT	Area	Height	USP Plate	USP Taililg
1	Eltrombopag	2.318	1333112	164078	2114.9	1.7
2	Eltrombopag	2.379	1355521	164511	2127.0	1.7
3	Romiplostim	5.535	44873	2931.4		1.7
4	Romiplostim	5.749	41056	2697.1		1.7

Linearity

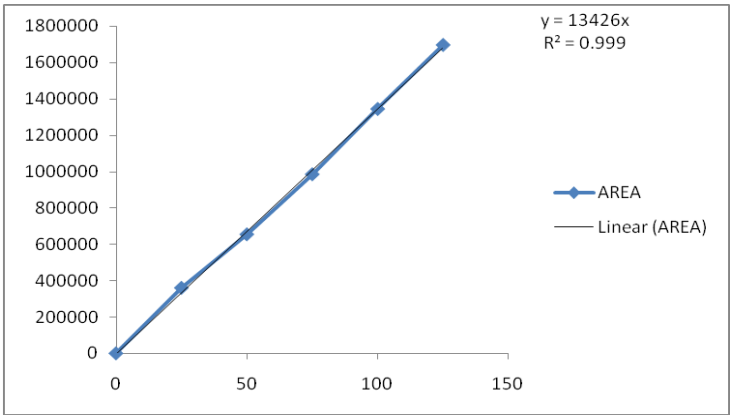

Figure 3 Calibration graph for Romiplostim at 274 nm

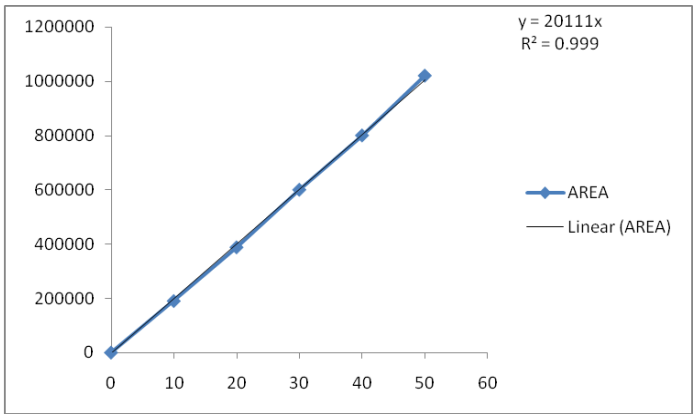

Figure 4 Calibration graph for Eltrombopag at 274 nm

Table 4 Analytical performance parameters of Romiplostim and Eltrombopag

Parameters	Romiplostim	Eltrombopag
Slope (m)	13644	8192
Intercept (c)	24221	14308
Correlation coefficient (R^2)	0.999	0.999

Accuracy

Table 5 DetailsofAccuracy 50 %

	Peak Name	RT	Area	Height
1	Eltrombopag	2.003	702873	86026
2	Eltrombopag	2.005	704987	85549
3	Eltrombopag	2.003	702008	84196
Mean		2.003	703289	
Std.Dev		0.001154	1532.51	
%RSD		0.05611	0.2179	

	Peak Name	RT	Area	Height
1	Romiplostim	5.065	239401	21744
2	Romiplostim	5.062	239865	21909
3	Romiplostim	5.065	239948	21382
Mean		5.064	239738	
Std.Dev		0.001732	294.786	
%RSD		0.03420	0.12296	

Table 6 DetailsofAccuracy100%

	Peak Name	RT	Area	Height
1	Eltrombopag	2.004	702873	86026
2	Eltrombopag	2.004	704997	85549
3	Eltrombopag	2.005	702108	84196
Mean		2.004	703326	
Std.Dev		0.000577	1496.82	
%RSD		0.028792	0.21282	

	Peak Name	RT	Area	Height
1	Romiplostim	5.067	239411	21744
2	Romiplostim	5.067	239855	21909
3	Romiplostim	5.062	239848	21382
Mean		5.065	239704.6	
Std.Dev		0.00288	254.346	
%RSD		0.05686	0.10610	

Table 7 DetailsofAccuracy150%

	Peak Name	**RT**	**Area**	**Height**
1	Eltrombopag	2.003	702973	86026
2	Eltrombopag	2.004	704977	85549
3	Eltrombopag	2.003	702008	84196
Mean		2.003	703319	
Std.Dev		0.000577	1514.496	
%RSD		0.02880	1.0684	

	Peak Name	**RT**	**Area**	**Height**
1	Romiplostim	5.057	239401	21744
2	Romiplostim	5.067	239865	21909
3	Romiplostim	5.057	239948	21382
Mean		5.060	239738	
Std.Dev		0.00577	294.786	
%RSD		0.11403	0.122961	

Table 8 Results of Accuracy

Sample concentration	Sample set no	Sample area		Amount added(amt found)		% Recovery	
		Eltrombopag	Romiplostim	Eltrombopag	Romiplostim	Eltrombopag	Romiplostim
50%	1	460064	276931	50 (49.8)	20 (20)	99.8	100
	2	460124	276694	50 (49.6)	20 (19.6)	99.6	99.6
	3	460216	276891	50 (49.8)	20 (19.6)	99.8	99.6
	Average Recovery					99.7%	99.7%
100%	1	923429	554156	100 (99.8)	40 (40)	99.8	100
	2	923654	554897	100 (99.6)	40 (39.8)	99.6	99.8
	3	923742	556371	100 (99.6)	40 (39.8)	99.6	99.8
	Average recovery					99.6%	99.8%
150%	1	1387901	828113	150 (149.8)	60 (60)	99.8	100
	2	1385360	828794	150 (149.8)	60 (59.8)	99.8	99.8
	3	1386984	828349	150 (149.6)	60 (59.8)	99.6	99.8
	Average recovery					99.7%	99.8%

Precision

Table 9 RepeatabilityresultsofEltrombopag and Romiplostim

	Peak name	RT	Area	Height
1	Eltrombopag	2.006	1313235	163051
2	Eltrombopag	2.005	1326776	162363
3	Eltrombopag	2.013	1347962	163866
4	Eltrombopag	2.007	1368872	163893
5	Eltrombopag	2.008	1363598	161294
Mean		2.007	1344088	
Std.Dev		0.003114	23777.655	
%RSD		0.155156	1.769050	

	Peak name	RT	Area	Height
1	Romiplostim	5.116	458218	46160
2	Romiplostim	5.124	452495	45294
3	Romiplostim	5.143	453221	44163
4	Romiplostim	5.136	457145	43079
5	Romiplostim	5.139	458898	43930
Mean		5.1316	455995.4	
Std.Dev		0.011238	2942.648	
%RSD		0.21899	0.64532	

Intermediate Precession (Ruggedness)

Table 10 Ruggednessresults

	Peak Name	RT	Area	Height
1	Eltrombopag	2.003	1366825	164933
2	Eltrombopag	2.002	1379095	163608
3	Eltrombopag	2.003	1375825	164628
4	Eltrombopag	2.003	1364299	164510
5	Eltrombopag	2.003	1395271	163964
6	Eltrombopag	2.003	1393763	166747
Mean		2.002	1379179	
Std.Dev		0.000408	13091.505	
%RSd		0.02037	0.9492245	

	Peak Name	RT	Area	Height
1	Romiplostim	5.054	484545	41393
2	Romiplostim	5.055	484511	40825
3	Romiplostim	5.053	480804	40865
4	Romiplostim	5.056	485023	40309
5	Romiplostim	5.056	484952	40213
6	Romiplostim	5.057	485203	41640
Mean		5.055	484173	
Std.Dev		0.001471	1672.903	
%RSd		0.029099	0.345517	

Limit of Detection for Romiplostim and Eltrombopag

Table 11 Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Romiplostim	56	176	3.14
Eltrombopag	56	154	2.75

Limit of Quantitation for Romiplostimin and Eltrombopag

Table 12 results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Romiplostim	56	563	10.05
Eltrombopag	56	558	9.96

Robustness

Table 13 DetailsofRobutnesslessflow

Peak name	RT	Area	USP Plate Count	USP Tailing
Eltrombopag	2.279	1690740	2158.1	1.8
Romiplostim	5.766	519208	3536.2	1.7

Table 14 DetailsofRobutnessmoreflow

Peak name	RT	Area	Height	USP Plate Count	USP Tailing
Etrombopag	2.789	1150303	165118	2069.9	1.7
Romiplostim	5.456	402322	43574	2713.8	1.7

Variation of mobile phase composition

Table 15 DetailsofRobutnessless organic composition

Peak name	RT	Area	Height	USP Plate Count	USP Tailing
Eltrombopag	2.171	1404976	159808	2910.4	1.8
Romiplostim	5.446	453297	27049	2840.1	1.7

Table 16 DetailsofRobutnessmore organic composition

Peak name	RT	Area	Height	USP Plate Count	USP Tailing
Eltrombopag	2.033	1378798	171546	2358.0	1.7
Romiplostim	5.067	499679	50843	2616.1	1.6

Assay

Table 17 Details of Eltrombopag and Romiplostim (Sample)

	NAME	**RT**	**AREA**	**HEIGHT**
1	Eltrombopag	2.004	1345687	1682364
2	Eltrombopag	2.005	1366422	1658232
Mean		2.004	1356054	
Std. Dev.		0.000707	14661.85	
% RSD		0.03527	1.08121	

	NAME	**RT**	**AREA**	**HEIGHT**
1	Romiplostim	5.068	466772	35426
2	Romiplostim	5.069	436124	34465
Mean		5.068	451448	
Std. Dev.		0.00070	2999.40	
% RSD		0.0138	0.6643	

	NAME	RT	AREA	HEIGHT
1	Eltrombopag	2.006	1345687	1682864
2	Eltrombopag	2.007	1365423	1658232
Mean		2.006	1355555	
Std. Dev.		0.000707	13955.459	
% RSD		0.0352	0.3204	

	NAME	RT	AREA	HEIGHT
1	Romiplostim	5.070	446272	36425
2	Romiplostim	5.071	436124	32467
Mean		5.070	441198	
Std. Dev.		0.000707	7175.71	
% RSD		0.013944	1.62641	

Table 18 Details of Eltrombopag and Romiplostim (standard)

S. No	Name of compound	Label claim(mg)	Amount taken(mg)	%purity
1	Eltrombopag	60	60.05	99.96
2	Romiplostim	100	99.35	97.18

	ELTROMBOPAG			ROMIPLOSTIM		
	Standard Area	Sample Area	Assay	Standard Area	Sample Area	Assay
Injection-1	1345687	1345687	100	466772	446272	95.60
Injection-2	1366422	1365423	99.92	436124	436124	100
Average Assay(%purity)			99.96			97.8

Stability Studies

Table no. 19 Results of Stress degradation studies

Stress Condition	Sample-1 (Eltrombopag)			Sample-2 (Romiplostim)		
	Area	%Assay	%Degradation	Area	%Assay	%Degradation
Acidic	120473	91.1	8.7	39575	92.4	8.3
Alkaline	124364	92.0	12.8	348779	81.7	12.8
Photolytic	113269	87.2	13.7	352292	87.4	12.4
Thermal	104474	96.3	14.5	352323	85.4	11.5
Oxidative	106734	94.3	11.2	392423	95.1	11.3

CONCLUSION:

On the basis of experimental results, the proposed method is suitable for the quantitative determination of Eltromobopag and Romiplostim in pharmaceutical dosage form. The method provides great sensitivity, adequate linearity and repeatability. The estimation of Eltromobopag and Romiplostim was done by RP-HPLC. The Phosphate buffer was pH 2.5 and the mobile phase was optimized which consists of Acetonitrile: Phosphate buffer mixed in the ratio of 80:20 % v/ v. A Symmetry C18 (4.6 x 150mm, 5µm, Make XTerra) column used as stationary phase. The

detection was carried out using UV detector at 274 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. The linearity range of Eltromobopag and Romiplostim were found to be from 10-50 µg/ml and 20-125µg/ml respectively. linear regression coefficient was not more than 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 97-102% of Eltromobopag and Romiplostim LOD and LOQ was found to be within limit.The proposed method is precise, simple and accurate to determine the amount of Eltromobopag and Romiplostim in formulation.High percentage of recovery shows that the method is free from the interference of excipients used in the formulation. So the method can be useful in the routine quality control of these drugs.

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