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A LC-MS compatible RP-HPLC method for the determination of ticagrelor in bulk

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ABSTRACT

A simple, rapid, accurate and precise LC-MS Compatible RP-HPLC method was developed and validated for the determination of Ticagrelor in bulk. Separation of the drug was achieved on Unisol C18 column (100 mm x 4.6 mm, 5 µ) as stationary phase with mobile phase consisting of ammonium acetate buffer pH 4.5 and acetonitrile in the ratio of 40: 60 V/V. The method showed a good linear response in the concentration range of 10-50 µg/mL with correlation coefficient of 0.99. The flow rate was maintained at 1.0 mL/min and effluents were monitored at 250 nm. The retention time was 3.88 min. The method was statistically validated for accuracy, precision, linearity, ruggedness, robustness, solution stability, selectivity. The results obtained in the study were within the limits of ICH guidelines and hence this method can be used for the determination of Ticagrelor in pharmaceutical dosage forms.

Keywords: Ticagrelor, LC-MS, HPLC, Validation, ICH guidelines

INTRODUCTION

Introduction [3-4]

Ticagrelor (Fig. 1) is an orally active antiplatelet agent, inhibitor of platelet activation and aggregation mediated by the P2Y12 ADP-receptor. Chemically, it is (1S,2S,3R,5S)-3-[7-{[(1R,2S)-2-(3,4-difluorophenyl) cyclopropyl] amino}-5-(propylthio)-3*H*-[1,2,3]-triazolo[4,5-d]pyrimidin-3-yl]-5- (2-hydroxyethoxycyclo pentane-1,2-diol. A recent study indicated that Ticagrelor reduce the rate of thrombotic cardiovascular events in patients with acute coronary syndrome. Some detailed mechanistic studies pointed out that Ticagrelor and its major metabolite reversibly interact with the platelet P2Y12 ADP-receptor to prevent signal transduction and platelet activation, which inhibits platelet aggregation and thrombus formation in atherosclerotic disease. Literature survey revealed that few HPLC, LC-MS and very few UV spectrophotometric methods have been developed and reported for the estimation of Ticagrelor. However no efforts have been reported for the UV spectrometric method of Ticagrelor in bulk form by using methanol and O-Phosphoric acid as a solvent,

which could be very economic and easily applicable as well. Hence an attempt has been made to develop and establish a novel, simple, rapid and sensitive LC-MS compatible RP-HPLC method in accordance with ICH guidelines for the estimation of Ticagrelor in bulk.

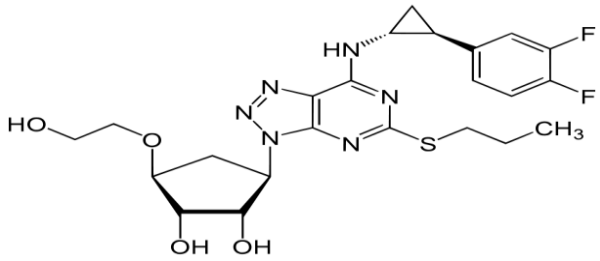

Figure [1]: Structure of Ticagrelor

MATERIALS AND METHODS [1,2- 29]

Instrumentation

To develop a high pressure liquid chromatographic method for quantitative estimation of Ticagrelor using Agilent 1260 infinity series HPLC system on Unisol C18 column (100 mm x 4.6 mm, 5 µ) was used. The instrument is equipped with an auto sampler and PDA detector. A Infinity auto sampler injector port was used for injecting the samples. Data was analyzed by using Open Lab software. Before injecting the samples into HPLC we ultrasonicated by using PCi Analytics Ultrasonicator of model no: 3.5L100 and filtered through Millipore Vaccum filter and 0.22um Millipore filter paper was used for filtration. Weighing of samples and buffer by using METTLER TOLEDO ME204 Balance and pH of the buffers can be adjusted by using EUTECH pH 700 meter.

Chemicals and Solvents

Ticagrelor was obtained as a gift sample from Raks Pharma, Visakhapatnam, India and was used without further purification. All chemicals and reagents used were of HPLC grade in HPLC method. HPLC grade Acetonitrile, Ammonium acetate and HPLC water is procured from Merck Pharmaceuticals Private Ltd., Mumbai, India.

Chromatographic conditions

A mixture of Ammonium acetate buffer pH 4.5 and acetonitrile in the ratio of 40: 60 V/V was found to be the most suitable mobile phase for ideal chromatographic separation of Ticagrelor. The solvent mixture was filtered through 0.22 µ membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1.0 mL/min. Injection volume was 5 µL and the column was maintained at a temperature of 20°C. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of the drug solution. The detection of the drug was monitored at 250 nm. The run time was set at 10 min.

Preparation of Ammonium Acetate buffer pH 4.5

0.77 grams of Ammonium Acetate was weighed and transferred into a 100 mL beaker, dissolved and diluted to 100 mL with HPLC water. pH was adjusted to 4.5 with glacial acetic acid.

Preparation of mobile phase and diluents

400 mL of the Ammonium acetate buffer was mixed with 600 mL of acetonitrile. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.22 µm filter under vacuum. The solvents acetonitrile and Ammonium acetate (60:40v/v) was used as diluent.

Preparation of standard solution

10 mg of Ticagrelor was accurately weighed, transferred to 10 mL volumetric flask and is dissolved in 7 mL of the diluent. Solution was sonicated for the complete dissolving of drug. Then it is filtered through 0.22 µ filter and the volume is made up to 10 mL with diluent to get a concentration of 1 mg/mL stock solution. Further pipetted 0.225 mL of the above stock solution into a 10 mL volumetric flask and diluted up to the

mark with diluent to obtain required concentrations.

Linearity

Several aliquots of standard solution of Ticagrelor was taken in different 10 mL volumetric flasks and diluted up to the mark with diluent such that the final concentrations of Ticagrelor were in the linearity range of 10-50 µg/mL. Evaluation of the drug was performed with UV detector at 250 nm, peak area was recorded for all the peaks. The response for the drug was linear and the regression equation was found to be y = 963770x + 5E+07 and correlation coefficient value of Ticagrelor was found to be 0.999. The results show that an excellent correlation exists between peak area and concentration of drug within the concentration range indicated.

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solution using the developed HPLC method. The LOD and LOQ for Ticagrelor were found to be 1.5µg/mL and 2.5µg/mL respectively.

System suitability

System suitability parameters like peak area, retention time, theoretical plates and tailing factor were calculated and compared with standard values.

Accuracy

The accuracy of the method was assessed by recovery study of Ticagrelor in the dosage form at three concentration levels. A fixed amount of pre analyzed sample was taken and standard drug was added at 50%, 100% and 150% levels. Each level was repeated three times. The content of Ticagrelor per tablet was calculated. The percentage recovery ranges from 98-102% and the mean recovery of Ticagrelor was 99.26% that shows there is no interference from excipients and the lower values of RSD of assay indicate the method is accurate.

Precision

The precision was determined for Ticagrelor in terms of intra-day and inter-day precision. For intra-day precision evaluation, a standard solution of fixed concentration was injected at various time intervals and %RSD for Ticagrelor was 0.06% (limit %RSD < 2.0%). In addition, the inter-day precision was studied by injecting the same concentration of standard solution on consecutive days and the %RSD for Ticagrelor was 0.01% (limit %RSD < 2.0%).

Ruggedness and Robustness

My method was not performed by any one so ruggedness will not be carried out. Robustness of the method was determined by making slight changes in the chromatographic conditions like changes in flow rate, mobile phase composition and we also checked by making the changes in pH of the buffer. It was observed that there were no marked changes in the chromatograms, which demonstrated that the HPLC method so developed is rugged and robust.

Solution stability

The stability of solution under study was established by keeping the solution at room temperature for fresh, 12, 24, 36, 48 hrs. The result showed no significant change in concentration and thus confirms the stability of the drug in the solvent used for the analysis.

RESULTS AND DISCUSSION

System Suitability studies

Table No. [1]: System suitability parameters

Injection number	Peak area	Theoretical plates	Tailing factor	Retention time
1	8876512	8478	1.05	3.88
2	8876513	8481	1.04	3.883
3	8876515	8484	103	3.83
4	8876518	8468	1.05	3.85
5	8876528	8469	1.05	3.87
6	8876530	8494	1.04	3.88

Mean	8876519	8479	1.043	3.865
%RSD	0.01	0.04	0.78	0.20
Acceptance criteria	NMT 2.0	NLT 2000	NMT 2.0	-

The obtained experimental values in system suitability trials (n=6) were found to be within the limits proposed by ICH guidelines.

Linearity

Table No. [2]: Linearity of detector response

Concentration(µg/ml)	Area
10	56325960
20	66430159
30	75965405
40	88156512
50	93651260
Correlation coefficient	0.99
Slope(m)	963770
Intercept(c)	5E+07

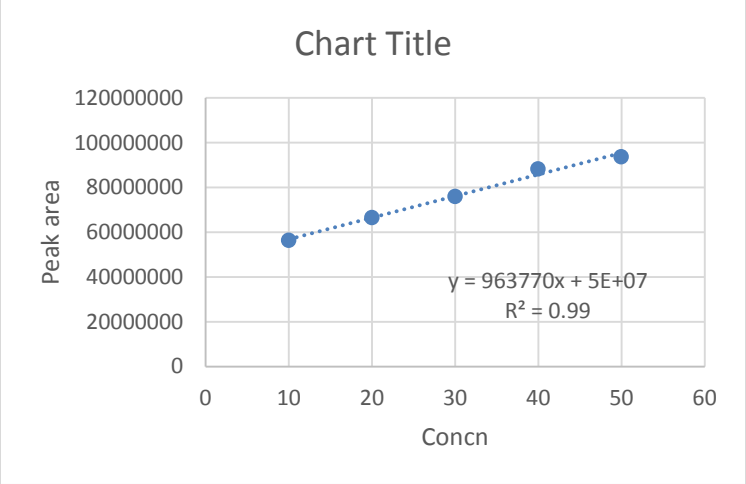

Figure No [2]: Linearity chart

The response was found to be linear and the correlation coefficient was found to be 0.99.

ACCURACY

Table No.[3]: Results for accuracy in HPLC

S.NO	% Level	Standard amount	Spiked amount	Amount found	% Recovery	Mean Recovery
1.	50 %	20	10	29.81	99.68	Mean=99.26
		20	10	29.65	99.41	SD = 0.5006 %RSD=0.50
		20	10	9.23	98.71	
2.	100 %	20	20	38.97	98.71	Mean =99. 20
		20	20	9.51	99.38	SD = 0.436 %RSD=0. 44
		20	20	39.63	99.53	

3.	150 %	20	30	9.13	99.13	Mean =99. 03
		20	30	8.96	98.96	SD =0. 087
		20	30	49.01	99.01	%RSD=0. 09

The results represent the high percent recovery values indicating that the proposed method is accurate.

Precision

Table No. [4]: Results intraday and interday in HPLC

S.No	Intraday peak area	Interday peak area
1	56325960	56326960
2	56345960	56345960
3	56335960	55335860
4	56385960	56485950
5	56425960	56385960
6	57325960	57385960
%RSD	0.06	0.01

The % RSD for Intraday precision and interday precision for Ticagrelor were found to be 0.06 and 0.01 which indicates the method is precise.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD value was found at 1.5 µg/ml concentration where the signal to noise ratio was 3:1 and the LOQ value was found at 2.5 µg/ml where the signal to noise ratio was 10:1.

ROBUSTNESS

Table No [5]: Results for Robustness

Parameter	Conditions	%RSD (NMT 2.0)	Tailing factor (NMT 2.0)	Theoretical plates (NLT 2000)
Flow Rate	0.8	0.01	1.03	5033
	1.0	0.08	0.99	5466
	1.2	0.06	1.07	5296
Buffer pH	4.5	0.02	1.2	5466
	5.0	0.07	0.9	4586
	5.5	0.01	1.0	4485
Mobile phase composition	50:50	0.02	0.9	5399
	60:40	0.01	1.07	5466
	65:35	0.44	1.02	5245

All the experimental values for robustness obtained fall into the acceptance criteria.

SOLUTION STABILITY STUDIES

Table No [6]: Results for solution stability in HPLC

S. No	Sampling time	Area obtained	Amount found	% Assay
1	Standard (fresh solution)	88156512	40	100
2	12 hrs	88156511	39.98	99.95
3	24 hrs	88156512	39.97	99.925
4	36 hrs	88156511	39.98	99.95
5	48 hrs	88156510	39.99	99.97

During study of the stability of the stored solutions of standard preparation for assay determination, the solutions were found to be stable for upto 48 hrs. Assay values obtained for the

above solutions were identical with the initial value without measurable loss.

Specificity

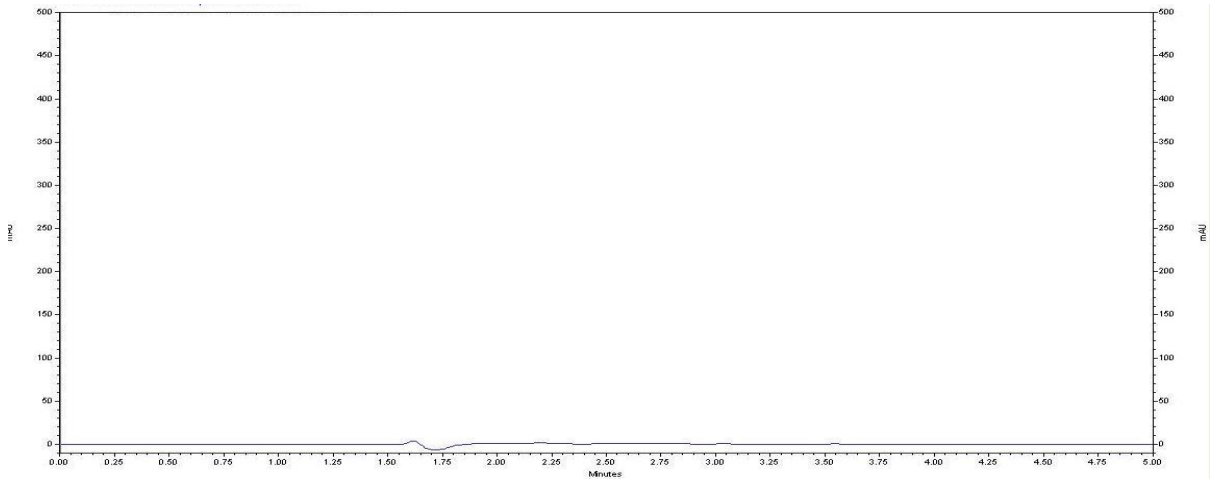

Fig.2 Blank chromatogram of Ticagrelor

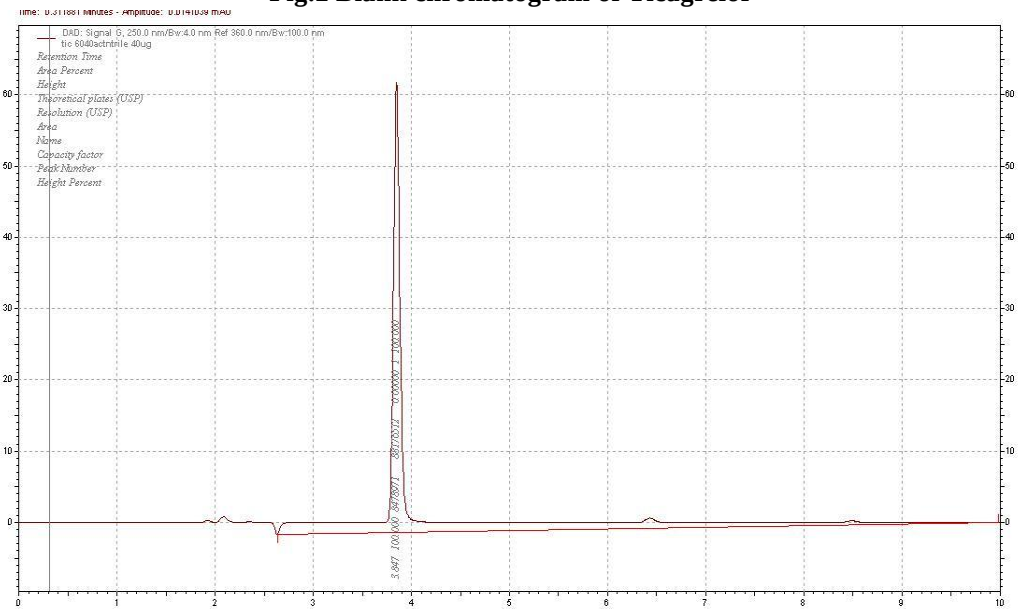

Fig.3 Standard chromatogram of Ticagrelor

Chromatograms of standard and blank were recorded and chromatogram of blank did not show any peak at the retention time of analyte. This shows that the method is specific.

CONCLUSION

A simple, precise, economic, accurate, robust and LC-MS compatible Reverse phase High Performance Liquid Chromatographic method was developed for the analysis of Ticagrelor. The initial trail was conducted with 20 minutes run time using Unisol reverse phase C18 column (150× 4.6 mm, 3µm, 20cm) at room temperature conditions, 1ml/min flow rate and isocratic elution mode. The detection wavelength was fixed by scanning the working standard solution and noting the maximum absorbance wavelength which was found to be 250 nm and the mobile phase composed of Acetonitrile: Ammonium acetate buffer (60:40).

The retention time was found at 3.88 mins. The calibration curve was linear with correlation coefficient of 0.999 over a concentration range of 10-50 µg/ml with linear regression equation y = 923770x + 5E+07. The limit of detection and limit of quantification were found at 1.5µg/ml and 2.5µg/ml respectively indicating the sensitivity of the method. Stability of the drug solution was

checked for a period of 48 hrs and from the results it was found that the drug solution is stable without undergoing degradation. The proposed method has been validated according to the ICH guidelines and can be successfully applied to estimate the levels of Ticagrelor in bulk form.

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