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ESTIMATION OF TICAGRELOR IN COMMERCIAL DOSAGE FORM USING A SENSITIVE VALIDATED RP- HPLC METHOD

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ABSTRACT

Objectives: The main purpose of this study was to develop a simple, precise, accurate RP-HPLC method for estimation of Ticagrelor HCl in bulk and formulation. **Materials and Methods:** A gift sample of Ticagrelor HCL with an assay value of 99.2% w/w was obtained as a gift sample. Methanol and water used are of HPLC grade. Ticagrelor HCl tablets of 90mg with a brand name **Brilinta**® were purchased from local market. An Enable C18 column (250 X 4.6mm, 5µm particle size), flow rate of 1mL/min and a detection wave length 255nm was used for separation. **Results:** The mobile phase consisted of Methanol and water in the ratio (90:10, v/v). Retention time was found be 3.414 min. At λ_{max} 255nm the method was found to be linear in the range of 5-30µg/mL with correlation coefficient (R^2=0.9984) and regression equation was found to be y=18511x+357545. The percentage recovery values ranged from 99.44%-100.67%. Validation parameters were all validated according to ICH guidelines. **Conclusion:** This method can be successfully applied for estimation of Ticagrelor HCl inbulk and formulation.

KEYWORDS: Ticagrelor HCl, Validation, RP-HPLC, Method development, Precision.

INTRODUCTION

Ticagrelor is chemically (1*S*,2*S*)-3-[7-[[(1*R*,2*S*)-2-(3,4-difluorophenyl) cyclopropyl] amino]-5-propyl sulfanyl triazolo [4,5-d] pyrimidin-3-yl] -5-(2-hydroxyethoxy) cyclopentane-1, 2diol [4];hydrochloride[1] It is an oral antiplatelet[2-3] drug that inhibits platelet aggregation, myocardial infarction and thrombus formation in atherosclerotic disease and reduces chances of cardiac arrest due to blockage. Literature review[4-8] for Ticagrelor revealed very few

methods based on varies techniques like UV Spectroscopy, LC-MS and HPLC for the estimation of Ticagrelor either in pharmaceutical formulation or in biological fluid. However only a few HPLC methodsare available for estimation of Ticagrelor, in this work the author developed a new HPLC method for estimation of ticagrelor.

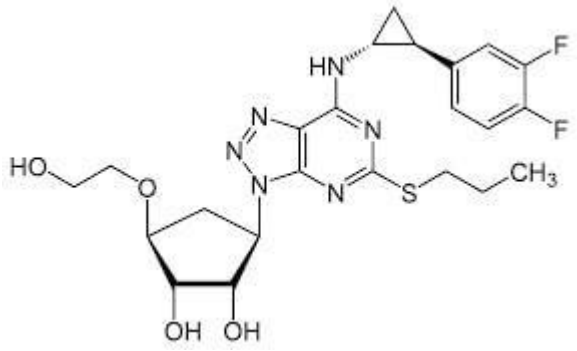

Figure 1: Structure of ticagrelor.

MATERIALS AND METHODS

Instruments and Materials

A gift sample of Ticagrelor HCL with an assay value of 99.2% w/w was obtained as a gift sample. ELITE analytical balance and Shimadzu LC- 20AD with binary pump, variable wavelength programable SPD- 20 detector was used. Rheodyne injector with a 20µL loop wasused and data was recorded using LC Solution software. Methanol and water used are of HPLC grade. Ticagrelor HCl tablets of 90mg with a brand name Brilinta® were purchased from localmarket.

Chromatographic conditions

An Enable C18 column (250 X 4.6mm, 5µm particle size), flow rate of 1mL/min and a detection wave length 255nm was used for separation. The mobile phase consisted of Methanol and water in the ratio (90:10, v/v). The mobile phase was filtered through Nylon 0.45µm, 47mm membrane filter and was degassed before use. The injection volume was 20µL and column temperature was maintained at ambient temperature. The column was equilibratedby pumping the mobile phase for at least 30min prior to sample injection. The wavelength of UV detector was set at 255nm.

Preparation of mobile phase

A mixture of Methanol and water in the ratio of 90:10(v/v) was prepared and sonicated for 30 minutes to remove gases.

Preparation of standard stock solution

A standard drug solution of ticagrelor HCl was prepared by adding 100mg of drug into a 100mL volumetric flask and made up to mark with ethanol to get a concentration of 1000µg/mL.

Preparation of working standard solution

From the above standard stock solution 10mL of sample was transferred to 100mL volumetric flask and made up to mark with ethanol to get a concentration of 100µg/mL.

Preparation of the sample solution

10 Tablets were weighed and powdered. The amount of powder equivalent to 100mg was weighed and transferred to a volumetric flask and dissolved in 100mL ethanol to get a concentration of 1000µg/mL. The solution was then subjected to filtration using Whattman filterpaper #44 From the above filtrate 0.2mL was transferred to a 10mL volumetric flask and made up to volume to get 20µg/mL solution and this solution was analyzed by UV VIS detector and chromatogram was recorded at 255nm.

Table 1: Optimized chromatographic conditions.

HPLC Instrument	Shimadzu LC solutions Software UV-Visible detector SPD-20A
UV-Visible spectrophotometer	Lab India (T60)
Column	Enable C18 Column (250 X 4.6mm 5 µm particle size)
Mobile phase	Methanol: Water (90:10)
Flow rate	1.0mL/min
Detection wavelength	255nm
Run time	7min
Retention time	3.414min

Method validation

Validation is the process of establishing documentary evidence demonstrating that a procedure, process, or activity carried produces results within predetermined specifications.[9] Parameters like Range, Linearity, Accuracy, Precision, Robustness, Ruggedness, Limit of Detection (LOD), Limit of Quantification (LOQ) were validated as per ICH guidelines.[10-12]

Linearity

The ability of an analytical procedure to produce test results that are directly proportional to concentration of analyte in the sample. Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration. For estimation of linearity at least 5 concentrations are required.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the valuewhich is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy is assessed by using 9 determinations covering a minimum of 3 concentrations. It is expressed in terms of % recovery which should be in between 98-102%.

Precision

The closeness of agreement between the obtained values by analyzing the same sample for multiple times under prescribed conditions.[13] There are 3 levels repeatability, intermediate precision, reproducibility (inter day precision).

Repeatability is a measure of the exactness under the same working conditions more than a short interim of time, that is, under ordinary working states of the scientific technique with thesame hardware.[14] It is also known as intraday precision.[15-16]

Precision is expressed in terms of % Relative Standard Deviation, which should be less than 2%.

Ruggedness

Ruggedness of an analytical procedure is the degree of reproducibility of results by analyzing same sample under variety of conditions like laboratories, instruments, analysts, reagents etc.

Robustness

Robustness of an analytical procedure is capacity to remain unchanged by small but deliberatechanges in parameters.

Sensitivity

Limit of detection (LOD) and Limit of quantification (LOQ) of the drug were calculated byusing equations according to ICH guidelines.

Limit of detection: It is the lowest amount of the drug in a sample that can be detected, but not necessarily quantitated.

$$LOD = \frac{3.3\times\sigma}{S}$$ Where =, s= Standard deviation

Limit of Quantification: It is an amount of analyte that can be quantitated with specified limitof accuracy and precision.

$$LOQ = \frac{10\times\sigma}{S}$$

System suitability

System suitability tests are an integral part of HPLC which were carried out to verify that the analytical system was working properly and could give accurate and precise result. Allow HPLC system to stable for 30 min and then inject six replicates of standard solution and chromatograms were recorded. %RSD of obtained 6 peak areas was calculated.

Table 2: System suitability data.

Parameters	Ticagrelor
Tailing factor	1.082
Retention time	3.209
Peak area	723911
Theoretical plates	16398

Specificity

Specificity is the ability to assess accurately the analyte in the presence of components which may be expected to be present in the sample matrix such as impurities or excipients. There should not be any interference of the diluents or placebo at retention time of drug substances.

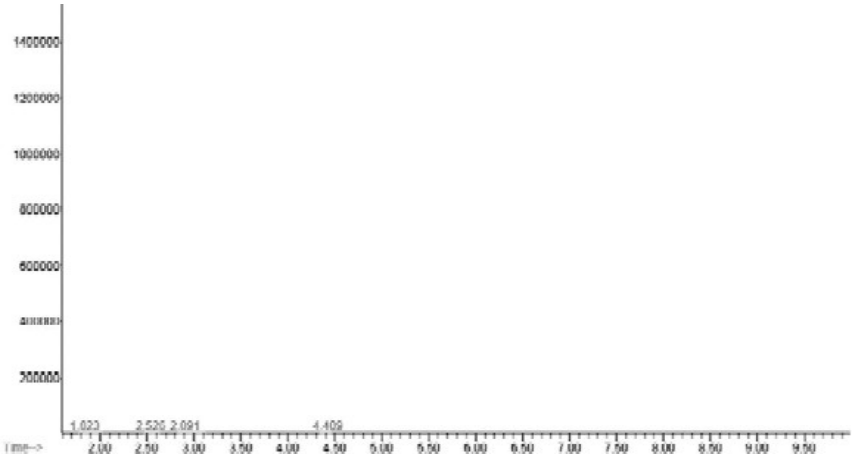

Figure 2: Chromatogram of blank.

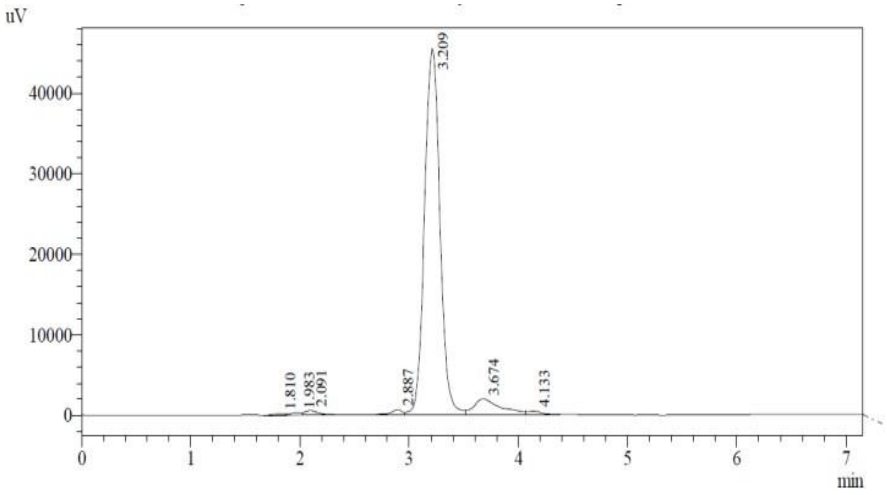

Figure 3: Chromatogram of standard.

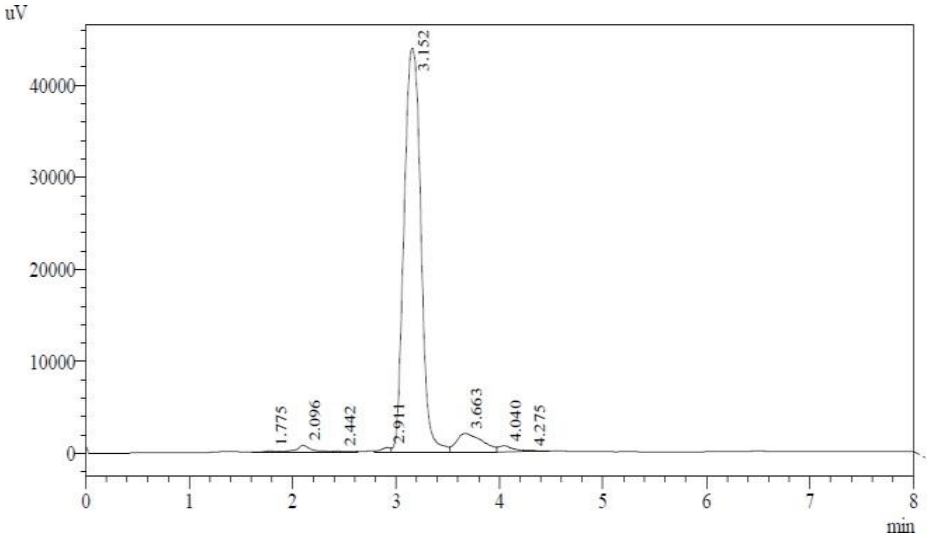

Figure 4: Chromatogram of sample.

Table 3: Specificity results of standard and sample.

Parameters	Standard	Sample
Retention time	3.209	3.152
Peak area	723911	709538
Theoretical plates	16398	14501
Tailing factor	1.082	1.029

Linearity

To test linearity series of solutions ranging from 5µg/mL-30µg/mL were prepared from standard stock solution and analyzed. Linearity was then evaluated by linear regression analysis. The linearity was shown in [Table 4].

Table 4: Linearity of working solutions.

S. No	Concentration (µg/mL)	Absorbance
1.	5	445010
2.	10	544673
3.	15	640084
4.	20	723911
5.	25	830742
6.	30	904480

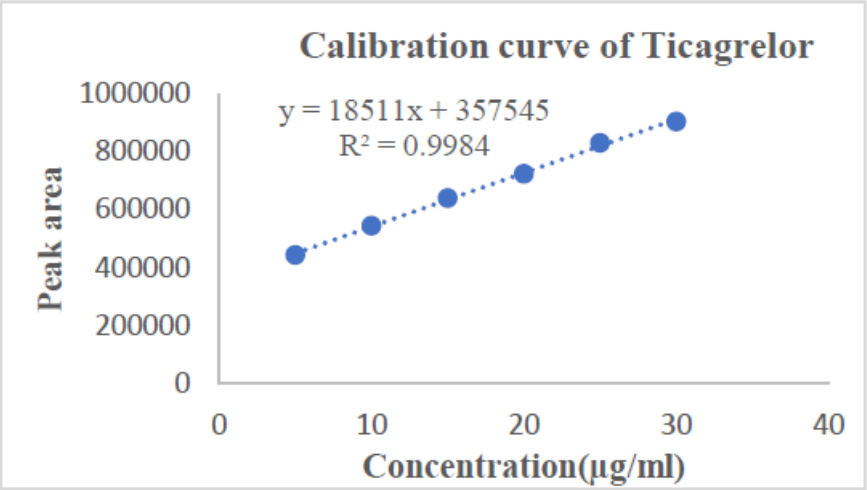

Figure 5: Calibration curve.

Accuracy

The accuracy of method was determined by preparing solutions of concentrations 80%, 100%, 120% in which the amount of marketed formulation **Brilinta®** to be added is kept constant that is 20µg/mL and the amount of pure drug to be added varied from 16µg/mL, 20µg/mL, 24µg/mL for 80,100, 120% respectively. The solutions for prepared in triplicate and accuracy was indicated by %RSD [Table 5].

Table 5: Accuracy data.

Ingredient	Level of addition (%)	Tablet amount (µg/mL)	Amount added (µg/mL)	Drug found (µg/mL)	% Recovery	Avg recovery ± (SD) %
Ticagrelor HCl	80	20	16	15.94	99.62	99.56% ± 0.311
	100	20	20	19.9	99.50	
	120	20	24	23.9	99.58	

Precision

In precision interday studies are carried out for solutions of concentrations (15µg/mL, 20µg/mL,25 µg/mL) They are analyzed 6times a day for 2 consecutive days and the peak areas were noted[Table 6- Table 8]. The results were indicated by %RSD.

Table 6: Interday precision (20µg/mL).

Concentration (µg/mL)	Peak Area (Day-1)	Peak Area (Day-2)
15	630742	652798
	632083	641704
	636810	648064
	639373	635251
	634342	648666
	635532	640084
	Avg: 634813.7 **SD:** 3147.89 **%RSD:** 0.497%	**Avg:** 644427.8 **SD:** 6507.62 **%RSD:** 1.009%

Table 7: Interday precision (15µg/mL)

Concentration (µg/mL)	Peak Area (Day-1)	Peak Area (Day-2)
20	723911	731824
	751924	745124
	745010	761121
	743390	743390
	744673	741479
	747655	756801
	Avg: 737760.5 **SD:** 11597.7 **%RSD:** 1.308%	**Avg:** 746623.2 **SD:** 10695.3 **%RSD:** 1.432%

Table 8: Interday precision (25µg/mL).

Concentration (µg/mL)	Peak Area (Day-1)	Peak Area (Day-2)
25	830742	828082
	832083	825713
	832373	826689
	841699	818677
	841432	818702
	841244	819849
	Avg: 836595.5 **SD**: 5357.26 **%RSD**: 0.640%	**Avg:** 822952 **SD**: 4332.942 **%RSD**: 0.526%

Ruggedness

The ruggedness of the method was carried out by analyzing the same sample using two different analysts, instruments, laboratories and environmental conditions etc. In this method ruggedness was carried out by analyzing the sample using two different analysts and respective chromatograms were recorded. The results are indicated in [Table 9].

Table 9: Ruggedness data.

Concentration (µg/mL)	Analyst 1		Analyst 2	
	Peak Area	**Statistical analysis**	**Peak Area**	**Statistical analysis**
20	723912	**Avg:** 737761.5 **%RSD**: 1.572%	731824	**Avg:** 746623.2 **%RSD**: 1.432%
	751925		745124	
	745011		761121	
	743391		743390	
	744674		741479	
	747656		756801	

Robustness

The **robustness** of an analytical method is measurement of its capability to remain unaffected by small but deliberate variations. Here it was carried out by analyzing the sample using two different wavelengths (±1 of lambda max) and also two flowrates that were (±1 of actual flow rate). The results are indicated in [Table 10].

Table 10: Robustness data.

Parameters		Theoretical Plates	Tailing Factor
Flow rate	**0.9**	10605.562	1.09
	1.1	10260.057	1.07
Wave length	**254**	6617.603	1.15
	256	10695.966	1.09

Sensitivity

Limit of detection (LOD) and Limit of quantification (LOQ) of the drug were calculated byusing equations according to ICH guidelines. Results are shown in [Table 11].

Table 11: LOD & LOQ.

Limit of Detection	Limit of Quantification
0.561µg/mL	0.924 µg/mL

RESULTS AND DISCUSSION

Proposed RP HPLC method was established for determination of Ticagrelor in bulk form as well as dosage form, which was developed and completely validated as per ICH guidelines. Todecide the detection wavelength scanning of the standard solution was performed over a range of 200- 400nm. Ticagrelor HCl in ethanol was found to be linear in the range 5-30 µg/mL [Figure 5]. The regression equation was found to be y= 18511x+357545, with 18511 as slope and 357545 as intercept. Correlation coefficient(R^2) was found to be 0.9984. The method developed Interday was carried out by analyzing the same sample 6 times for 6

consecutive days and was found to be precise as % RSD was found to be less than 2%. The method was also found to be accurate, indicated by % recoveries ranging from 99.44%-100.67%. LOD & LOQ were found to be 0.561 and 0.924 respectively indicating that the method is sensitive.

Robustness was carried out by analyzing the sample at 2 different wavelengths and 2 different flowrates for 2 consecutive days, and there was no much difference observed in system suitability parameters. Ruggedness was carried out by 2 different analysts using the same sample for 6 times and was found to be rugged as the % RSD was found to be less than 2%.

All the parameters validated were shown in [Table 12].

Table 12.

Parameters	Results
Absorption maxima (nm)	255 nm
Linearity range	5-30 µg/mL
Regression equation	y= 18511x+357545
Correlation coefficient(R^2)	0.9984
LOD(µg/mL)	0.561
LOQ(µg/mL)	0.924
Accuracy (% Recovery± SD)	99.56% ± 0.311
Inter day Precision (% RSD) Day 1 Day 2	 1.308 1.432

CONCLUSION

All the above parameters conclude that the proposed method is linear, accurate, precise, robust and rugged and also cost effective and can be successfully applied for estimation of Ticagrelor HCl in bulk and formulation.

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