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AN IMPROVED AND VALIDATED RP HPLC METHOD DEVELOPMENT FOR THE QUANTITATIVE ESTIMATION OF TICAGRELOR

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ABSTRACT

A simple, precise and accurate reverse phase high performance liquid chromatography (RP- HPLC) method was developed and validated for the determination of ticagrelor using Unisol C18 (150 mm × 4.6 mm, 3 µ) with a mobile phase consisting of 700 mL of HPLC grade methanol and 300 mL of triethanolamine buffer of pH 4.5. The detection was carried out at 240 nm and retention time of ticagrelor was found to be 3.247 min. Linearity was observed from 10 to 120 µg/mL (coefficient of determination R^2 was 0.998) with equation, y = 3878x + 6460. The method was statistically validated for accuracy, precision, linearity, ruggedness, robustness, solution stability, selectivity as per ICH guidelines. The results obtained in the study were within the limits of ICH guidelines and hence this method can also be used for the determination of ticagrelor in pharmaceutical dosage forms.

Keywords *– Ticagrelor, Triethanolamine buffer, RP HPLC, Validation, ICH guidelines*

1. INTRODUCTION

Ticagrelor chemically known as (1S,2S,3R,5S)-3-(7-{[(1R,2S)-2-(3,4-difluorophenyl)cyclopropyl]amino}-5-(propylsulfanyl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-5-(2-hydroxyethoxy)cyclopentane-1,2-diol is an orally active antiplatelet drug [1].

An adenosine triphosphate analogue and reversible P2Y12 purinoreceptor antagonist that inhibits ADP (adenosine diphosphate) mediated platelet aggregation. It is used for the prevention of thromboembolism by patients with acute coronary syndrome or a history of myocardial infarction. A platelet aggregation inhibitor which is used for prevention of thromboembolic events in patients with acute coronary syndrome. It has a role as a platelet aggregation inhibitor and a P2Y12 receptor antagonist. It is a member of triazolopyrimidines, an organofluorine compound, an aryl sulfide, a secondary amino compound and a hydroxyether. No efforts were made till now using the triethanolamine buffer hence a simple, precise, and accurate RP-HPLC method was developed [2].

Venous thromboembolism is a condition in which a blood clot (a thrombus) forms in a vein and then dislodges to travel in the blood (an embolus) [3]. The three major therapies available for the myocardial treatment purpose are anti-platelet drugs,

thrombolytic agents, and primary coronary angioplasty [4]. This study includes that the following method has been validated according to the ICH guidelines [5] and are successfully applied to estimate the levels of ticagrelor.

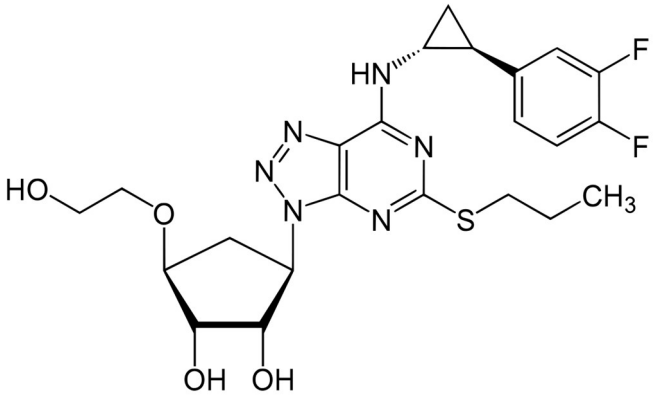

Fig. 1: Structure of ticagrelor [6]

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

Ticagrelor was obtained as a gift sample from Raks Pharma Pvt. Ltd., Visakhapatnam, India in sufficient quantity. The other reagents and chemicals used in the method are of HPLC grade. HPLC grade methanol was provided by Merck life sciences Pvt ltd. Triethanolamine was provided by Merck life sciences Pvt ltd. HPLC grade water was provided by Merck life sciences Pvt ltd. Othophosphoric acid was provided by Qualigens, India.

2.2 Instrumentation

To develop an accurate and precise HPLC method for estimation of ticagrelor, Agilent 1260 Infinity series HPLC system and the column used was Unisol C18 column (150 mm × 4.6 mm, 3 µ) was used. Open lab software was used for analyzing the data obtained and the HPLC also has an autosampler injection port for injecting the samples. It is also equipped with PDA detector. The process of degassing was done by using PCi Analytics ultrasonicator of model no: 3.5 L100 and filtered through Millipore vacuum filter and 0.22 µm 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.

2.3 Chromatographic conditions

A mixture of methanol: triethanolamine buffer of pH 4.5 in the ratio of 80:20 was taken as an ideal mobile phase based on the pKa and solubility of ticagrelor according to the literature survey [7,8] and it was filtered through 0.22 µ membrane filter and well degassed for 15 minutes before its use. A flow rate of 0.8 mL/min was maintained at 25°C. Ticagrelor was estimated through UV detector at 250 nm. Injection volume was 10µL and the run time was allowed up to 10 minutes.

2.4 Preparation of triethanolamine buffer of pH 4.5

50 mL of triethanolamine solution was taken in a 500 mL beaker and it was dissolved in HPLC grade water and the remaining volume was made up to 500 mL by using HPLC grade water and the pH was adjusted to 4.5 with orthophosphoric acid.

2.5 Preparation of Mobile phase and diluents

Various mobile phases were tried by trial and error method and the optimized mobile phase ratio was 30:70 (v/v) of buffer: methanol. The solvents methanol: HPLC grade water in the ratio of 80:20 was used as diluents.

2.6 Preparation of stock solution

10 mg of ticagrelor was accurately weighed and it was transferred to a 10 mL volumetric flask. To this 5 mL of methanol was added and the drug was dissolved in it completely by vigorous shaking and then make the remaining volume up to the mark by using methanol to get a concentration of 1 mg/mL stock solution.

2.7 Preparation of standard solution

1 mL of stock solution of ticagrelor was pipette out into a 10 mL of volumetric flask and it was made up to the mark by using diluents to produce 100 µg/mL of concentrated ticagrelor solution.

2.8 Preparation of sample solution

Ten tablets were weighed, and the average weight was calculated. The tablets were crushed with a mortar and pestle for 10 minutes. A portion of powder equivalent to the weight of one tablet was accurately weighed and transferred to a 100 volumetric flask. Approximately 50 mL diluent was added and sonicated for 30 minutes. The contents were restored to room temperature and diluted to final volume with the diluent to furnish stock solution. The stock solution was filtered through 0.45 µ nylon filter and 10 mL of the filtered solution was transferred to 100 mL volumetric flask and diluted to volume with diluent to give test solution containing 60 µg/mL.

2.9 Optimized chromatographic conditions

Table 1: Parameters and conditions of optimized chromatographic method

Parameters	Conditions
Column	Unisol C18 (150 mm × 4.6 mm, 3µ)
Mobile phase	70:30
Flow rate	0.8 mL/min
Injection volume	10 µL
Detector wavelength	240 nm
Retention time	3.247 mins
Column temperature	27°C
Run time	10 ins

2.10 Assay of Ticagrelor Tablets

Ticagrelor standard solution and ticagrelor sample solution were prepared as discussed in 2.7 and 2.8. The samples were analysed using the developed chromatographic method and the % content of the drug in the tablets was determined. % assay was calculated using the below formula.

$$\% \text{ Assay} = \frac{\text{peak area of sample}}{peak\ area\ of\ standard} \times \frac{concentration\ of\ standard}{concentration\ of\ sample} \times \frac{label\ claim}{average\ weigh\ \ of\ tablets} \times \frac{potency}{100} \times 100$$

2.11 Method Validation

2.11.1 Linearity

Several aliquots of standard solution of ticagrelor were 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 20-120 µg/mL as shown in the Table 4. UV detector was used for evaluation of the drug at 240 nm, peak area was recorded for all the peaks. The response of the drug was linear, and the regression equation was found to be y = 3878.x + 6460 and correlation coefficient value was found to be 0.998 as shown in the Figure 2. The results show that correlation between peak area and concentration of drug was excellent within the concentration range.

2.11.2 Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) of the method developed were determined by injecting serially low concentrations of the standard solution using the developed HPLC method. The LOD and LOQ for ticagrelor were found to be 0.14 µg/mL and 0.42 µg/mL, respectively.

2.11.3 System suitability

System suitability parameters like peak area, retention time, theoretical plates and tailing factor were calculated and were compared with the standard values as shown in the Table 3.

2.11.4 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 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 98-101% and the mean recovery of ticagrelor was 99% that shows there is no interference from excipients and the lower values of % RSD of assay indicate the method is accurate as shown in the Table 5.

2.11.5 Precision

The precision was determined in terms of intra-day and inter-day precision. In intra-day precision evaluation, a standard solution of fixed concentration was injected at various time intervals and % RSD for ticagrelor was 0.018% (limit % RSD < 2.0%). Similarly, the inter-day precision was studied by injecting the same concentration of standard solution on successive days and the % RSD for ticagrelor was 0.025% (Limit % RSD< 2.0%) as shown in the Table 6.

2.11.6 Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions like changes in flow rate, mobile phase composition and by making changes in pH of the buffer. No marked changes were observed in the chromatograms as shown in the Table 7, which states that the HPLC method developed is robust [9-14].

3. RESULTS AND DISCUSSION

3.1 Assay

Analysis of ticagrelor tablets was performed by the proposed method and the percent assay of the formulation was calculated in triplicate. The assay percentage of ticagrelor was found to be within the limits of 98-102% and the results are shown in the Table 2.

Table 2: Data of assay of ticagrelor tablets

S. No	Label claim(mg)	Amount found(mg)
1	60	58.34
2	60	59.55
3	60	59.02
Mean	60	58.97
%Assay	98.3	

3.2 System suitability studies

Table 3: System suitability parameters

Injection number	Peak area	Theoretical plates	Tailing factor	Retention time
1	452904	7102	1.03	3.247
2	452827	7098	1.05	3.251
3	452825	7208	1.02	3.249
4	452913	7127	1.04	3.238
5	452738	7254	1.01	3.256
6	453006	7168	1.02	3.279
Mean	452868.8	7159.5	1.02	3.253
SD	92.51	62.44	0.01	0.013
% RSD	0.020	0.87	1.43	0.427
Acceptance criteria	NMT 2	NMT 2	NMT 2	NMT 2

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

3.3 Linearity

Table 4: Linearity of ticagrelor

Concentration (mcg/mL)	Peak area
20	13503216
40	22312650
60	30173910
80	38256891
100	45290461
120	52250321

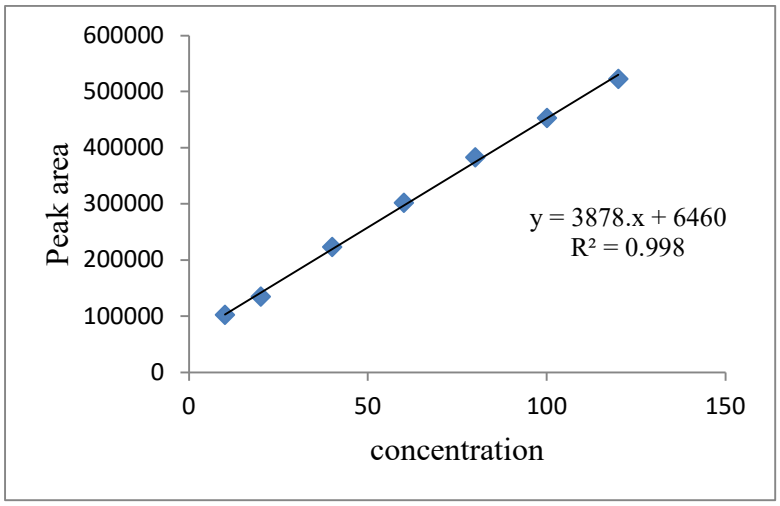

Fig. 2: Linearity chart of Ticagrelor

The response was found to be linear and the correlation coefficient was found to be 0.998.

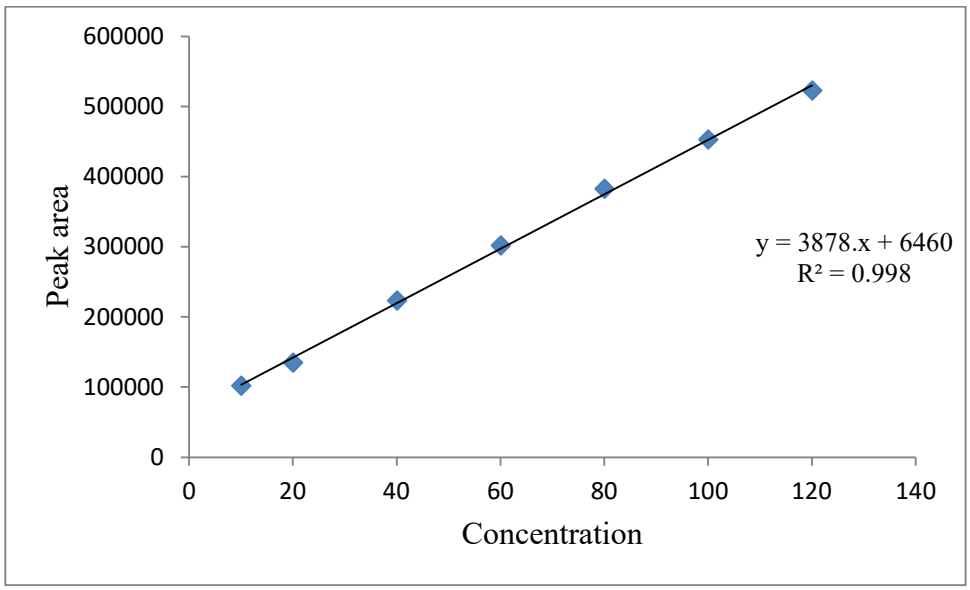

Fig. 2: Linearity chart of Ticagrelor

The response was found to be linear and the correlation coefficient was found to be 0.998.

3.4 Accuracy

Table 5: Data of accuracy in HPLC

% Level	Std Amount	Amt Added	Amt Found	% Recovery	Mean Recovery
50%- 1	30	14.95	44.72	99.49	
2	30	14.97	44.14	98.15	99.23
3	30	14.93	44.96	100.06	
100	30	29.42	59.34	99.86	
	30	29.43	58.58	98.57	99.59
	30	29.45	59.66	100.35	
150	30	44.12	74.54	100.56	
	30	44.44	73.75	99.07	99.93
	30	44.86	74.98	100.16	

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

3.5 Precision

Table 6: Results of intraday and interday in HPLC

	Intraday peak	Interday peak
	452904	452706
	453006	452913
	452825	452827
	452984	452738
	452804	452987
	452887	452945
Mean	452901.66	452852.66
SD	81.61	114.48
% RSD	0.02	0.02

The % RSD for Intraday precision and interday precision for ticagrelor were found to be 0.018 and 0.025 which indicates the method is precise.

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

The LOD value was found at 0.14 µg/ml concentration where the signal to noise ratio was 3:1 and the LOQ value was found at 0.42 µg/ml where the signal to noise ratio was 10:1.

3.7 Robustness

Table 7: Results for robustness

	Conditions	Tailing factor (NMT 2.0)	Theoretical plates (NLT 2000)
Flow Rate	1	1.03	7098
	0.8	0.99	7102
	1.2	0.82	7208
Buffer pH	4.5	1.04	7117
	5	1.01	7014
	5.5	1.02	7168
Mobile Phase Composition	30:70	0.84	7124
	40:60	1.05	7116
	20:80	1.02	7254

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

3.8 Specificity

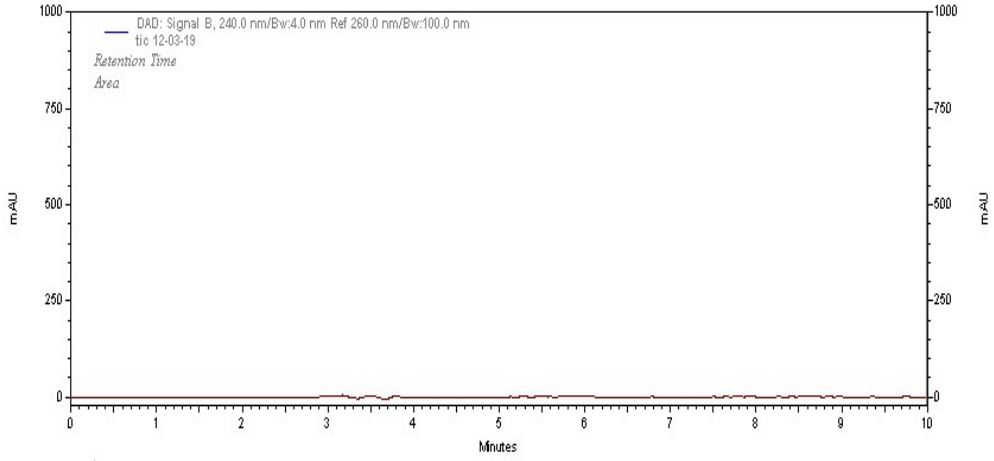

Fig. 3: Blank chromatogram of Ticagrelor

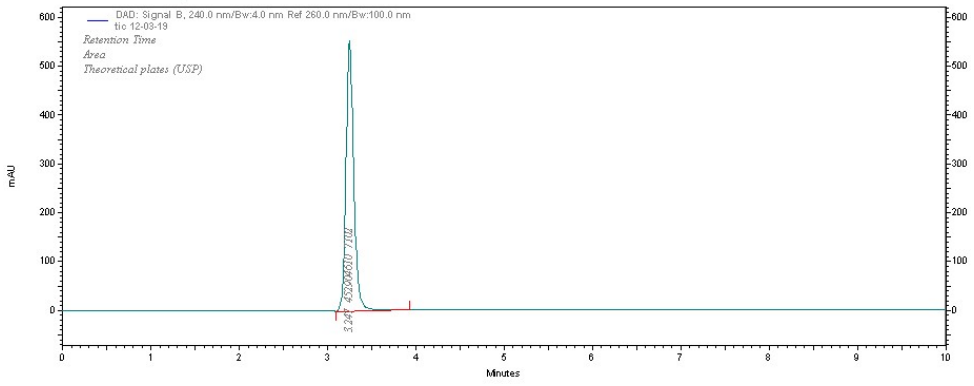

Fig. 4: Standard chromatogram of ticagrelor

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.

4. DISCUSSION

Here we had developed a simple, precise, and improved validated method for quantitative estimation of Ticagrelorthrough reverse phase High Performance Liquid Chromatography (RP HPLC). All variable parameters were selected on basis of different trials performed and through literature survey and were optimized to yield the best possible results. The reported analytical parameters were selected after evaluating different conditions which may affect results of HPLC analysis, viz. column, ratio of aqueous and organic components of mobile phase, detection wavelength, diluents, concentration of analytes, etc.

The Unisol C18 (150 mm × 4.6 mm, 3µ) was selected owing its advantages of high resolution and reproducibility with relatively low-back pressure and tailing. For the selection mobile phase, initial trials were performed using mobile phases of different composition containing water adjusted to acidic pH (1.8 - 4.0) through the addition of o-phosphoric acid and methanol, 0.1% o-phosphoric acid with methanol, 0.1% formic acid in combination with methanol, etc. However, all these trials resulted in poor peak shapes. Ultimately methanol and triethanolamine buffer of pH 4.5 that offered improved acceptable peak shape. After selection, the proportion of mobile phase components was optimized to reduce retention times and fine tune the peak shape.

To decide the detection wavelength, scanning of standard solution over the range of 190-350 nm was performed on the PDA detector. Detection wavelength of 240 nm was selected, as scans on 240 nm provides results with good response and linearity. This newly developed method was validated as per ICH guidelines. This furnishes evidence that the method is appropriate for its intended use.

5. CONCLUSION

A simple accurate and precise RP HPLC method was developed for analysis of Ticagrelor. The initial trial was conducted with the Unisol reverse phase C18 column (150×4.6 mm, 3µ) at 27°C, 0.8 mL/min flow rate and isocratic elution mode. The detection wavelength was fixed by scanning the working standard solution and observing the maximum absorbance wavelength which was found to be 240 nm and the mobile phase composed of Methanol: Triethanolamine buffer of pH 4.5 (70:30). The retention time was found at 3.247 mins. The calibration curve was linear with correlation coefficient of 0.998 over a concentration range of 10-120 µg/ml with linear regression equation y = 3878.x + 6460. The limit of detection and limit of quantification were found at 0.14µg/ml and 0.42µg/ml respectively indicating the sensitivity of the method. The proposed method has been validated according to the ICH guidelines and can be successfully applied to estimate the levels of Ticagrelor.

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REFERENCES

1. https://www.drugbank.ca/drugs/DB08816
2. https://pubchem.ncbi.nlm.nih.gov/compound/Ticagrelor
3. https://www.ncbi.nlm.nih.gov/books/NBK132796/
4. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2014343/
5. https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf
6. https://en.wikipedia.org/wiki/Ticagrelor#/media/File:Ticagrelor.svg
7. Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC method development. 2nd edition. New York. John wiley. 1997, 233-291.

8. https://www.applichem.com/fileadmin/Broschueren/BioBuffer.pdf
9. Pawar A.K.M., Harini U. A LC-MS compatible RP-HPLC method for the determination of ticagrelor in bulk. International Journal of Pharmacy and Analytical Research, 2017, 6(2), 423-430.
10. Nazare lobo john shane et al., Method development and validation for the estimation of ticargrelor in bulk and comparison with other published methods. Journal of Global Pharma Technology, 2016, 12(8), 01-06.
11. Vegesnaswetha, S. V. U. M. Prasad, Y. Akhila. Analytical method development and validation of stability indicating assay method of ticagrelor tablets by using RP-HPLC. World Journal of Pharmaceutical and Medical Research, 2017, 3(10), 235-241.
12. Parida AK, Rao KS, Patnaik AK. A Novel Validated RP-HPLC Method for the estimation of Ticagrelor in Bulk and Pharmaceutical Dosage Forms. Research Journal of Pharmacy and Technology. 2018;11(3):867-72.
13. Tabassum K, Sarvesh R. Analytical Method Development and VALIDATION Studies OF Ticagrelor Tablets by RP-HPLC. International Journal of Applied Pharmaceutics. 2017;9(4), 10-21.
14. Ambasana MA, Kapuriya NP, Mangtani KM, Ladva KD. An improved assay method for the estimation of ticagrelor hydrochloride by reverse phase liquid chromatography. International Journal of Pharmaceutical Sciences and Analytical Research. 2016, 7(5), 2009-2014.