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RP-HPLC Method Development and Validation for Estimation of Ticagrelor in Bulk and Pharmaceutical Dosage Form

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Abstract: Ticagrelor is a selective Adenosine diphosphate (ADP)-receptor antagonist which is prescribed in the form of tablets and acts as an oral antiplatelet for the prevention of further thrombotic events in patients with Acute coronary syndrome (ACS) and those undergoing Percutaneous coronary intervention (PCI). Therefore, accurate and reliable determination of Ticagrelor in bulk and in dosage forms is vital for clinical consideration. The objective of the method is to develop a new, simple, sensitive, accurate, and economical analytical method for the determination of assay of Ticagrelor Tablet and to perform a forced degradation study of Ticagrelor Tablet by RP-HPLC. Chromatographic separation was achieved on an UHPLC equipped with reverse phase C-18 (250 × 4.6mm, 5µ) with a mobile phase composed of acetonitrile and buffer solution (75:25) at a flow rate of 1.10 ml/min. The effluents were detected at a wavelength of 256 nm. The retention time of Ticagrelor was found to be at 3.579 min. The correlation coefficient for Ticagrelor was found to be 0.997. Recovery of Ticagrelor in the formulation was found to be 98%-102%. LOD and LOQ values of Ticagrelor were found to be 1.31 and 3.98, respectively. The method was proven to be precise (%RSD=2%), accurate (>90%), and specific for the measurement of Ticagrelor in tablets. Thus, being simple, accurate, precise, and rapid, the newly developed RP-HPLC method is recommended for the estimation of Ticagrelor in nature. Due to its high sensitivity and specificity, it is a suitable choice for identifying Ticagrelor in drugs and other products and differentiating hid similarities. However, this method can effectively be used for routine assay and stability study, which can help manage cardiovascular diseases since the quality and efficacy of Ticagrelor-containing products are significant to patients' condition.

Introduction

Ticagrelor belongs to the Category Anti-Platelet Agent. Ticagrelor is a P2Y12 receptor antagonist (Tao L et al., 2022). The IUPAC name is (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 (Kumar et al., 2016). Ticagrelor is a P2Y12 receptor antagonist (Nikitha and Ajitha, 2016). Literature survey revealed estimation of Ticagrelor by several techniques, such as Stability-Indicating RP-HPLC Method for the Determination Ticagrelor in Pharmaceutical Dosage Form (Patel and Patel, 2014; Parida et al., 2018; Pappula et al., 2021). The RPHPLC method was developed and validated to estimate Ticagrelor in Bulk and The Pharmaceutical Tablet (Meena et al., 2021; Mankar et al., 2023; Patil and Godge, 2024). The Formulations and stability are indicating the UPLC method and the the estimation of ticagrelor in bulk and its tablet are used in dosage form (Kulkarni and Gajare, 2016; Omaima et al., 2019; Sri Ranjani et al., 2021; Deshpande et al., 2023; Farooq and Khan, 2023; Gosavi et al., 2023). The quantitative determination of Ticagrelor in pharmaceutical dosage forms was done using the Ultra

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Performance Liquid Chromatographic (UPLC) method (Joshy et al., 2016; Omaima et al., 2019; Iyer et al., 2021; Ahmad et al., 2023).

Based on the less complication and easy availability of HPLC, an estimation of ertugliflozin along with sitagliptin in the tablet dosage form was done by Mankar et al. (2023). Siddheshwar et al. (2023) also revolve around creating and validating a cheap RP-HPLC method for determining the amount of Pazopanib HCl in the raw material and tablets.

This study attempted to develop a rapid and economical Reverse-phase HPLC (RP-HPLC) method for estimating Ticagrelor in bulk and pharmaceutical formulation with better sensitivity, precision and accuracy.

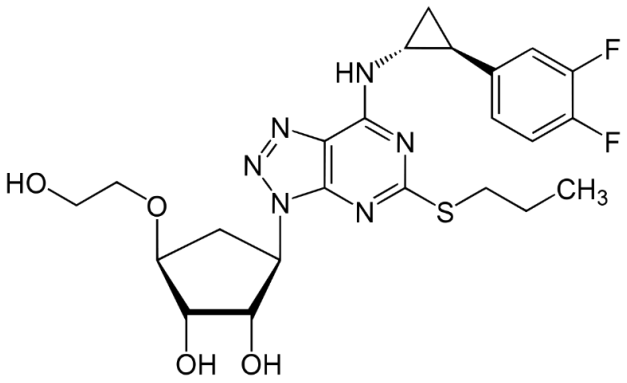

Figure 1. Structure of Ticagrelor.

Materials and Methods

Reagents and chemicals

Ticagrelor was received from the Swapnaroop Research Pvt. Ltd. Ticagrelor Tablet 60 mg was purchased from a market manufactured by Sun Pharma Pvt. Limited. HPLC grade acetonitrile and HPLC grade water (Finar), Ortho Phosphoric acid and Potassium dihydrogen phosphate (Merck) were available at Pravara Rural College of Pharmacy, Pravaranagar. The reagents and chemicals which are used for RP-HPLC were filtered through 0.45-µm filter paper.

Instrumentation and Chromatographic conditions

The analysis of Ticagrelor was carried out on UHPLC equipped with reverse phase C-18 (250 × 4.6mm, 5µ) and UV detection system and running on Lab Solution, Version DB 6.110 software. At 25°C chromatographic separation was attained with a mobile phase consisting of acetonitrile and buffer solution (Dissolved 6.8 gm of Potassium dihydrogen phosphate in 1000 ml of HPLC grade water; Adjusted pH of this solution to 2.90 with ortho phosphoric acid) in the ratio of 75: 25, respectively and utilises an ultrasonic bath to degas. The column used was C18, 250 mm x 4.6 mm, 5µ equilibrated by pumping the mobile phase through the column for 30 minutes before injecting the drug solution. The injection volume was 20 µL and the flow rate was maintained at 1.10 mL/min. At 256 nm, UV detection was achieved.

Preparation of standard stock solution

Standard solution of 20 ppm concentration of Ticagrelor was prepared by weighing the 100 mg of Ticagrelor standard into 100 mL volumetric flask. Add 70 mL of mobile phase, sonicate for 5 minutes to dissolve, and then make up to 100 mL with mobile phase and mix. Further, dilute 2.0 mL of this solution to 100 mL with mobile phase and mix. Further, sonicate the solution to degas.

Preparation of sample solution

Sample solution of 20 ppm concentration was prepared by accurately weighing the amount of the finely powdered tablets equivalent to 100 mg of Ticagrelor was taken and transferred into a 100 ml volumetric flask containing 60ml of mobile phase and sonicated with occasional shaking for 10 min and dilute up to the mark with mobile phase. The resultant solution was filtered through a 0.22 µl syringe filter. 2 ml was diluted to 100 ml with mobile phase. This was filtered through 0.20µm PTFE membrane filter. 20µl volume of final sample solution was injected in duplicate into HPLC and peak areas were measured under optimized chromatographic conditions.

Method

The mobile phase with acetonitrile and buffer pH 2.90 (75:25%v/v) was employed in isocratic mode at a flow rate of 1.10 ml/min. The run time was 6 mins and 20µL of the sample was injected for every run into the column. The wavelength of the UV detector was set at 256 nm. Above chromatographic conditions and mobile phase used were found to be the most suitable for peak shape and system suitability parameters for Ticagrelor. It was pumped through the column at a flow rate of 1.10 mL/min. The column was maintained at the ambient temperature and the injection volume was 20µL. Prior to injecting the drug solution, the column was equilibrated by pumping the mobile phase through it for 30 minutes. Equal volumes of the sample solution, the standard solution, and the blank (diluent) were separately injected. Measure the Peak area and record the chromatograms.

Results and Discussion

Method validation

System Suitability

For the purpose of analysing the existence of interfering components in the Ticagrelor working solution, specificity and selectivity have been studied. The data demonstrates that the system's suitability is

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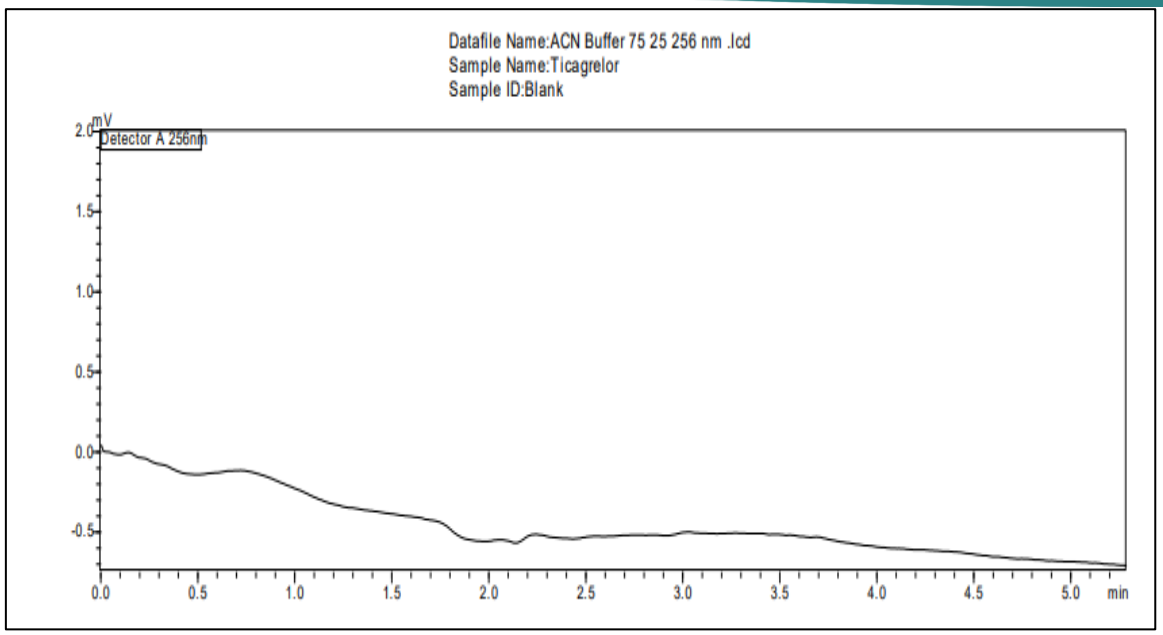

Figure 2. Chromatogram of Blank.

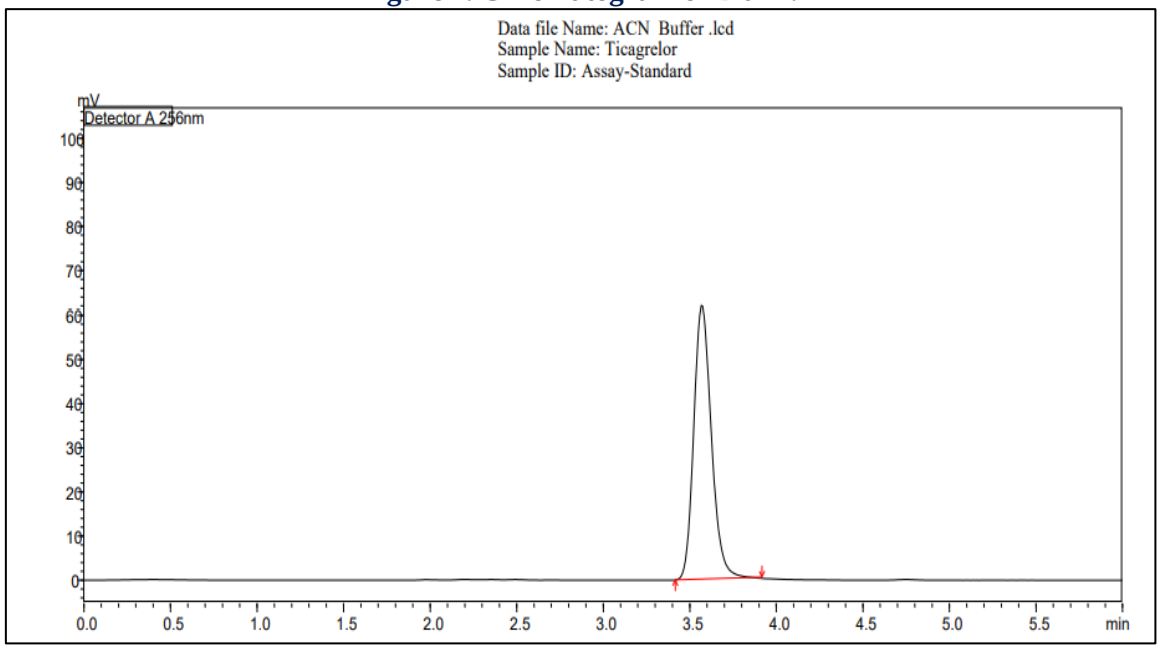

Figure 3. Chromatogram of Standard.

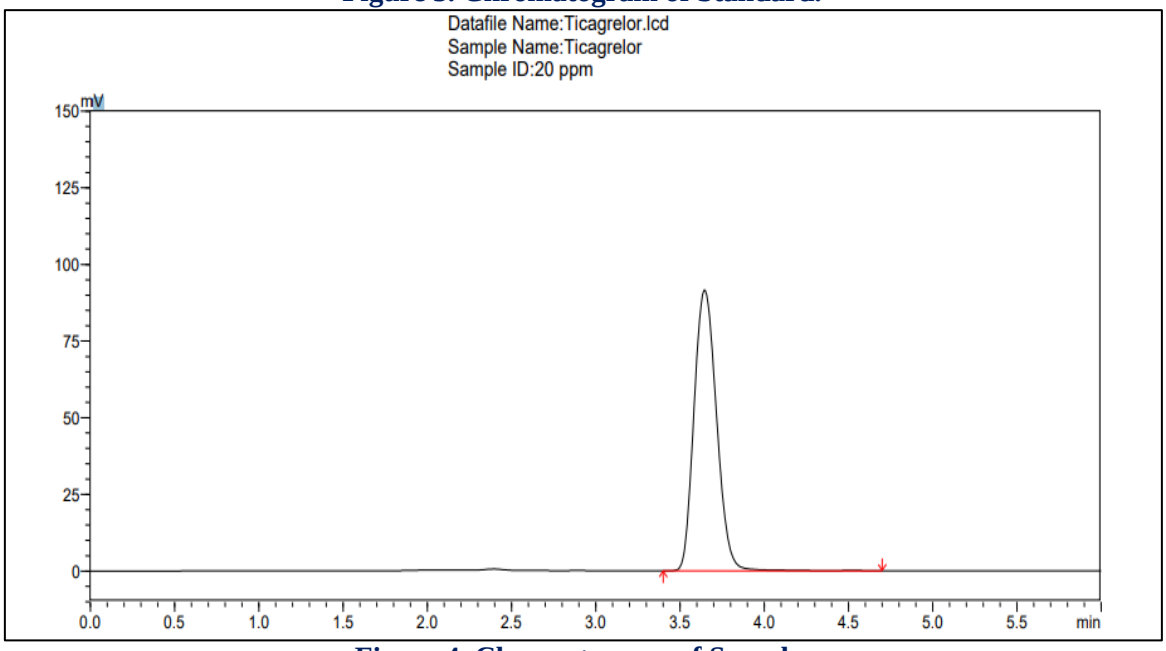

Figure 4. Chromatogram of Sample.

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within the acceptance criteria. Thus, the system is suitable. The tailing factor should not be more than 2.0 from the injection of the standard solution. The theoretical plates for ticagrelor peak should not be less than 1500 from the first injection of the standard solution.

Table 1. System Suitability Test of Ticagrelor.

Parameters	Result
Peak Area	746134
Retention Time	3.579
Theoretical plates	5137
Tailing factor	1.2

Specificity: (Identification, Interference & Peak Purity)

Inject Blank (Diluent), standard solution and sample solution. The data demonstrates that retention time in standard and sample is the same for Ticagrelor peak. The data demonstrates that there is no interference in Blank and Placebo at the retention time of Ticagrelor peak. The standard and sample solution obtained a peak purity match in both chromatograms.

Table 2. Specificity (Identification and Interference).

Component	Retention time (min)	Area	Theoretical Plates	Asymmetry
Blank	-		-	-
Standard solution	3.590	724572	5297	1.207
Sample solution	3.606	723195	5318	1.209

Linearity

To attain solutions in the range of 50 to 150% (i.e., 50%, 75%, 100%, 125%, and 150%) of the working concentration, linearity solutions were prepared by quantitatively diluting the stock solution of the Ticagrelor standard. The correlation coefficient should not be less than 0.999, is considered as acceptable, and was obtained.

% Recovery

Accuracy was assessed for ticagrelor at three levels: 50%, 100%, and 150% of the working concentration. Ticagrelor's 20 ppm working concentration level. Every level is prepared in triplicates. The data shows that individual recovery for 50% to 150% is in the range of 97.0% - 103.0% and the Mean recovery for 50% to 150% is in the range of 98.0%-102.0%.

Robustness

Robustness was performed regarding flow rate and wavelength and chromatograms were recorded. System suitability criteria should be fulfilled. The % difference for Area obtained in each modified condition should not be more than 2.0 when compared to the control and was obtained.

Precision

Precision of the method was established for intra-day (repeatability) and inter-day Precision was studied using 17, 27 and 37µg/ml working solutions. All of these solutions were injected under predetermined chromatographic conditions, and the results were within the acceptable RSD range i. e., ≤ 2%. Intermediate precision was performed on the same sample on different days by two different analyses and the value of RSD is found to be <2%.

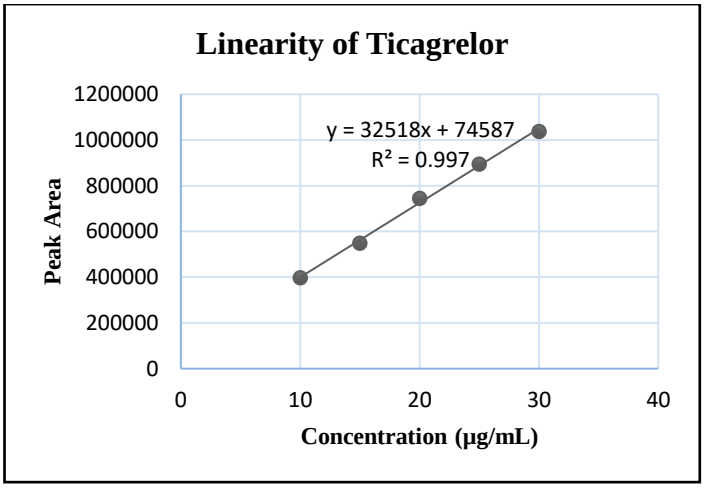

Figure 5. Linearity of Ticagrelor.

Table 4. % Recovery for Ticagrelor.

Theoretical Conc.(t)	Area obtained (m)	Recover conc. in µg/ml(x)	% Recovery
30 µg/ml	1047126	30.7	102.4%
40 µg/ml	1405552	41.2	103.1%
50 µg/ml	1734573	50.9	101.8%

Table 5. Robustness for Ticagrelor.

Changes in Parameter	Values	Retention time of Ticagrelor	Area	% Difference
Control	As per method	3.568	839856	NA
Change in Flow rate (± 0.1 mL/min)	1.0 mL/min	3.917	817317	1.5
	1.2 mL/min	3.303	815855	1.7
Change in wavelength(± 2 nm)	254 nm	3.583	833553	-0.4
	258 nm	3.582	836538	-0.8

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Intraday Precision

Table 6. %RSD in Intraday Precision

Sr. No.	Conc (µg/ml)	Area			RT			Mean		%RSD	
		Set I	Set II	Set III	Set I	Set II	Set III	Area	RT	Area	RT
1	17	609459	612861	618133	3.580	3.576	3.576	613484	3.577	0.71	0.06
2	27	992735	996529	995192	3.576	3.575	3.575	995192	3.575	0.21	0.02
3	37	134103	131832	133143	3.565	3.566	3.569	133143	3.566	0.88	0.06

Intermediate precision

Table 7. %RSD in Intermediate Precision.

Sr. No.	Conc (µg/ml)	Area			RT			Mean		%RSD	
		Set I	Set II	Set	Set I	Set II	Set	Area	RT	Area	RT
1	17	60945	61515	62121	3.580	3.576	3.584	61527	3.580	0.96	0.11
2	27	99273	99835	99108	3.576	3.575	3.568	99405	3.572	0.38	0.11
3	37	13349	13344	13385	3.565	3.565	3.564	13359	3.565	0.17	0.02

LOD and LOQ

An analytical method's limit of detection (LOD) is the lowest quantity of analytes in a given sample, which can be simply detected but not quantified to the given value. The limit of quantification (LOQ) of an analytical method is the lowest amount of analyte in a given sample, which can be simply quantified with appropriate accuracy and precision. LOD and LOQ were calculated and are given in Table and the values indicated that the method was susceptible to quantify and detect the drug.

Table 8. LOD and LOQ of Ticagrelor

Sl. No.	LOD	LOQ
1.	1.31 (µg/ml)	3.98 (µg/ml)

Forced Degradation Studies

The degrading behaviour of ticagrelor, which has been studied under various stress conditions, including acidic, basic and thermal conditions, is described in the present study. The result of the assay of Ticagrelor shows that the degradation product does not interfere with the analytical procedure quantitatively when this drug is analysed. Thus, this analytical methodology is also helpful for determining Ticagrelor in sample. From the table below, it was observed that Ticagrelor was found stable under Thermal conditions. Degradation observed in acid and alkali stress conditions. The current method can be used to analyze ticagrelor in pharmaceutical quality control since it effectively separates the degraded products and the method is stability indicating.

Table 9. Forced Degradation Studies.

Reagents	Conditions	% Assay	Degradation%
Acid degradation	With 1 N HCl, 80°C, 4 Hrs. in water bath	76.708%	23.292%
Base degradation	With 1 N NaOH, 18 Hrs. at bench top	71.229%	28.771%
Thermal degradation	80°C for 24 hr	98.499%	1.501%

Conclusion

According to the results, the method has good reproducibility, and the stability RP-HPLC method is accurate, precise, specific, reproducible, and sensitive. The RP-HPLC method can also be used to analyse the Ticagrelor tablet's single dose formulation. No interference of additives, matrix etc. is encountered in these methods. Various validation parameters were carried out: specificity, linearity, precision, LOD, LOQ, accuracy, and forced degradation. The linearity was obtained with correlation factor (r^2) 0.997 in the concentration range of 10 µg/ml–30 µg/ml. The retention time of Ticagrelor was found to be at 3.579 min. The %RSD for all the parameters was found to be within the limits, that is, <2% and % recovery was also found to be within the limit (97%-103%). From the studies, it can be concluded that this RP-HPLC technique can be successfully used to estimate the Ticagrelor in tablet formulations.

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Conflict of Interests

There are no conflicts of interest as declared by the authors.

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