RESEARCH PAPER

Development and Validation of an RP-HPLC Method for Estimation of Ticagrelor in Bulk Drug and Pharmaceutical Formulation

[1]Borade B.A.*, [2]Dighade S.J. and [3]Ganjiwale R.O.

[1]Institute of Pharmaceutical Education and Research, Borgaon, Meghe, Wardha, Maharashtra
[2]Institute of Pharmacy and Research, Badnera, Amravati, Maharashtra
[3]Institute of Pharmaceutical Education and Research, Borgaon Meghe, Wardha, Maharashtra
**E-mail: bhagyashri.borade22@gmail.com, principaliopr@gmail.com*

Received: 24th April, 2026; Revised: 20th May 2026; Accepted: 29th August, 2026; Available Online: 31st August, 2026

ABSTRACT
A simple, accurate, and stability-indicating reverse-phase HPLC method was developed and validated for the quantitative estimation of Ticagrelor in bulk drug and tablet dosage forms according to ICH guidelines. Chromatographic separation was achieved using a mobile phase comprising acetonitrile and water containing 0.1% formic acid and 0.1% triethylamine (60:40, v/v), with detection at 256 nm. The optimized method demonstrated satisfactory system suitability, a retention time of 5.63 minutes, excellent linearity (R^2 = 0.9955), high accuracy (99.46–99.69% recovery), good precision, and adequate sensitivity with low detection and quantification limits. The method was successfully applied to marketed and expired Ticagrelor tablets, which complied with acceptable assay specifications despite a slight reduction in drug content after expiry. Forced degradation studies confirmed greater susceptibility under acidic and alkaline conditions, while oxidative and neutral environments caused minimal degradation. Evaluation of tablet quality indicated that expired tablets exhibited increased hardness and slower disintegration and dissolution, suggesting gradual changes in performance during storage while remaining within pharmacopoeial limits.

Keywords: *(Ticagrelor; RP-HPLC; Method Validation; Stability-Indicating Method; ICH Guidelines; Forced Degradation Studies; Pharmaceutical Quality Control; Tablet Evaluation.)*
How to cite this article: Borade B.A., Dighade S.J. and Ganjiwale R.O., Development and Validation of an RP-HPLC Method for Estimation of Ticagrelor in Bulk Drug and Pharmaceutical Formulation. Int J Drug Deliv Technol. 2026;16(79s):740-764. DOI: 10.25258/ijddt.16.79s.139
Source of support: Nil.
Conflict of interest: None

INTRODUCTION

The creation of a reliable analytical method is a critical stage in pharmaceutical research because it provides precise identification and quantification of drug components during formulation development, quality control, and stability testing. Reverse Phase high-Performance Liquid Chromatography (RP-HPLC) is a popular analytical technique because to its great sensitivity, precision, reproducibility, and ability to separate chemicals from contaminants and degradation products. It has become the preferred approach for regular pharmaceutical analysis due to its ease of use, speed, and cost-effectiveness.

Ticagrelor is a cyclopentyl-triazolo-pyrimidine derivative that acts as a reversible antagonist of platelet P2Y12 receptors. It is commonly recommended to avoid thrombotic cardiovascular events in patients with acute coronary syndrome and those having percutaneous coronary intervention. Ticagrelor has a low water solubility, which makes formulation development and analytical evaluation difficult. As a result, a sensitive and reliable analytical method is required for accurate estimate of Ticagrelor in bulk drugs, pharmaceutical dosage forms, and innovative drug delivery systems such as nanocrystals.

Analytical methods used in pharmaceutical analysis should provide precise and reproducible results while minimizing analysis time and solvent consumption. RP-HPLC fulfills these requirements by offering excellent resolution, short run times, and compatibility with a wide range of pharmaceutical compounds. The selection of suitable chromatographic conditions, including stationary phase, mobile phase composition, pH, flow rate, and detection wavelength, plays a critical role in obtaining sharp and symmetrical peaks with satisfactory system suitability parameters.

Method development involves the systematic optimization of chromatographic variables to achieve adequate separation and consistent performance. During this process, different mobile phase compositions and operating conditions are evaluated to obtain acceptable retention time, peak symmetry, theoretical plate count, and resolution. Once optimized, the analytical method must be validated to demonstrate that it is suitable for its intended purpose.

**Author for Correspondence: bhagyashri.borade22@gmail.com*

According to the International Council for Harmonisation (ICH) guideline Q2(R2), analytical method validation is performed by evaluating parameters such as specificity, linearity, accuracy, precision, detection limit, quantitation limit, robustness, and system suitability. Validation confirms that the developed RP-HPLC method consistently produces reliable and reproducible results under specified operating conditions, thereby ensuring confidence in analytical data generated during routine quality control and research applications.

In the present study, an RP-HPLC method was developed and optimized for the estimation of Ticagrelor. The developed method was designed to provide accurate, precise, rapid, and reproducible quantification of the drug while complying with ICH validation requirements. The validated method can be effectively applied for routine analysis of Ticagrelor in bulk drug, pharmaceutical formulations, dissolution samples, and nanocrystal formulations during formulation development and quality assessment.

EXPERIMENTAL

Material and Methods:

API	Procured from	
Ticagrelor IP	Maithri Laboratories Private Limited (Telangana, India)	
List of Chemicals and Reagents	**Procured from**	**Grade**
Acetonitrile	Dange Treding Company (Amravati, Maharashtra)	HPLC
Water	Dange Treding Company (Amravati, Maharashtra)	HPLC
Methanol	Dange Treding Company (Amravati, Maharashtra)	HPLC
Formic acid	Dange Treding Company (Amravati, Maharashtra)	AR
Triethylamine	Dange Treding Company (Amravati, Maharashtra)	AR
Potassium dihydrogen orthophosphate	Dange Treding Company (Amravati, Maharashtra)	AR
Sodium Dihydrogen phosphate	Dange Treding Company (Amravati, Maharashtra)	AR
Orthophosphoric acid	Dange Treding Company (Amravati, Maharashtra)	AR

General Procedure and Detection Method:

Determination of wavelength for Ticagrelor

A standard stock solution of Ticagrelor was prepared by accurately weighing 10 mg of the drug and transferring it into a 10 mL volumetric flask. Acetonitrile was added, followed by sonication, and the solution was then diluted with a suitable diluent to achieve a final concentration of 1000 µg/mL. From this stock solution, a further dilution was prepared to obtained a 10 µg/mL solution. To determine the maximum wavelength (λ max), the 10 µg/mL solution was analyzed using a UV spectrophotometer over a wavelength range of 200–400 nm, with acetonitrile as the blank. The recorded UV spectrum showed a maximum absorbance at 256 nm, which was selected as the detection wavelength for the HPLC study

Preparation of standard solutions

Stock solution for Ticagrelor: Ticagrelor 10 mg was precisely weighed, dissolved in Acetonitrile, and diluted to 10 ml in a volumetric flask to achieve a concentration of 1000 mg/ml.

Working standard for Ticagrelor: A 1.0 ml aliquot of the stock solution was diluted to 10 ml with the mobile phase, resulting in a concentration of 100 µg/ml.

HPLC method development of ticagrelor in bulk and in tablet dosage form:

Preparation of Standard Solution:

An accurately weighed 10 mg of ticagrelor was transferred to a 10 mL volumetric flask and dissolved in acetonitrile. The solution was then sonicated, and the volume was adjusted to the mark with acetonitrile to obtain a final concentration of 1000 µg/mL. To prepare the working standard, appropriate aliquots of the stock solution were pipetted out and diluted with acetonitrile to achieve a concentration of 10 µg/mL.

Selection and Detection of Wavelength:

A standard stock solution of Ticagrelor (1000 µg/mL) was prepared by dissolving 10 mg of the drug in acetonitrile, followed by sonication and dilution. A 10 µg/mL solution was then obtained from this stock. UV spectrophotometric analysis (200–400 nm) using acetonitrile as a blank identified 256 nm as the maximum absorbance wavelength, which was selected for the HPLC study.

Selection of mobile phase:

The working standard solution of Ticagrelor was prepared and analyzed using the HPLC system with different solvents and their combinations. Various mobile phases, such as water: methanol, acetonitrile: water, and different buffers, were tested to achieve optimal peak stability. Finally, a mobile phase of acetonitrile and water (60:40 v/v) with 0.1% formic acid and 0.1% triethylamine was chosen, as it produced sharp, symmetrical peaks and suitable retention times for Ticagrelor.

Chromatographic conditions:

Column	Solar C18 Column ((150×4.6mm,5umparticle size)
Mobile Phase	Acetonitrile and water (60:40 v/v) with 0.1% formic acid and 0.1% triethylamine
Detection wavelength	256
Injection volume	20 µl
Flow rate	1.2 ml/min
Retention time	5.6min
Run time	8 min
Detector	PDA

Validation of Proposed Method

System Suitability Test Parameters

For the system suitability test, six replicate injections of the Ticagrelor working standard solution (10 µg/mL) were analyzed under optimized chromatographic conditions to evaluate the system's applicability. By injecting the standard solution into the chromatographic system, key parameters such as resolution, peak asymmetry, and theoretical plate count were evaluated.

Linearity

A standard solution of Ticagrelor (1000 µg/mL) was used to prepare a calibration curve. One milliliter of this solution was transferred into a 10 mL volumetric flask, and the volume was adjusted with acetonitrile to obtain a 100 µg/mL concentration. From this solution, seven aliquots (0.5, 1.0, 1.5, 2.0, and 2.5 mL) were further diluted separately to 10 mL with acetonitrile, resulting in a concentration range of 5–25 µg/mL. Each prepared solution was analyzed using the standard chromatographic conditions, and the peak areas were recorded. Five replicate injections were performed for each concentration, and the chromatograms were recorded. A calibration curve was constructed by plotting peak area against drug concentration, demonstrating a linear response within the 5–25 µg/mL range.

Accuracy

The accuracy of the analytical method was evaluated using recovery studies via the standard addition method at 80%, 100%, and 120% levels. Tablet powder equivalent to 5 mg of Ticagrelor was spiked with standard Ticagrelor solution (30%, 50%, and 70%) in triplicate. Samples were dissolved in acetonitrile, sonicated for 20 minutes, filtered (0.45 µm Nylon), and diluted to obtain final concentrations of 15, 20, and 25 µg/mL.The solutions were analyzed under optimized chromatographic conditions. Recovery percentages were determined using calibration curves, ensuring method reliability.

Precision

Intra-day and Inter-day Precision

The precision was determined through intraday and interday studies. For intraday precision, a 10 µg/mL ticagrelor solution was prepared and injected three times within the same day. For interday precision, the solution was analyzed over three consecutive days. The percentage relative standard deviation (%RSD) was calculated, with lower %RSD indicating higher precision.

A ticagrelor standard solution (1000 µg/mL) was prepared by dissolving 10 mg of ticagrelor in ACN, sonicated, and diluted to 10 µg/mL. It was injected six times into the HPLC system, and the %RSD confirmed the method's precision. The study was conducted by two analysts following the standard test method.

Repeatability

10 mg of ticagrelor was weighed and dissolved in acetonitrile (ACN) to prepare a 1000 µg/mL stock solution. From this, a 10 µg/mL working solution was obtained. This solution was injected six times into the HPLC system, and the %RSD of the peak areas confirmed good repeatability.

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

Limit of Detection (LOD)

The Limit of Detection (LOD) is the lowest concentration of an analyte that can be detected but not necessarily measured accurately. It helps determine whether a substance is present in a sample, even if the exact amount cannot be quantified. LOD is calculated using the formula:

$$LOD = \frac{3.3 \times \sigma}{S}$$

Where, σ = standard deviation of the response

S = Slope of the calibration curve

Limit of Quantification (LOQ)

The Limit of Quantification (LOQ) is the lowest concentration of an analyte that can be measured with accuracy and precision. It ensures that the analyte can be reliably quantified in a sample.

Robustness

The robustness of the analytical method was evaluated by making deliberate variations in chromatographic conditions to assess its reliability. The following parameters were modified:

- **Flow Rate:** The flow rate was adjusted by ±0.2 mL/min, with tested values of 0.8 mL/min, 1.0 mL/min, and 1.2 mL/min.

- **Wavelength:** The detection wavelength was varied by ±2 nm, evaluating measurements at 219 nm, 221 nm, and 223 nm.
- **Injection Volume:** The injection volume was altered by ±2 µL, with tested values of 18 µL, 20 µL and 22 µL.

These variations were conducted to ensure the method's consistency and reliability under different conditions.

Ruggedness

The ruggedness of the method was tested to ensure its reliability under different conditions. The analysis was performed by different analysts to check if variations in personnel affected the results. Additionally, the method was tested at different room temperatures to see if temperature changes had any impact on accuracy and precision. These results from these tests conducted help to determine whether the method remained consistent and reliable, regardless of differences in analysts or room temperature.

Assay

Preparation of Sample and Standard Solutions

Twenty marketed ticagrelor tablets (90 mg each) were weighed, and the average weight per tablet was calculated. A quantity equivalent to 50 mg of ticagrelor was accurately transferred into a 50 mL volumetric flask. To this, 25 mL of the acetonitrile was added, and the solution was sonicated for 10 minutes to ensure proper dissolution. The volume was then made up to 50 mL with the acetonitrile and filtered using HPLC filters, yielding a 1000 µg/mL stock solution. From this, 0.1 mL was diluted to 10 mL with the mobile phase to obtain a 10 µg/ml solution.The same process was followed for the expired tablet sample. Both standard and sample solutions were injected into the HPLC system for analysis. To calculate the percentage purity, the first step was to determine the concentration of the drug found in milligrams (mg).

Table 1. Acceptance criteria for method validation parameters and assay

Sr.No	Parameter	Acceptance Criteria
1	System Suitability	%RSD ≤ 2% Theoretical Plates(N)≥2000 Tailing Factor(T) ≤ 2.0 Resolution (Rs): > 2.0
2	Linearity	Correlation Coefficient (r^2): ≥ 0.995
3	Accuracy	Mean Recovery: 98.0% – 102.0% %RSD of Recovery: ≤ 2.0%
4	Precision	Repeatability (%RSD): ≤ 2.0% Intermediate Precision (%RSD): ≤ 2.0%
5	Robustness	%RSD: ≤ 2.0% for each variation
6	Ruggedness	%RSD: ≤ 2.0% for each variation
7	Assay	Drug Content: 90.0% – 110.0% of label claim %RSD of Assay Results: ≤ 2.0% Tailing Factor (T): ≤ 2.0

Force degradation study of Ticagrelor (API)

Acid hydrolysis

Ticagrelor API (25.0 mg), 90 mg marketed tablet powder equivalent to 25.0 mg, and expired tablet powder equivalent to 25.0 mg were each dissolved in sufficient acetonitrile in separate 25 mL volumetric flasks. The API was directly dissolved, while the marketed and expired tablet powders were sonicated for 10 minutes to facilitate dissolution. In all cases, the volume was adjusted to the mark with 0.1 N HCl to obtain a final concentration of 1.0 mg/mL. The solutions were then refluxed in round-bottom flasks on a heating mantle for 4 hours and allowed to stand at room temperature for 8 hours.

Base hydrolysis

Ticagrelor API (25.0 mg), 90 mg marketed tablet powder equivalent to 25.0 mg, and expired tablet powder equivalent to 25.0 mg were each dissolved in a suitable amount of acetonitrile in separate 25 mL volumetric flasks. The API was directly dissolved, while the marketed and expired tablet powders were sonicated for 10 minutes to facilitate dissolution. In all cases, the volume was made up to the mark with 0.1 N NaOH to achieve a concentration of 1.0 mg/mL. The solutions were then refluxed in round-bottom flasks on a heating mantle at 50°C for 5 hours and subsequently left to stand at room temperature for another 5 hours.

Neutral hydrolysis

Ticagrelor API (25.0 mg), 90 mg marketed tablet powder equivalent to 25.0 mg, and expired tablet powder equivalent to 25.0 mg were each dissolved in a sufficient amount of acetonitrile in separate 25 mL volumetric flasks. The API was directly dissolved, while the marketed and expired tablet powders were sonicated for 10 minutes to facilitate dissolution. In all cases, the solutions were appropriately diluted with water to achieve a concentration of 1.0 mg/mL. The prepared 25 mL solutions were then refluxed in round-bottom flasks on a heating mantle for 8 hours and then allowed to stand at room temperature for another 8 hours.

Oxidative degradation

Ticagrelor API (25.0 mg), along with accurately weighed quantities of marketed and expired tablet powders equivalent to 25.0 mg of Ticagrelor each, were individually dissolved in adequate amounts of acetonitrile in separate 25 mL volumetric flasks. While the dissolved directly, the marketed and expired

tablet powders were sonicated for 10 minutes to ensure proper dissolution. The final volume in each flask was made up to the mark using a 3% H_2O_2 solution to achieve a concentration of 1.0 mg/mL. All prepared solutions were then stored at room temperature in a dark environment for 24 hours. All the above stress sample were diluted appropriately with acetonitrile to give 10µg/m1 concentration.

Thermal degradation

A sufficient amount of Ticagrelor (approximately 50 mg) was evenly spread in a covered petri dish and placed in a hot air oven at 70°C for varying time intervals. A 10 mg sample was withdrawn at different time points on days 1, 3, 7, and 15. In a similar procedure, marketed Ticagrelor tablets (90 mg) were powdered, and an amount equivalent to 50 mg of Ticagrelor was used. Expired Ticagrelor tablets (90 mg) were also powdered, and a quantity equivalent to 50 mg of Ticagrelor was placed in the oven under the same conditions.

Photo degradation

A sufficient quantity of Ticagrelor (approximately 50 mg) was uniformly spread in a covered petri dish and exposed to sunlight. A 10 mg sample was withdrawn periodically for analysis. For marketed Ticagrelor tablets (90 mg), the powder equivalent to 50 mg was used and exposed to the same conditions. Similarly, expired Ticagrelor tablets (90 mg) were powdered, and the 50 mg equivalent was also exposed to sunlight under the same conditions. For both thermal and photodegradation studies, the samples were dissolved and diluted with acetonitrile to 1 mg/mL, then further diluted to a final concentration of 10 µg/mL. A mixed stress sample of Ticagrelor was prepared by combining 1 mL of each acidic, alkaline, oxidative, and photo-stressed sample at 100 µg/mL. The same was done for marketed and expired Ticagrelor tablets (90 mg), using 100 µg/mL for each condition.

RESULTS AND DISCUSSION

An RP-HPLC method for the estimation of Ticagrelor was successfully developed and optimized using a mobile phase consisting of acetonitrile and water containing 0.1% formic acid and 0.1% triethylamine (60:40, v/v). The optimized chromatographic conditions produced a sharp, symmetrical peak with a retention time of **5.63 min** at a detection wavelength of **256 nm**.

The developed method complied with all ICH validation requirements. System suitability parameters were within acceptable limits, confirming the efficiency of the chromatographic system. Linearity was established over the selected concentration range with a correlation coefficient (**R^2 = 0.9955**), indicating a strong linear relationship between concentration and peak area.

The accuracy of the method was evaluated by recovery studies at different concentration levels. The percentage recovery ranged from **99.46% to 99.69%**, demonstrating excellent accuracy. Precision studies showed low variability, confirming the reproducibility of the method. The method exhibited high sensitivity with a **Limit of Detection (LOD) of 0.185 µg/mL** and a **Limit of Quantification (LOQ) of 0.562 µg/mL**.

The assay of commercial Ticagrelor tablets revealed a drug content of **98.14**

Forced degradation studies demonstrated that Ticagrelor was most susceptible to degradation under acidic and alkaline conditions. Moderate degradation was observed under thermal and photolytic stress, whereas neutral hydrolytic and oxidative conditions produced minimal degradation.

System Suitability:

The system suitability of the HPLC method was evaluated by injecting the sample solution of Ticagrelor six times as per the test procedure. The standard chromatograms were analyzed to determine the percentage relative standard deviation (%RSD) of retention times, theoretical plates, tailing factor, and peak area.

Table 2. System suitability test of parameters of Ticagrelor

Sr. No	Retention Time (mins.)	Tailing Factor	Area	No. of Plates
1	5.638	1.16	289729	7522
2	5.637	1.17	290065	7452
3	5.636	1.16	292101	7611
4	5.632	1.15	293426	7563
5	5.636	1.16	295100	7703
6	5.638	1.16	290459	7608
Mean	5.636167	1.16	291813.3333	7576.5
±SD	0.002229	0.00632	2131.710925	85.83414
%RSD	0.0395%	0.54%	0.73%	1.13%

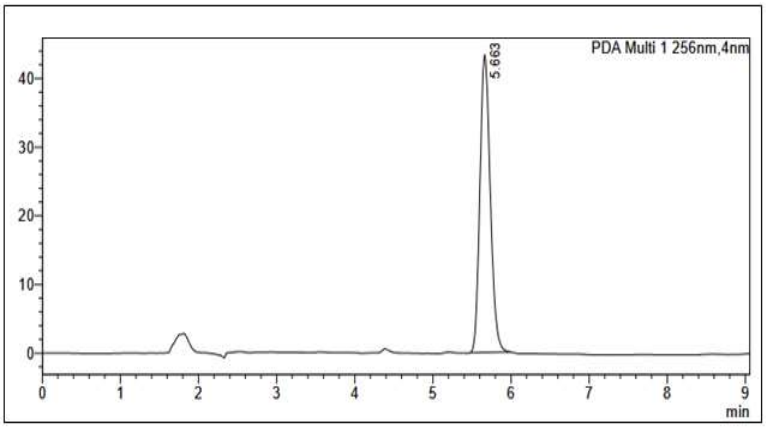

Figure 1. Chromatogram of System suitability

Specificity

The specificity of the method was evaluated by comparing the chromatograms of a blank solution and a ticagrelor standard solution. The retention times observed are recorded below

.

Table 3. Interference from drug sample with blank solution and standard solution

Sr. No	Solution	Retention time (min)
1	Blank	No peak observed
2	Ticagrelor standard	5.663

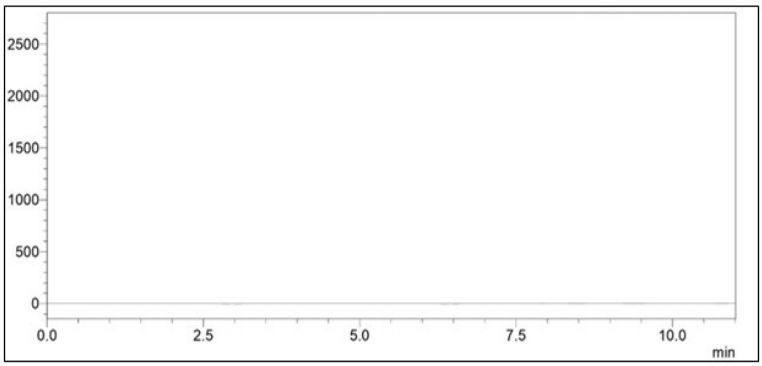

Figure 2. Chromatogram of blank

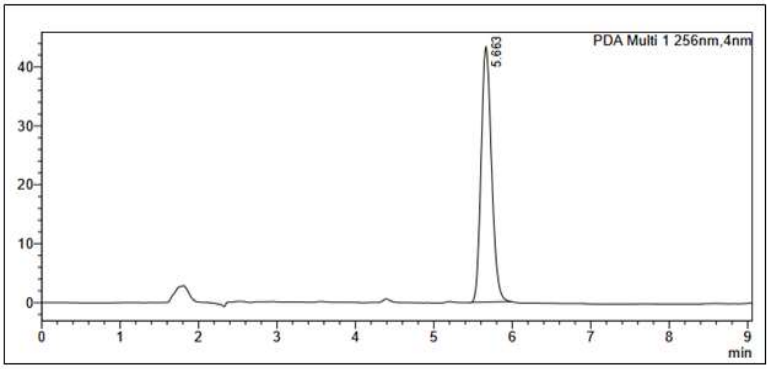

Figure 3. Chromatogram of standard Ticagrelor

Result of linearity

The linearity of the method was evaluated by plotting a graph of the average peak area at each concentration level (µg/mL). The resulting calibration curve was observed to be a straight line, demonstrating the method's linearity. The regression equation was determined as **y = 35755x + 32689**, with a correlation coefficient (R^2) of **0.9955** for Ticagrelor.

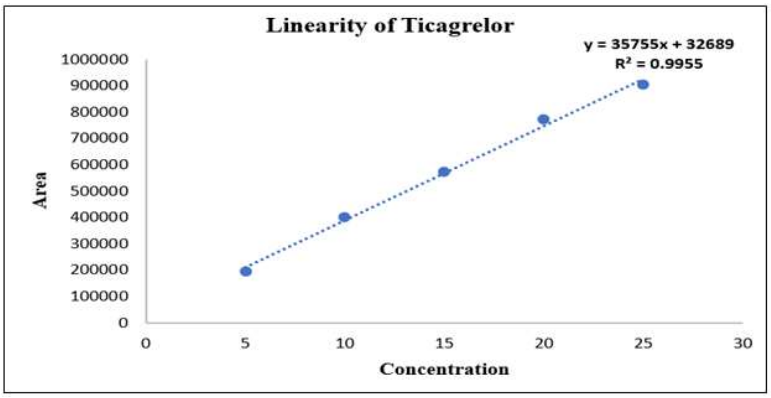

Figure 4. Linearity of Ticagrelor

Table 4. Linearity data of Ticagrelor

Sr. No	Concentration (µg/ml)	Peak Area
1	5	195824
2	10	400696
3	15	572610
4	20	771761
5	25	904160
Correlation coefficient (R^2)		0.995
Regression Equation		y = 35755x + 32689

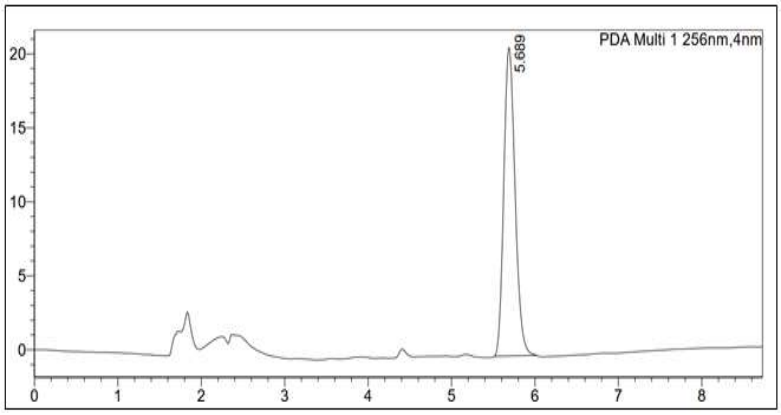

Figure 5. Linearity chromatogram of 5 µg/ml concentration

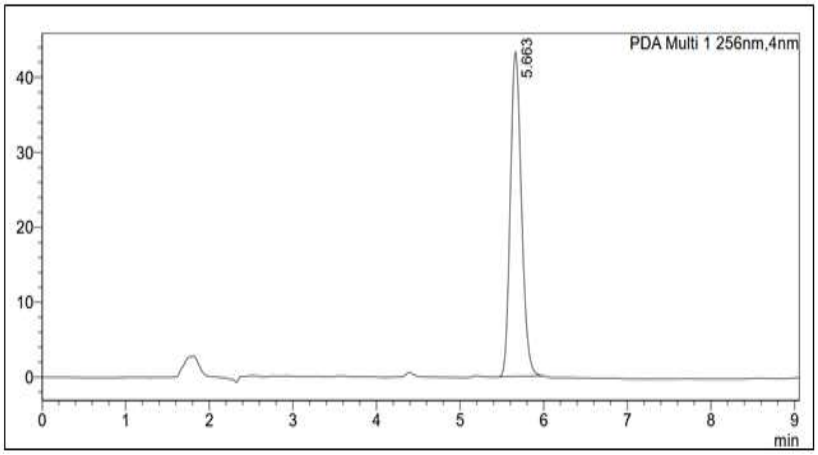

Figure 6. Linearity chromatogram of 10 µg/ml concentration

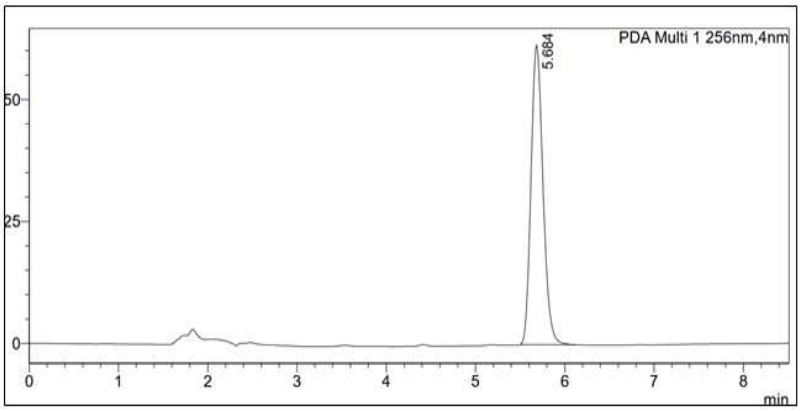

Figure 7. Linearity chromatogram of 15 µg/ml concentration

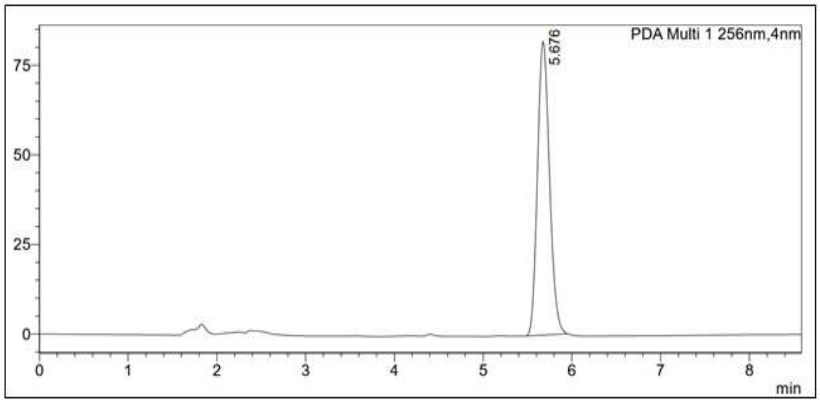

Figure 8. Linearity chromatogram of 20µg/ml concentration

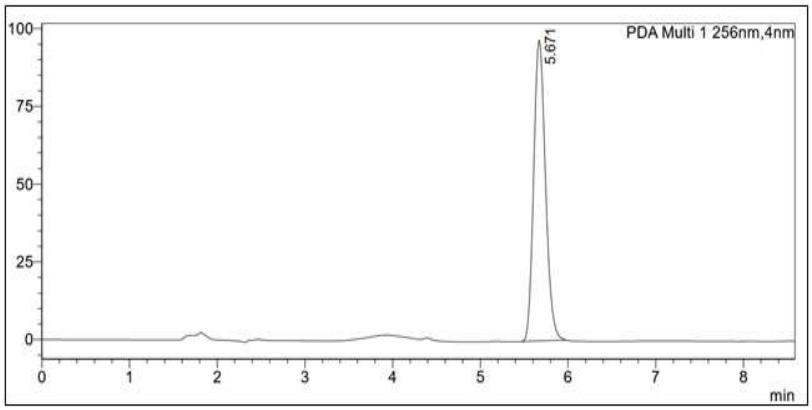

Figure 9. Linearity chromatogram of 25 µg/ml concentration

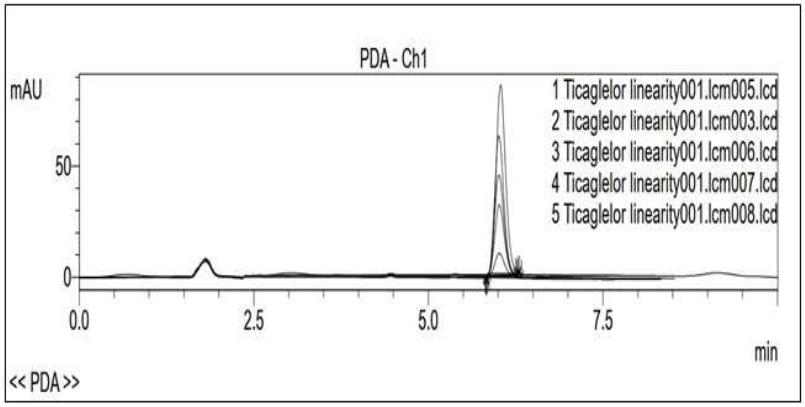

Figure 10. Superimposed Linearity peaks of Ticagrelor

Accuracy

To evaluate the accuracy, a recovery study was conducted using the standard addition method at 80%, 100%, and 120% of the test concentration. Accurately weighed pre-analyzed tablet powder equivalent to 5 mg of Ticagrelor (10% of the label claim) was transferred into nine separate 10 mL volumetric flasks. Standard Ticagrelor in solution form was then added at 30%, 50%, and 70% levels in triplicate to achieve a concentration range of 80–120% of the label claim. The contents were dissolved in an adequate amount of acetonitrile using ultrasonication for 20 minutes, followed by filtration through a 0.45 µm Millipore Nylon filter. The volume was then adjusted to the mark with acetonitrile. One milliliter of each filtrate was further diluted to 10 mL with the mobile phase, and solutions of 15, 20, and 25 µg/ml were prepared. The final solutions were filtered and analyzed using optimized chromatographic conditions The following results are reported under the Table no. 5,6 and 7.

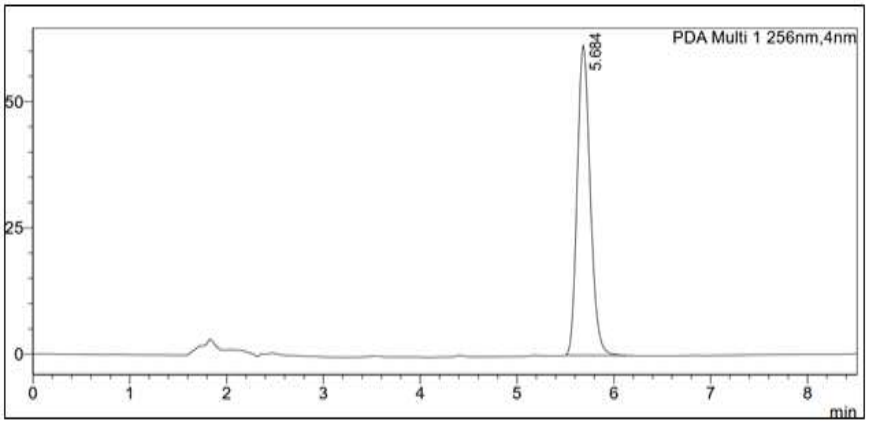

Figure 11. Chromatogram of 80% recovery – 01

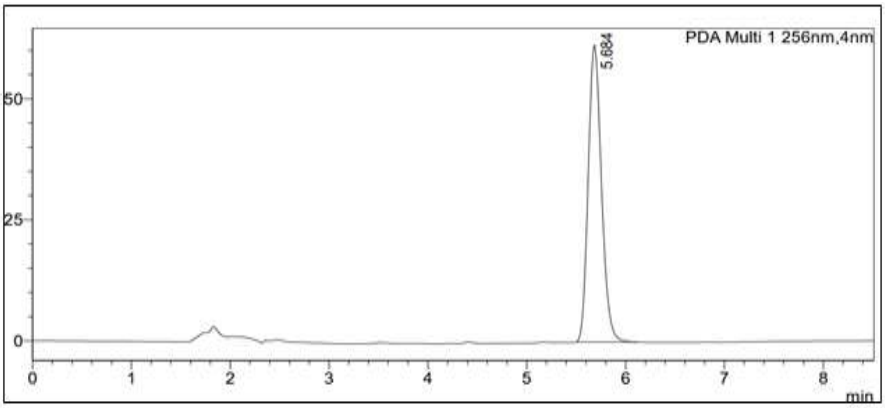

Figure 12. Chromatogram of 80% recovery -02

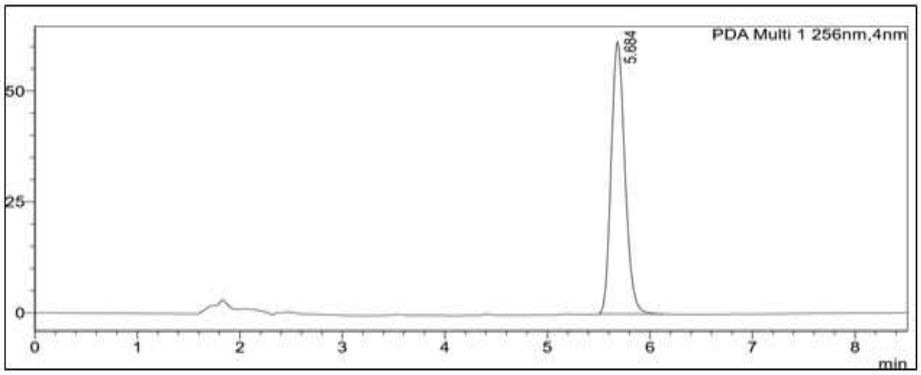

Figure 13. Chromatogram of 80% recovery -03

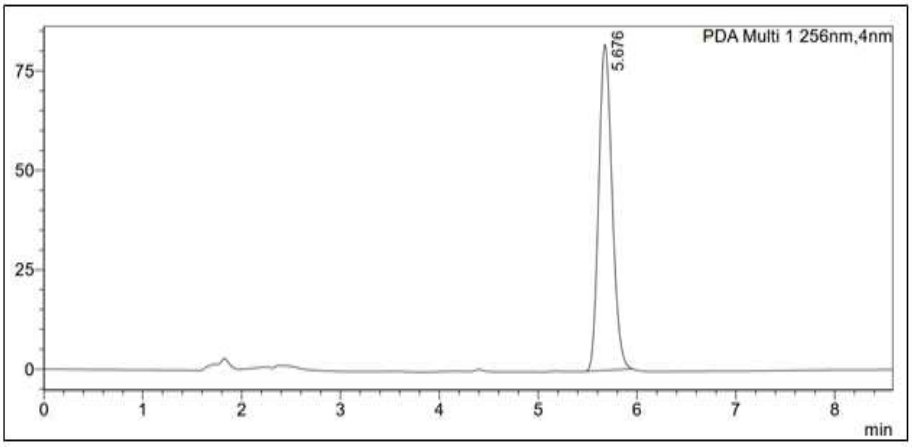

Figure 14. Chromatogram of 100% recovery -01

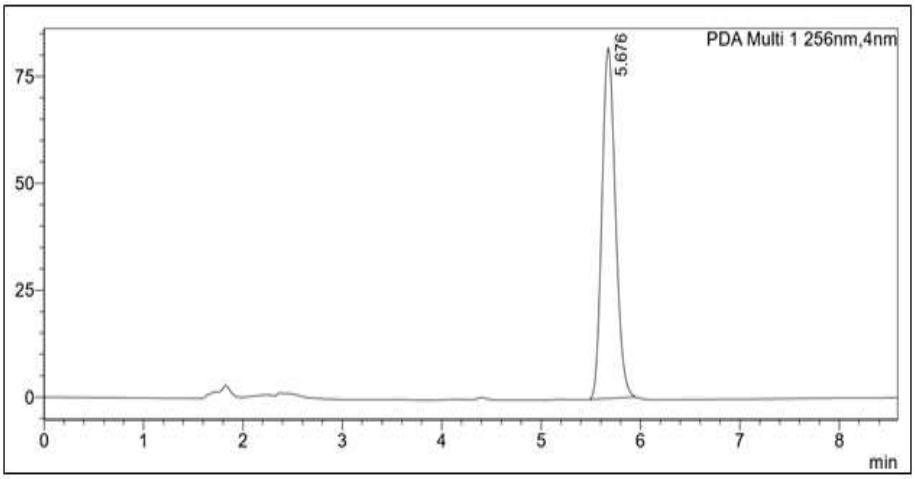

Figure 15. Chromatogram of 100% recovery -02

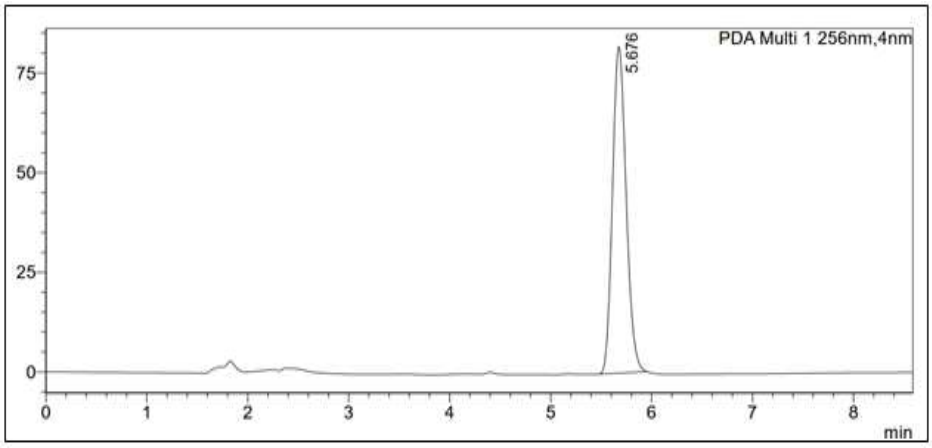

Figure 16. Chromatogram of 100% recovery -03

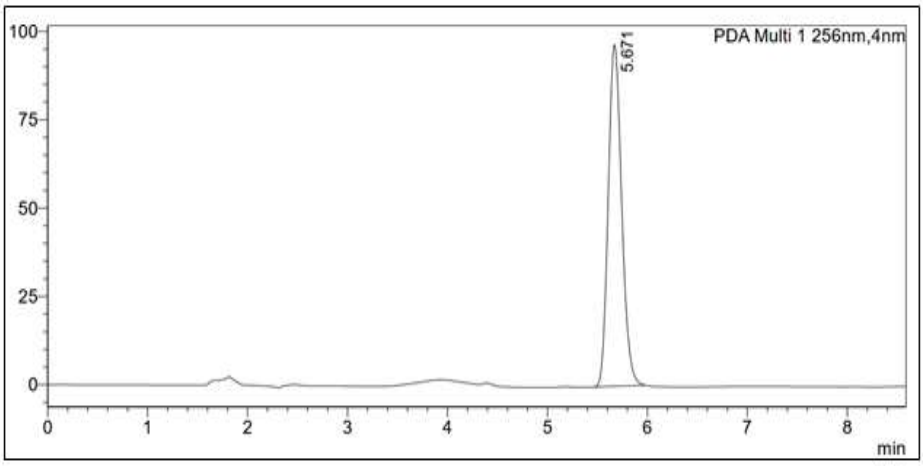

Figure 17. Chromatogram of 120% recovery -01

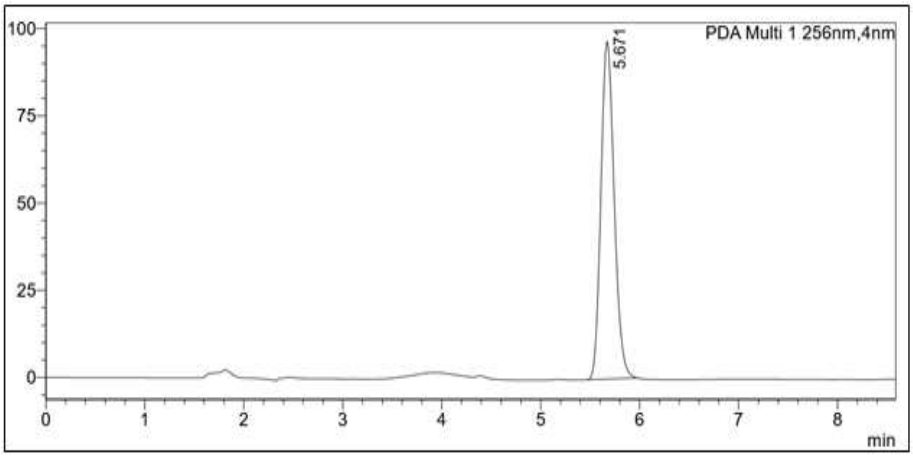

Figure 18. Chromatogram of 120% recovery -02

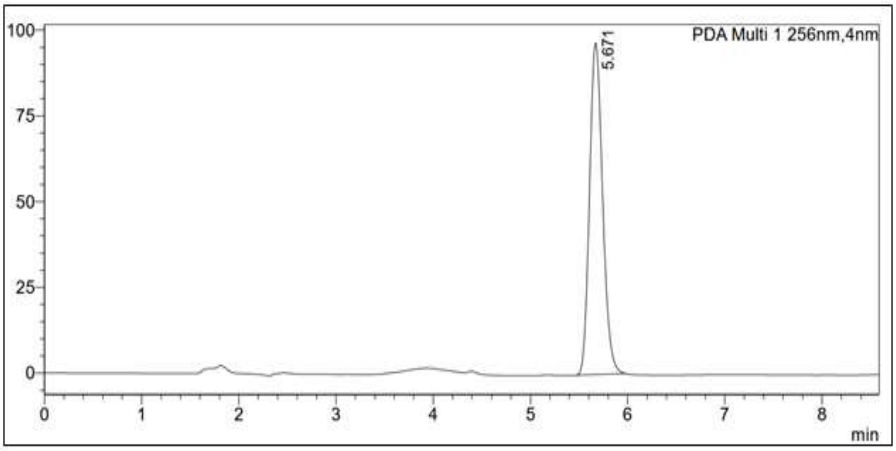

Figure 19. Chromatogram of 120% recovery -03

Table 5. Results of 80% recovery studies

Sr. No	Wt. of powder taken equivalent to 5mg	Amt. of pure Drug added(mg)	Sample peak area	Amt. of estimated drug (mg)	Amt. of recovered drug	%Recovery
1	17	3	572610	8.00	3.00	100.00
2	17	3	571812	7.99	2.99	99.63
3	17	3	570287	7.97	2.97	98.92
Mean						99.52
Standard deviation						0.549
%RSD						0.552

Table 6. Results of 100% recovery studies

Sr. No	Wt. of powder taken equivalent to 5mg	Amt. of pure Drug added(mg)	Sample peak area	Amt. of estimated drug (mg)	Amt. of recovered drug	%Recovery
1	17	5	771626	10.00	5.00	99.97
2	17	5	767572	9.95	4.95	98.91
3	17	5	769854	9.98	4.98	99.51
Mean						99.46
Standard deviation						0.5266
%RSD						0.5295

Table 7. Results of 120% recovery studies

Sr. No	Wt. of powder taken equivalent to 5mg	Amt. of pure Drug added(mg)	Sample peak area	Amt. of estimated drug (mg)	Amt. of recovered drug	%Recovery
1	17	7	901360	11.96	6.96	99.47
2	17	7	904130	12.00	7.00	100.00
3	17	7	902097	11.97	6.97	99.61
Mean						99.69
Standard deviation						0.272
%RSD						0.2728

Precision

Ticagrelor (10 µg/mL) was analyzed six times for repeatability, tested thrice in one day for intra-day precision, and over three days for inter-day precision. The following results are given in table no. 8,9 and 10.

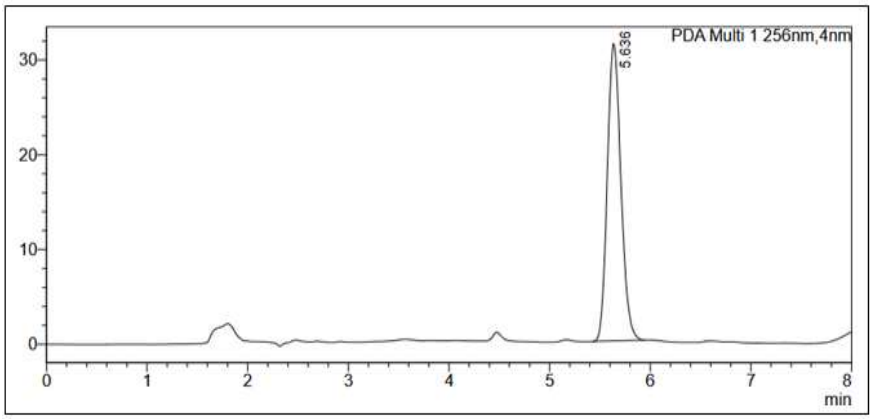

Figure 20. Precision graph

Intraday Precision

Table 8. Intraday precision on study of Ticagrelor

Sr. No	Concentration(µg/mL)	Area	Mean peak area	Standard deviation	%RSD
1	10	295362	294639.3	949.46	0.32%
	10	294992			
	10	293564			
2	10	292981	292382.3	1081.38	0.36%
	10	293032			
	10	291134			
3	10	294061	295108.7	946.77	0.32%
	10	295362			
	10	295903			

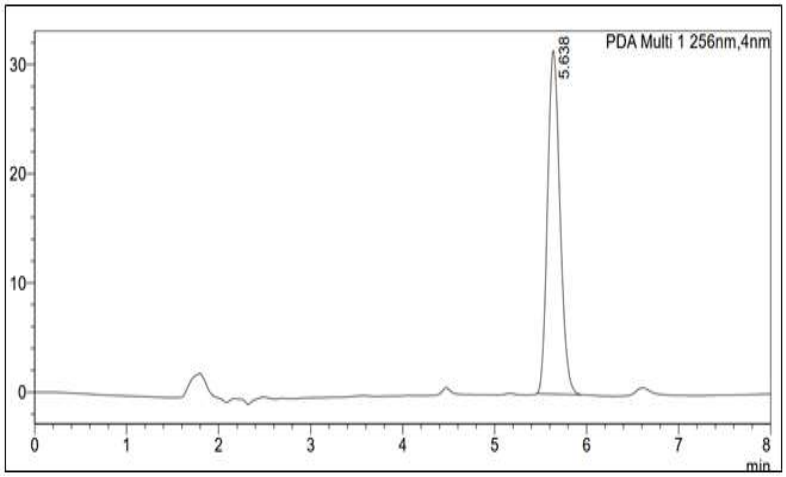

Figure 21. Chromatogram of intraday precision-01

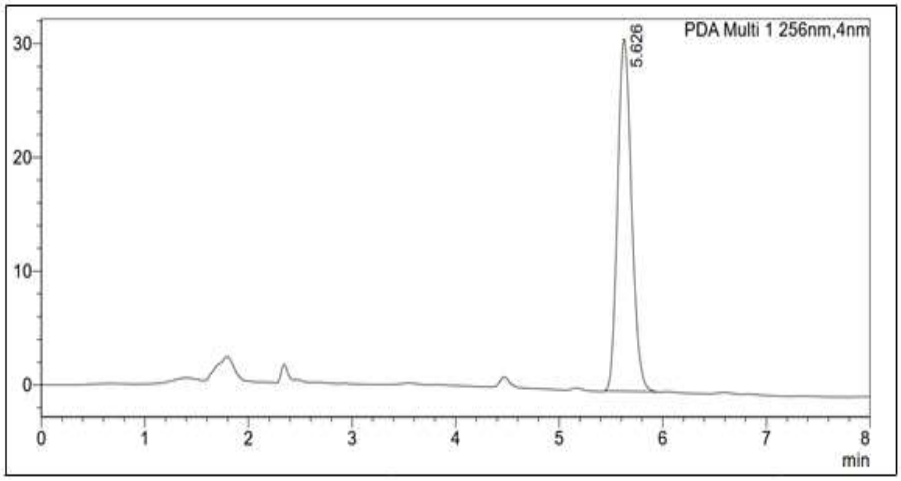

Figure 22. Chromatogram of intraday precision-02

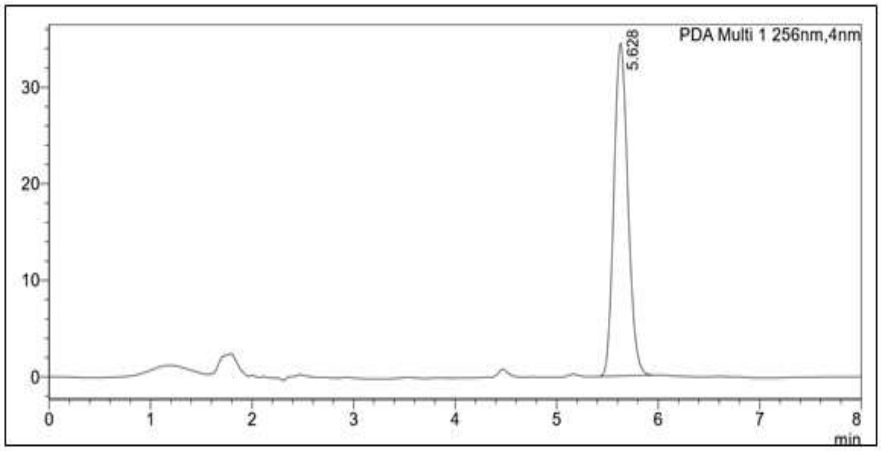

Figure 23. Chromatogram of intraday precision-03

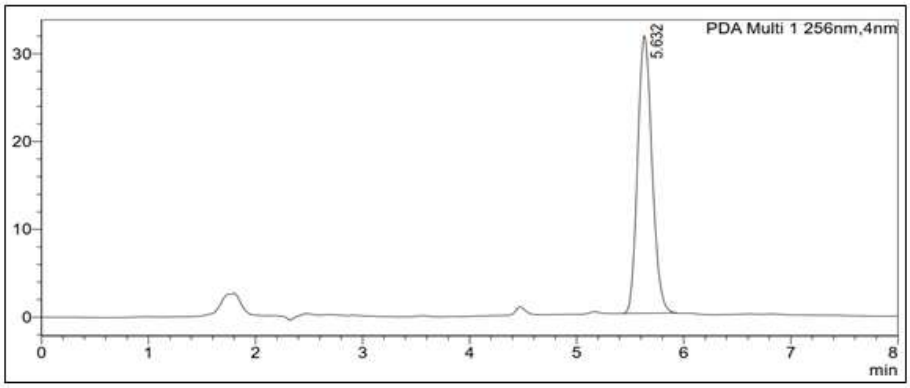

Figure 24. Chromatogram of intraday precision-04

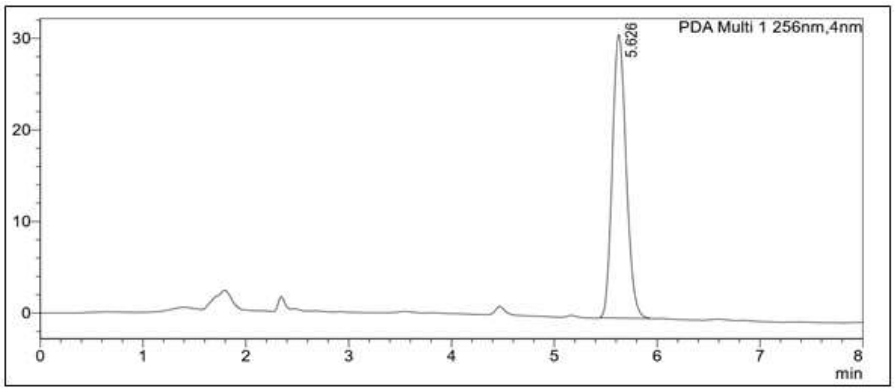

Figure 25. Chromatogram of intraday precision-05

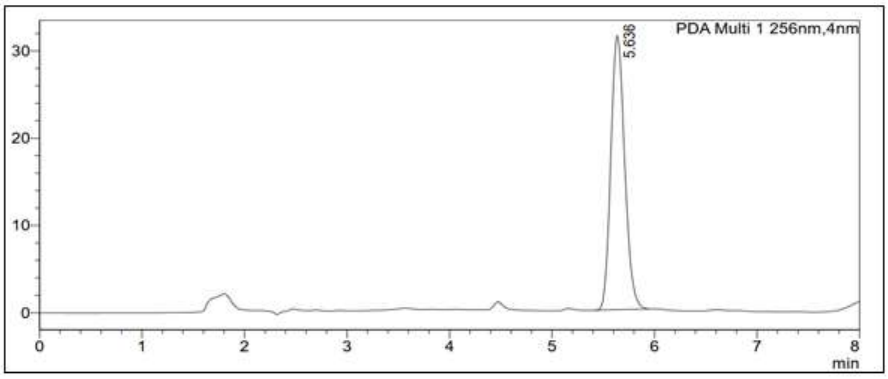

Figure 26. Chromatogram of intraday precision-06

Interday Precision

Table 9. Interday precision on study of Ticagrelor

Sr. No	Concentration(µg/mL)	Area	mean peak area	standard deviation	%RSD
1	10	295338	294187.7	1721.93	0.58%
	10	295017			
	10	292208			
2	10	293579	293055.33	2160.63	0.73%
	10	294906			
	10	290681			
3	10	295377	293706.3	2380.21	0.81%
	10	294761			
	10	290981			

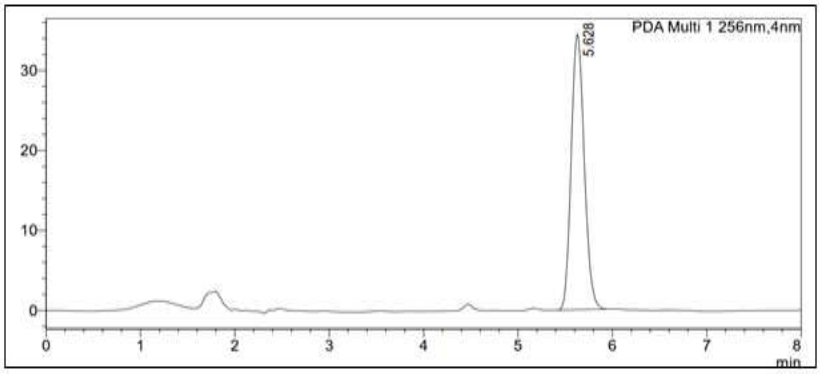

Figure 27. Chromatogram of interday precision-01

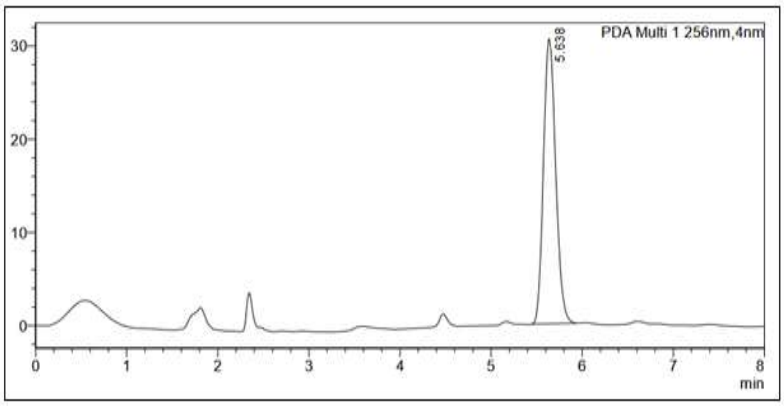

Figure 28. Chromatogram of interday precision-02

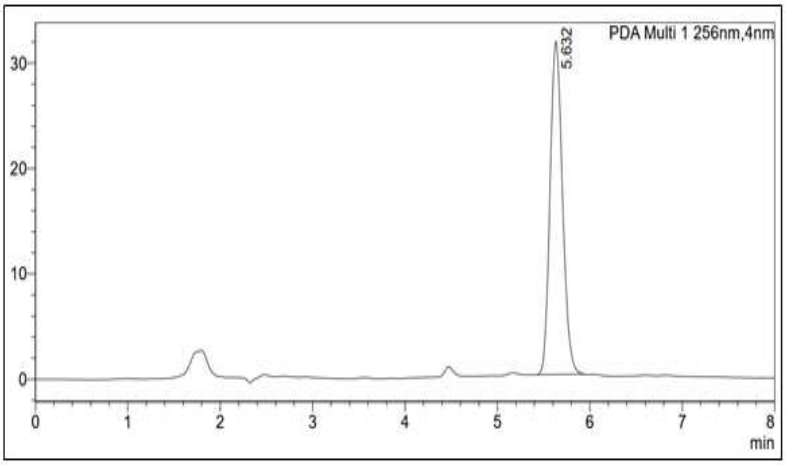

Figure 29. Chromatogram of interday precision-03

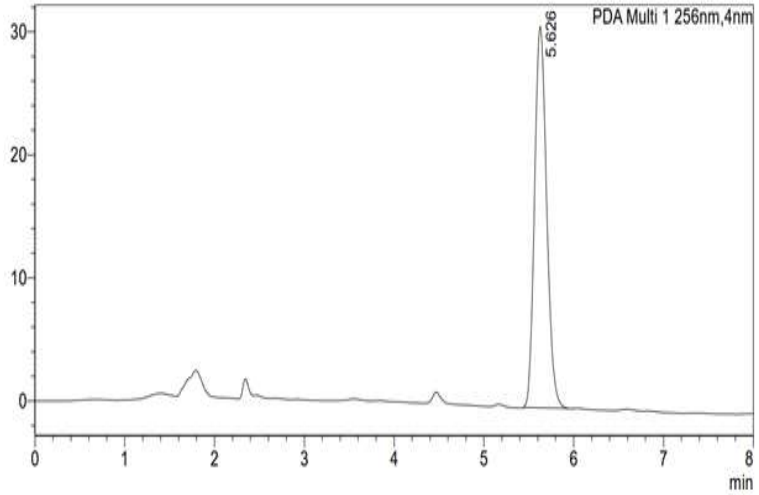

Figure 30. Chromatogram of interday precision-04

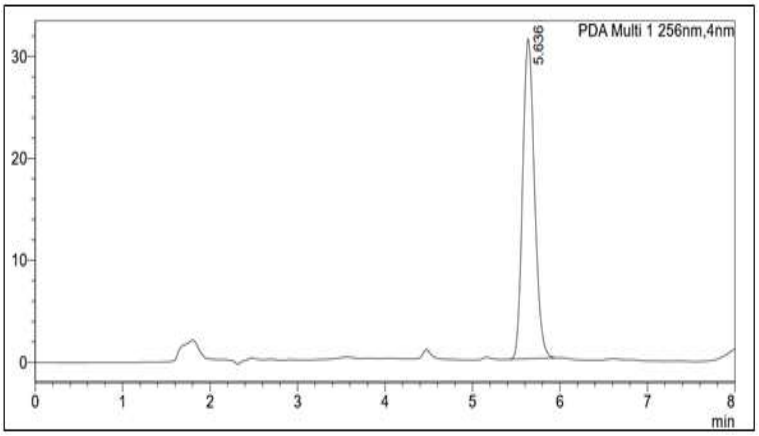

Figure 31. Chromatogram of interday precision-05

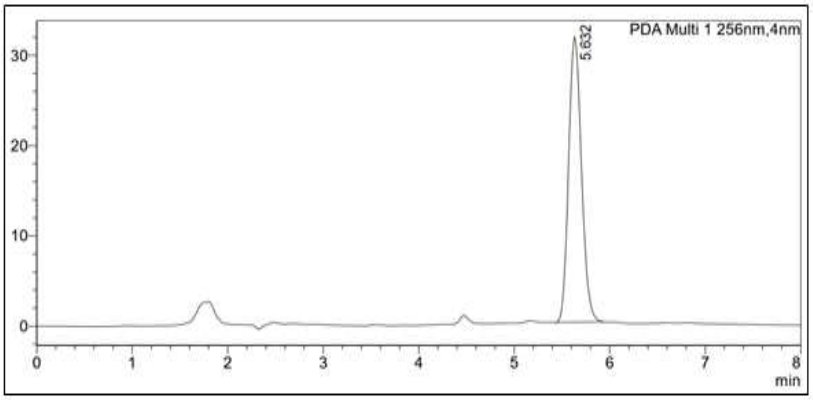

Figure 32. Chromatogram of interday precision-06

Repeatability

Table 10. Repeatability of Ticagrelor

Sr. No	Concentration(µg/mL)	Peak Area of Ticagrelor
1	10	289845
2	10	290162
3	10	292085
4	10	293337
5	10	294918
6	10	290534
Mean		2918123.5
SD		2013.56
%RSD		0.69%

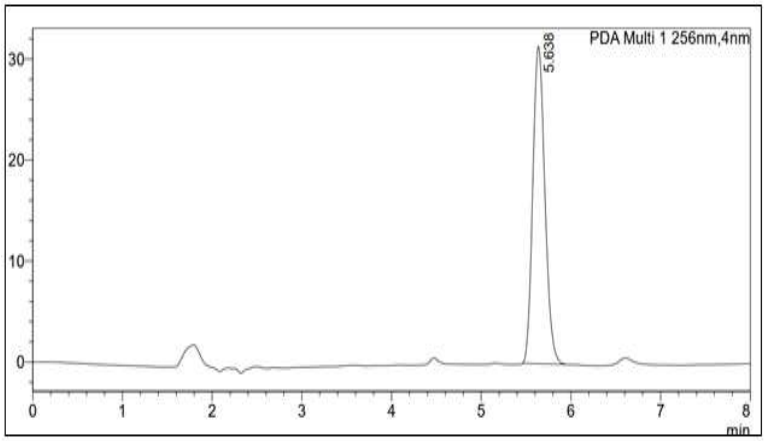

Figure 33. Chromatogram of repeatability -01

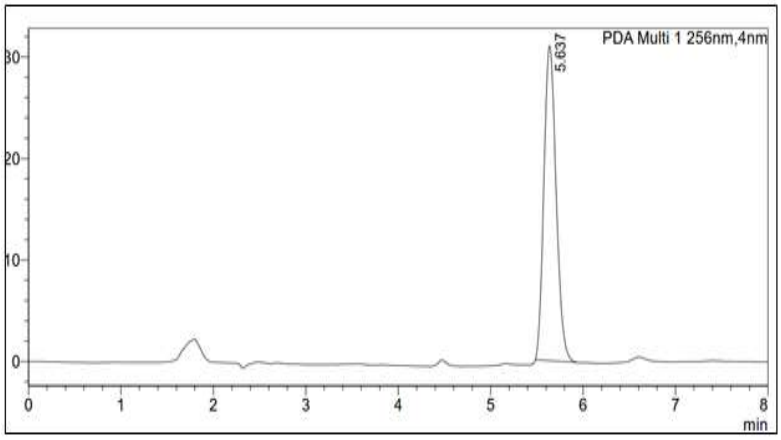

Figure 34. Chromatogram of repeatability -02

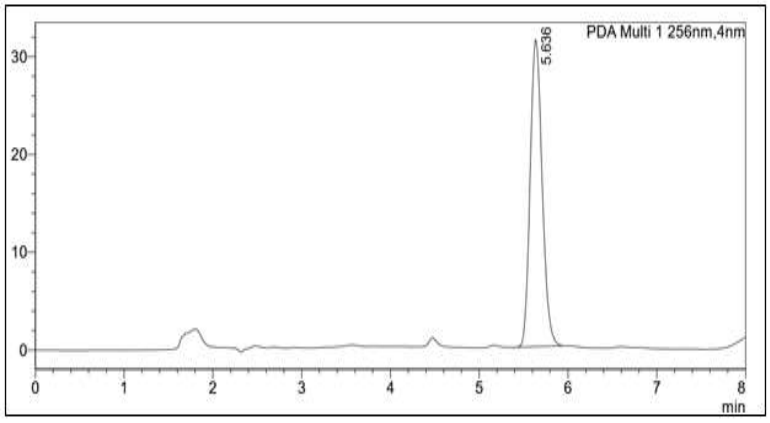

Figure 35. Chromatogram of repeatability -03

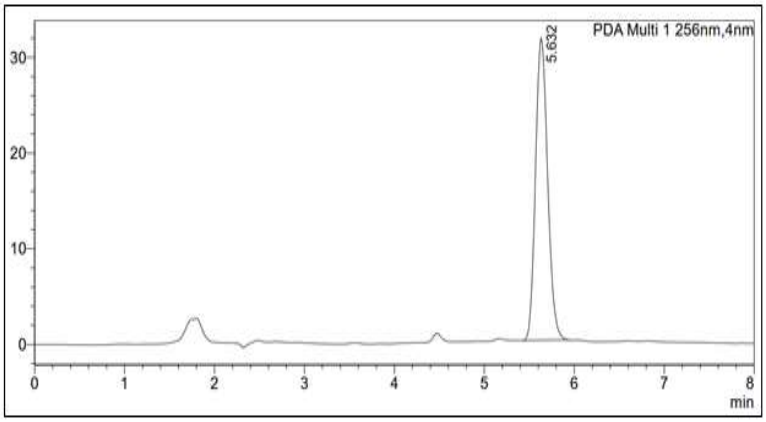

Figure 36. Chromatogram of repeatability -04

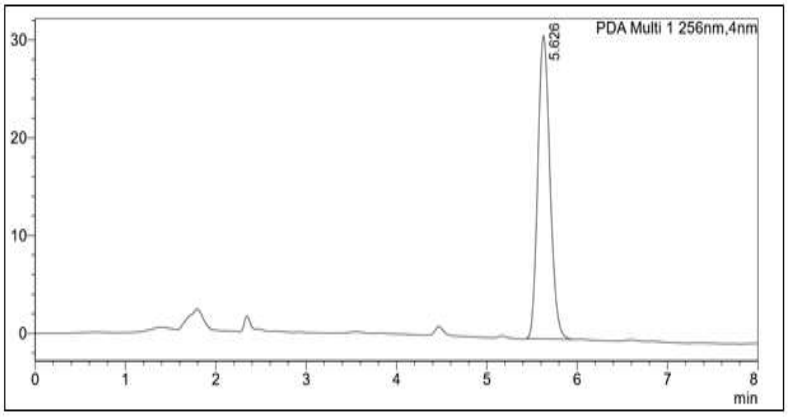

Figure 37. Chromatogram of repeatability -05

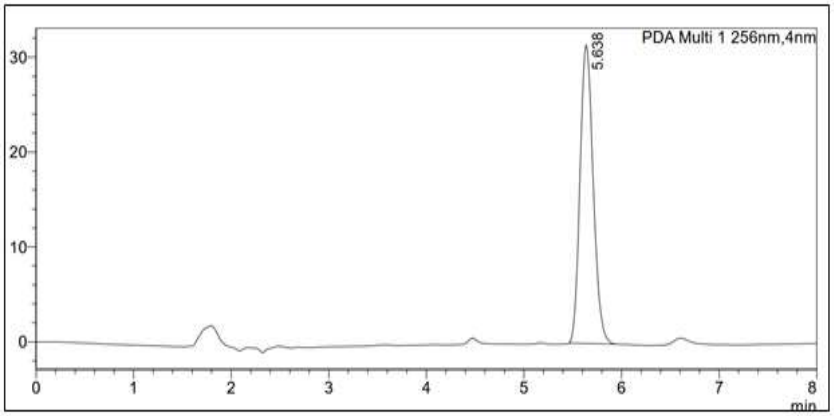

Figure 38. Chromatogram of repeatability -06

Limit of detection (LOD) and Limit of quantification (LOQ)

The Limit of Detection (LOD) is the minimum concentration of an analyte that can be detected but may not be precisely measured. The Limit of Quantification (LOQ) is the lowest concentration at which the analyte can be accurately and reproducibly quantified with confidence.

Table 11. Table for limit of detection and limit of quantification

LOD	0.185µg/ml
LOQ	0.562µg/ml

The limit of detection (LOD) for ticagrelor was found to be **0.185 µg/mL**, while the limit of quantification (LOQ) was determined to be **0.562 µg/mL**. These values indicated the sensitivity of the analytical method used. The low LOD suggested that even trace amounts of ticagrelor could be detected, while the LOQ ensured accurate and precise quantification.

Robustness

The robustness of the analytical method was evaluated to

determine its stability and reliability under slight variations in experimental conditions. Minor deliberate changes were made to method parameters such as flow rate, detection wavelength, and injection volume. The aim was to observe whether these small variations affected the performance of the method. The results showed that the method remained consistent and accurate, confirming its robustness for routine analysis.

Table 12. Results for robustness

Parameter	Variation	Area	Mean	SD	% RSD
Flow rate (ml/min)	1.1	285981	287055	1768.36	0.616%
	1.2	289096			
	1.3	286088			
Injection Volume (uL)	18	307578	294260.3	13901.27	0.047%
	20	295362			
	22	279841			
Wavelength (nm)	254	291056	293476.3	2233.85	0.761%
	256	295459			
	258	293914			

Ruggedness

The ruggedness was carried out under different conditions, such as variations in analysts and room temperatures. The study aimed to verify result consistency and reliability. Data analysis was performed to determine the influence of these factors, ensuring the method remained robust and accurate under various condition.

Table 13. Result of ruggedness

Parameter	Variations	Area	Mean	SD	%RSD
Different analyst	1	295274	297331.3	4727.58	1.59%
	2	293981			
	3	302739			
Room temperature	20	295578	292834.3	4057.90	1.38%
	25	288173			
	30	294752			

Assay

To calculate the percentage purity, the first step was to determine the concentration of the drug found in milligrams (mg).

Table 14. Calculation table for % purity of Pharmaceutical Dosage Form

Sample Type	Label claim (mg)	Weight taken equivalent to 50mg	Peak area	Tailing factor	theoretical plate	Standard peak area	%purity
Pharmaceutical Formulation	90	0.183	370845	1.16	7422	329788	98.14%

The assay of marketed ticagrelor tablet showed **98.14%** purity, meeting Indian Pharmacopoeia (IP) standards. Expired tablets had purity of **94.5%**, which was still within the acceptable 90.0–110.0% range but indicating drug degradation over time, which may impact potency and efficacy.

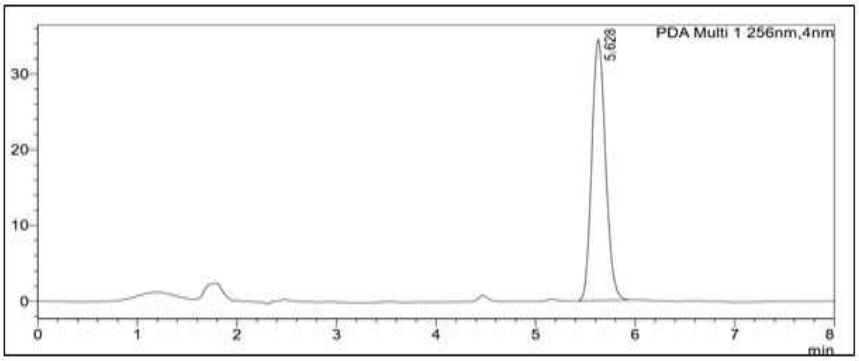

Figure 39. Chromatogram of marketed Ticagrelor STD

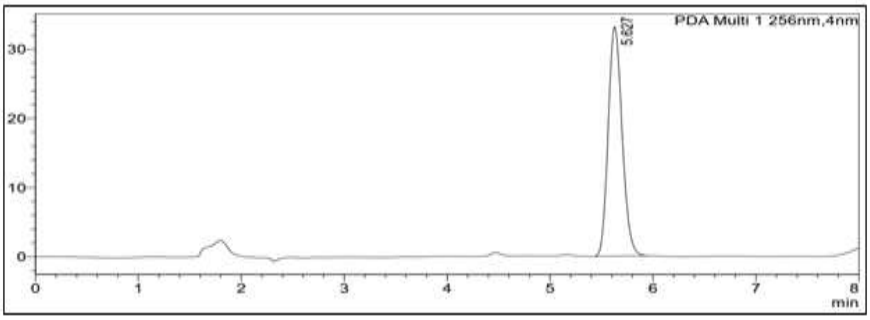

Figure 40: Chromatogram of marketed Ticagrelor STD

Force Degradation Study of Ticagrelor (API)

A forced degradation study was carried out on Ticagrelor in its API form, marketed tablet, and expired tablet under various stress conditions. The results showed that Ticagrelor degraded the most under acidic and basic conditions, with the highest degradation seen in the expired tablets. Moderate degradation was observed under photolytic and thermal conditions, while neutral hydrolysis caused only slight degradation. Oxidative stress showed the least impact on stability across all samples. Overall, Ticagrelor was found to be least stable in acidic conditions and most stable under oxidative stress. The expired tablets showed slightly higher degradation in all conditions compared to the marketed ones, indicate impact of product shelf life on chemical stability. The detailed results for each stress condition across all three forms are presented in the following table.no: 23,24 and 25.

Table 15. Force degradation study of Ticagrelor API

Sr. No	Sample and condition of exposure (API)	% Estimation	% Degradation
1	Neutral degradation (Refluxed for 8 h)	96.05%	3.95%
2	Basic degradation (At room temp. 8h)	88.82%	11.18%
3	Acidic degradation (Refluxed for 4 h)	85.95%	14.05%
4	Photo degradation(7days)	89.93%	10.07%
5	Thermal degradation (At 100 ^{0}C for 24 hours)	90.95%	9.05%
6	Oxidative degradation (At room temp 5h)	96.75%	3.25%

Table 16. Force degradation study of Pharmaceutical Dosage Form

Sr. No	Sample and condition of exposure (Pharmaceutical Dosage Form)	% Estimation	% Degradation
1	Neutral degradation (Refluxed for 8 h)	95.95%	4.05%
2	Basic degradation (At room temp. 8h)	88.15%	11.85%
3	Acidic degradation (Refluxed for 4 h)	84.21%	15.79%
4	Photo degradation(7days)	89.50%	10.50%
5	Thermal degradation (At 100 ^{0}C for 24 hours)	90.22%	9.78%
6	Oxidative degradation (At room temp 5h)	95.33%	4.67%

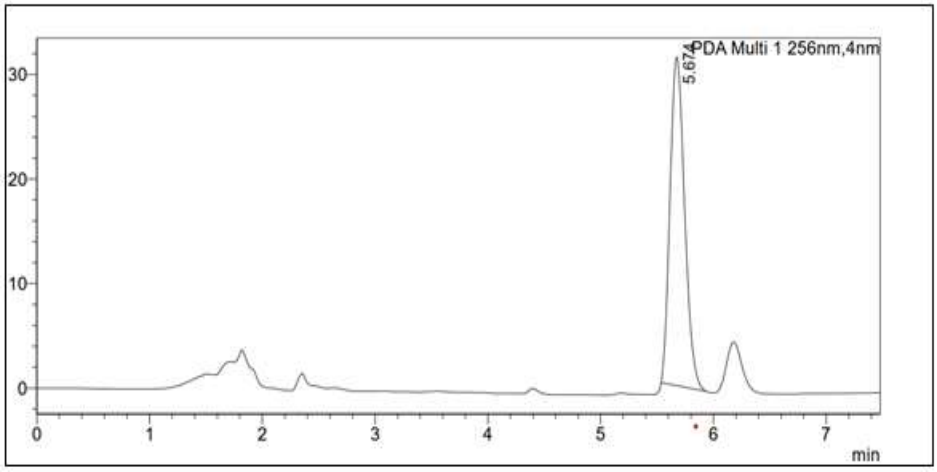

Figure 41. Chromatogram of ticagrelor (Neutral)

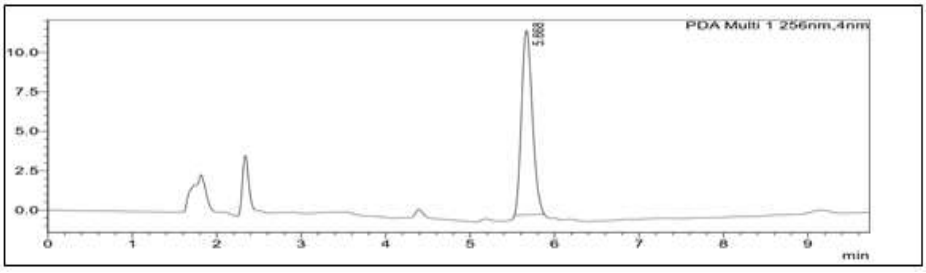

Figure 42. Chromatogram of ticagrelor (Basic degradation)

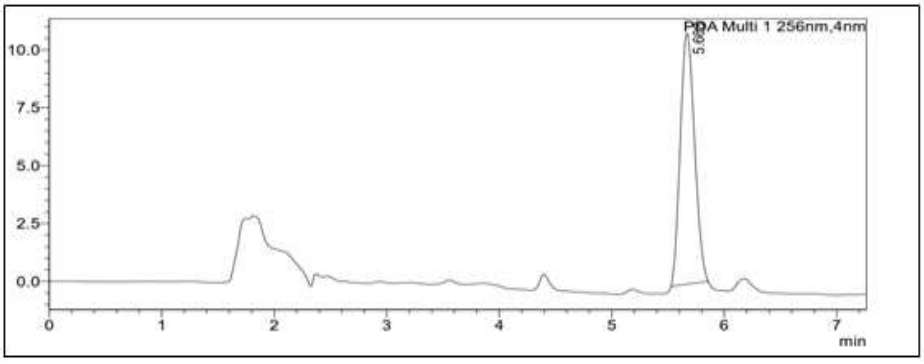

Figure 43. Chromatogram of ticagrelor (Acidic degradation)

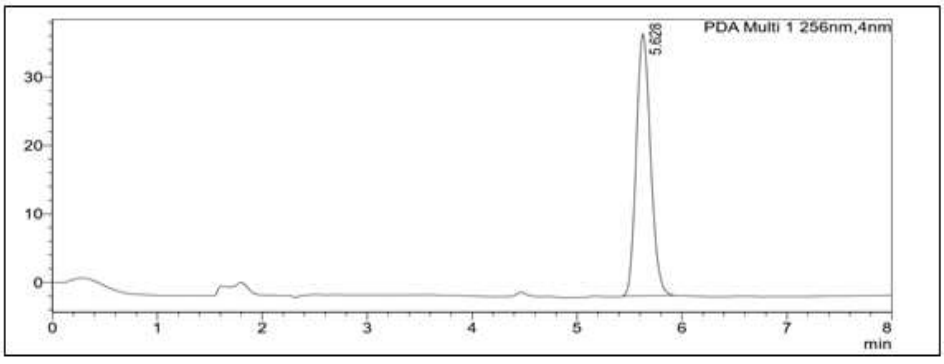

Figure 44. Chromatogram of ticagrelor (Photo degradation)

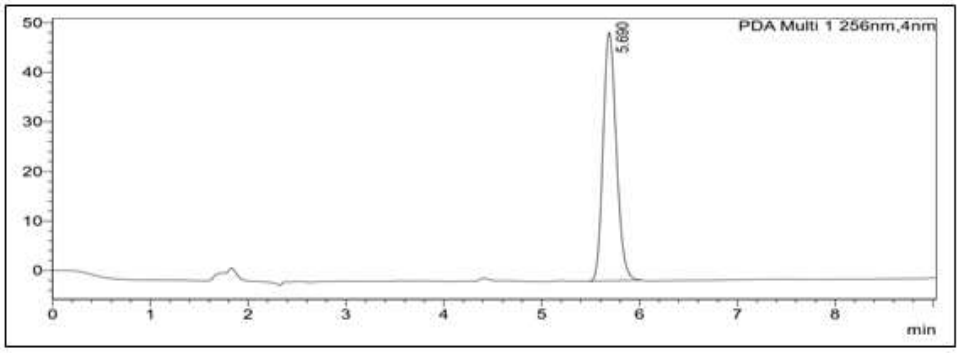

Figure 45. Chromatogram of ticagrelor (Thermal Degradation)

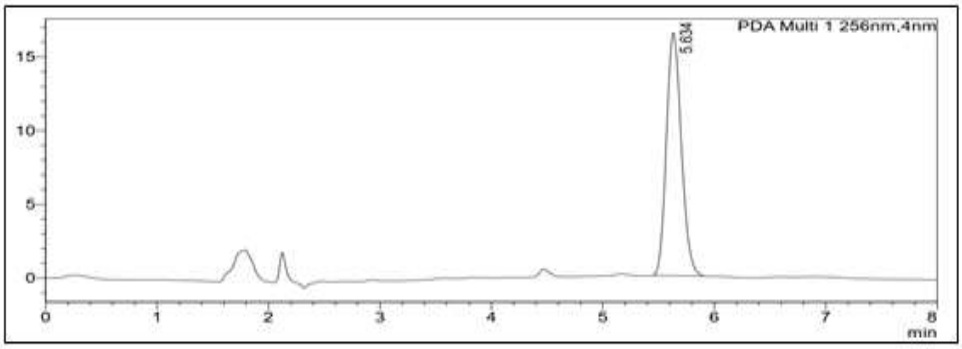

Figure 46. Chromatogram of ticagrelor (Oxidative degradation)

DISCUSSION

The optimized RP-HPLC method provided satisfactory chromatographic performance with a well-resolved Ticagrelor peak and a short retention time of 5.63 minutes, making it suitable for routine pharmaceutical analysis. The selected mobile phase composition ensured good peak symmetry and reproducible chromatographic behavior.

Validation results confirmed that the method fulfilled the requirements recommended by ICH guidelines. The excellent linearity (R^2 = 0.9955) demonstrated that the method is capable of accurately measuring Ticagrelor over the selected concentration range. Recovery values close to 100% indicated that the method is highly accurate and free from interference by excipients. Similarly, the low variability observed during precision studies confirmed the reproducibility and reliability of the analytical procedure.

The low LOD and LOQ values demonstrated that the method possesses sufficient sensitivity to detect and quantify small amounts of Ticagrelor, making it applicable for routine quality control, formulation development, and stability studies.

Forced degradation studies confirmed that Ticagrelor is more vulnerable to acidic and alkaline environments, indicating possible hydrolytic degradation under extreme pH conditions. The comparatively lower degradation under oxidative, neutral, thermal, and photolytic conditions suggests that the drug possesses acceptable stability under normal storage conditions but should be protected from excessive heat, moisture, and inappropriate pH environments.

Overall, the developed RP-HPLC method proved to be accurate, precise, sensitive, and stability-indicating, making it suitable for routine quality control.

CONCLUSION

A simple, rapid, accurate, precise, and stability-indicating RP-HPLC method was successfully developed and validated for the estimation of Ticagrelor in bulk drug and tablet dosage forms according to ICH guidelines. The optimized chromatographic conditions provided excellent peak resolution with a retention time of 5.63 minutes and satisfactory system suitability characteristics.

The validation results demonstrated excellent linearity, accuracy, precision, and sensitivity, confirming the suitability of the developed method for routine pharmaceutical analysis. Forced degradation studies established that the method can effectively separate Ticagrelor from its degradation products, demonstrating its stability-indicating capability.

Therefore, the developed RP-HPLC method can be confidently employed for routine quality control, assay determination, stability testing, and pharmaceutical research involving Ticagrelor formulations.

REFERENCES

1. Allen L, Ansel HC. *Ansel's pharmaceutical dosage forms and drug delivery systems*. Philadelphia: Lippincott Williams & Wilkins; 2013.
2. Zarmpi P, Flanagan T, Meehan E, Mann J, Fotaki N. Eur J Pharm Biopharm. 2017;111:1-15.
3. Chauhan YS, Nex R, Sevak G, Rathore MS. Stability testing of pharmaceutical products. Res J Pharm Dos Forms Technol. 2021;13(4):317-328.
4. Faya P, Seaman JW Jr, Stamey JD. Stat Med. 2018;37(17):2599-2615.
5. Food and Drug Administration. Guidance for industry: stability testing of drug substances and drug products. USA: Food and Drug Administration; 1998. Available from: http://www.fda.gov/ohrms/dockets/98fr/980362gd.pdf
6. Waterman KC, Adami RC. Accelerated aging: prediction of chemical stability of pharmaceuticals. Int J Pharm. 2005;293(1-2):101-125. doi:10.1016/j.ijpharm.2004.12.013.
7. Robards K, Ryan D. *Principles and practice of modern chromatographic methods*. London: Academic Press; 2021.
8. Ermer J, Nethercote PW, editors. *Method validation in pharmaceutical analysis: a guide to best practice*. Chichester: John Wiley & Sons; 2025.
9. Chavda H. Drug Dev Ind Pharm. 2021;47(9):1373-1391.
10. International Conference on Harmonisation. Q1A(R2): stability testing of new drug substances and products. Geneva: ICH; 2003.
11. Williams H. Predictive stability testing utilizing accelerated stability assessment program (ASAP) studies. In: Bajaj S, Singh S, editors. *Methods for stability testing of pharmaceuticals*. New York: Humana; 2018. p. 213-232.
12. Sharma S, Sharma AK, Moe HW, Khanapure A, Mohan P. Med J Armed Forces India. 2022;78:S194-S200.
13. Sridharan K, Jain SK. Do drugs really expire beyond the expiry date? Evaluation of expired (30 years from the manufacturing date) acetaminophen suspension and captopril tablet preparations. Pharm Pract (Granada). 2024;22(1):2906.
14. Swaroop AP, Varun D. Pharmanest Int J Adv Pharm Sci. 2011;2(5-6):423-433.
15. Kudari DRK, Rao CP, Prasanna CL, Kumar MS, Sulthana S. NeuroQuantology. 2022;20(15):7178-7196.
16. Barroso J, Sandelowski M, Voils CI. J Eval Clin Pract. 2006;12(4):454-462.
17. Beery S, Miller C, Sheridan D. Nursing2023. 2019;49(8):17.
18. González-González O, Ramirez IO, Ramirez BI, O'Connell P, Ballesteros MP, Torrado JJ, et al. Pharmaceutics. 2022;14(11):2324.
19. Brion G, Bunkers L. Expiration dating and National Drug Code rules. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2026.
20. Khan SR, Kona R, Faustino PJ, Gupta A, Taylor JS, Porter DA, et al. J Pharm Sci. 2014;103:1331-1336.
21. Gikonyo D, Gikonyo A, Luvayo D, Ponoth P. Drug expiry debate: the myth and the reality. Afr Health Sci. 2019;19(3):2737-2739.
22. Arioua A, Shaw D. JMA J. 2024;7:1-4.
23. Sarla GS. Egypt J Intern Med. 2019;31:431-434.
24. Sharma MK, Murugesan M. J Chromatogr Sep Tech. 2017;8:349.
25. Blessy MRDP, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs—A review. J Pharm Anal. 2014;4(3):159-165.
26. Iram F, Iram H, Iqbal A, Husain A. J Anal Pharm Res. 2016;3:73.
27. Chew YL, Khor MA, Lim YY. Choices of chromatographic methods as stability indicating assays for pharmaceutical products: a review. Heliyon. 2021;7(3):e06553.
28. Janzen H. Forced degradation studies—comparison between ICH, EMA, FDA and WHO guidelines and ANVISA's resolution RDC 53/2015. Bonn: University of Bonn; 2016. Master's thesis.
29. Shinde NG, Bangar BN, Deshmukh SM, Sulake SP, Sherekar DP. Asian J Res Pharm Sci. 2013;3:178-188.
30. Lyon RC, Taylor JS, Porter DA, Prasanna HR, Hussain AS. J Pharm Sci. 2006;95:1549-1560.
31. Bodas M, Yuval L, Zadok R, Hess Z, Haran B, Kaplan M, et al. Biosecur Bioterror. 2012;10:182-187.
32. Sutar SV, Yeligar VC, Patil SS. A review: stability indicating forced degradation studies. Res J Pharm Technol. 2019;12(2):885-890. doi:10.5958/0974-360X.2019.00152.5.
33. Singh S, Bakshi M. Guidance on conduct of stress tests to determine inherent stability of drugs. Pharm Technol. 2000;24(9):1-14.
34. Moldoveanu SC, David V. *Essentials in modern HPLC separations*. Amsterdam: Elsevier; 2022.

35. Rastegar L, Mighani H, Ghassempour A. Anal Biochem. 2018;557:123-130.

36. Sunil A, Anju G, Rajat V. Org Med Chem Int J. 2018;6:143-146.

37. Isane SP, Waghmare SA, Kamble HV. A review on method development, validation, optimization and applications of HPLC. Int J Res Appl Sci Eng Technol. 2022;10:1860-1867.

38. Chavan SD, Desai DM. World J Adv Res Rev. 2022;16:389-402.

39. Indian Pharmacopoeia Commission. *Indian pharmacopoeia*. Vol. 1. Ghaziabad: Indian Pharmacopoeia Commission; 2018. p. 324-325.

40. Kabil MF, Dena ASA, El-Sherbiny IM. Profiles Drug Subst Excip Relat Methodol. 2022;47:91-111.

41. Marin LM, Sîrbu I, Ozon EA, Skotnicki M, Lulek J, Chivu RD, et al. Ticagrelor—mechanism of action and its impact on drug efficacy. Farmacia. 2024;72(2):273-279. doi:10.31925/farmacia.2024.2.4.