Application of Stability-indicating RP-HPLC Method for quantification of Ticagrelor In Oral dispersible tablet dosage form

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Abstract

A simple, rapid, accurate and precise stability-indicating HPLC method was developed and validated for the determination of Ticagrelor in oral dispersable tablets. Separation of the drug was achieved on Shimadzu HPLC comprising of LC- 20 AD binary gradient pump, a variable wavelength programmable SPD-20A detector and SCL system controller. C18G column (250 mm x 4.6 mm, 5 μ) as stationary phase with mobile phase consisting of methanol and water in the ratio of 25: 75 v/v. The method showed a good linear response in the concentration range of 10-90 μg/ml with correlation coefficient of 0.999. The flow rate was maintained at 1.0 ml/min and effluents were monitored at 254 nm. The retention time was 5.786 min. The method was statistically validated for accuracy, precision, linearity, ruggedness, robustness, solution stability, selectivity and forced degradation studies. Stress conditions including acid, alkali hydrolysis, water stress, oxidation, photolysis, and heat were applied. The degradation products did not interfere with the detection of Ticagrelor, thus the method can be considered as a stability indicating method. The results obtained in the study were within the limits of ICH guidelines and hence this method can be used for the determination of Ticagrelor in oral dispersible tablets.

Keywords: Ticagrelor; HPLC; Stability-indicating; Oral Dispersable Tablets; ICH

1. Introduction

Ticagrelor (1S,2S,3R,5S) – 3 · (7-{{(1R,2S) – 2 -(3,4difluoro phenyl) cyclopropyl]amino}-5-(propylsulfanyl)-3H-[1,2,3] triazolo[4,5-d]pyrimidin-3-yl]-5-(2-hydroxyethoxy) cyclopentane-1,2-diol. (TCG) is a platelet aggregation inhibitor used for the prevention of thrombotic events (myocardial infarctions, strokes) in patients with acute coronary syndromes. It belongs to the class of triazolopyrimidines which are polycyclic aromatic compounds containing triazole ring fused to a pyrimidine ring. Ticagrelor and its major metabolite reversibly interact with the platelet P2Y12 ADP-receptor to prevent signal transduction and platelet activation. It is a white crystalline powder with an aqueous solubility of approximately 10μg/ml at room temperature. It has a log P of 2.30, pKa of strong acidic function 12.94 and strong basic function 2.90. It prevents platelet aggregation and thrombus formation in atherosclerotic disease which reduces chances of cardiac arrest due to blockage. The marketed formulation of ticagrelor replaced dopiporgelcontaining formulations due to higher efficacy and lower side effects. It was approved for use in the European Union by the European Commission on December 3, 2010, by the US Food and Drug Administration on July 20, 2011. It is marketed under the name Brilinta® in the USA and Brilique® in the EU. The Brilinta® is available as tablet dosage form and Brilique® is available in orodispersible tablet containing 90 mg ticagrelor. The orodispersible tablets may be used as an alternative to Brilique 90 mg film-coated tablets for patients who have difficulty swallowing the tablets whole or for whom there is a preference for orodispersible tablets. The tablet should be placed on the tongue, where it will rapidly disperse in saliva.

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Literature survey reveals various analytical methods reported for estimation of Ticagrelor in tablets dosage forms includes spectrophotometry[1-5] and HPLC[6-7] There are some chromatographic methods reported for estimation of ticagrelor in presence of its impurities. Very few stability indicating RP-HPLC methods[8-9] reported of estimation of Ticagrelor in tablets dosage form. Ticagrelor is also available in orodispersible tablets (Brilique®) but to date no stability indicating RP-HPLC methods reported of estimation of Ticagrelor in orodispersible tablets dosage form. There were accurate and sensitive analytical methods available in literature for other drugs.[10-70] According to Current Good Manufacturing Practices, all drugs must be tested with a stability-indicating assay method before release. It was felt necessary to develop a stability-indicating liquid chromatography (LC) method for the determination of Ticagrelor as bulk drug and as pharmaceutical dosage forms and to separate the drug from the degradation products under the International Conference on Harmonization (ICH) suggested conditions[71] (hydrolysis, oxidations, photolysis and thermal stress). Therefore, the principal objective of this study was to develop a new, simple, economical, precise, and reproducible stability indicating RP-HPLC method with a wide linear range and good sensitivity for assay of Ticagrelor in the bulk drug and in the pharmaceutical dosage forms (orodispersible tablets).

2. Materials and methods

2.1. Chemicals

Ticagrelor pure drug (purity >99.8%) was obtained as gifted sample from RA Chem Pharma Ltd(Hyderabad,India), HPLC grade water and acetonitrile was from MERCK India Ltd. HPLC grade methanol was from standard reagent Pvt Ltd Hyderabad. Analytical grade hydrochloric acid, sodium hydroxide and hydrogen peroxide was from SD Fine chemicals Mumbai, India. Nylon membrane filters 0.2 µm and 0.45 µm were from PALL life sciences Mumbai, India.

2.2. Equipment

The instruments used in the study were electronic balance (Apex,India), sonicator (LAB india Ltd Mumbai, 3.5 L), hot air oven (Accumaxindia), and digita pH meter (Elico LI 120). HPLC (Shimadzu, Kyoto, Japan) was monitored and integrated using LC solutions software. Additionally, syringe [Hamilton (Rheodyne-20 μL)] and syringe filter [Himedia Syringe-driven filters (0.22 μm)] were used.

2.3. Chromatographic conditions

The chromatographic system used for the method development and validation consisted of Shimadzu HPLC comprising of LC-20AD binary gradient pump, a variable wavelength programmable SPD-20A detector and an SCL 20A system controller. A Rheodyne injector 7725i fitted with a 20 μL loop was used and data were recorded and evaluated by use of LC solutions software version 5.0. Separation was performed on a Enable C18G (250 × 4.6 mm i.d.,5µ) at ambient temperature. A mixture of methanol and water in the ratio of 20: 80 v/v was found to be the most suitable mobile phase for ideal chromatographic separation of Ticagrelor. The solvent mixture was filtered through 0.45 μ membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1.0 mL/min. Injection volume was 20 μL and the column was maintained at room temperature. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of the drug solution. The detection of the drug was monitored at 254 nm. The run time was set at 10 min

2.4. Preparation of mobile phase

Mobile phase was prepared by mixing 200 mL of HPLC grade methanol with 800 mL of HPLC grade water. The mobile phase was sonicated for 10 min and filtered through the 0.45 μm membrane filter.

2.5. Preparation of standard stock solutions

The standard stock solutions of 100 μg/mL of the drug were prepared by dissolving 50 mg of pure drug in the methanol in a 50 mL volumetric flask and the volume was made up to the mark Resulting solutions were further diluted with mobile phase to obtain a final concentration of 100 μg/mL and stored under refrigeration. Aliquots of standard stock solutions were put in a 10 mL volumetric flask and diluted up to the mark with mobile phase. In such a way, the final concentrations of the drug were in the range of 10–90 μg/mL.

2.6. Preparation of sample solution

Ten tablets were accurately weighed and tablet powder equivalent to 5 mg of Ticagrelor was taken and dissolved in 50 ml of methanol. The resulting solution (5 ml) was transferred to a 10 ml volumetric flask and diluted up to the mark with mobile phase. The final solution was filtered through 0.45μ membrane filter using injection filter.A 20 μL of the
filtrate was injected into chromatographic system. The peak area of the Ticagrelor was determined and concentration was found using linear regression equation obtained from calibration curve.

2.7 Method validation
The developed method was validated by evaluating linearity, accuracy, precision, robustness, ruggedness, detection limit, quantification limit and stability. Coefficients of variation and relative errors of less than 2% were considered acceptable, except for the quantification limit, for which these values were established at 2%, as recommended in the literature.

2.7.1 System Suitability Test
Before performing validation experiments, system suitability test (SST) has to be applied to indicate that HPLC system and method are capable of providing data with admissible quality. SST was performed by investigating capacity factor, tailing factor, theoretical plates number, resolution and also relative standard deviation (RSD) of the peak areas.

2.7.2 Stability
Stability was assessed by analyzing QC standard solutions after keeping them at room temperature for 48 h. Obtained results were investigated as recovery values and compared to the freshly prepared solutions.

2.7.3 Linearity
A stock solution of ticagrelor of 1000 μg/mL was prepared with methanol. From it, various working standard solutions were prepared in the range of 10 to 120 μg/ml using mobile phase as diluent and injected into HPLC. It was shown that the selected drug had linearity in the range of 10–90 μg/mL. The calibration plot (peak area of Ticagrelor versus Ticagrelor concentration) was generated by replicate analysis (n=9) at all concentration levels and the linear relationship was evaluated using the least square method within Microsoft Excel® program.

2.7.4 Accuracy
The accuracy of the method was carried out using one set of different standard addition methods at different concentration levels, 80%, 100% and 120%, and then comparing the difference between the spiked value (theoretical value) and actual found value.

2.7.5 Precision
The precision of the method was ascertained from the peak area obtained by actual determination of six replicates of a fixed amount of the drug (50 μg/mL). The precision of the assay was also determined in terms of intra- and inter-day variation in the peak areas of a set of drug solutions on three different days. The intra- and inter-day variation in the peak area of the drug solution was calculated in terms of relative standard deviation (RSD).

2.7.6 Robustness
Robustness of the proposed method for Ticagrelor was carried out by the slight variation in flow rate, pH and mobile phase ratio. The percentage recovery and RSD were noted for Ticagrelor.

2.7.7 Ruggedness
The test solutions were prepared as per test method and injected under variable conditions. Ruggedness of the method was studied by different analysts.

2.7.8 Detection limit and quantification limit
The limit of detection (LOD) and limit of quantification (LOQ) were established based on the calibration curve parameters, according to the following formulas:

\[ \text{LOD} = 3.3 \times \text{SD} / \text{slope} \]
\[ \text{LOQ} = 10 \times \text{SD} / \text{slope} \]

or detection limit=3.3σ/s, quantification limit=10σ/s, where σ is the standard deviation of y-intercept of regression line, and s is the slope of the calibration curve.
2.8. Forced degradation studies

The specificity of the method can be demonstrated through forced degradation studies conducted on the sample using acid, alkaline, oxidative, thermal, photolytic, and ultra violet (UV) degradations. The sample was exposed to these conditions, and the main peak was studied for the peak purity, thus indicating that the method effectively separated the degradation products from the pure active ingredient.

2.8.1. Hydrolytic degradation

Hydrolytic stress testing was performed to force the degradation of the drug substance to its primary degradation products by exposure to neutral, acidic and basic conditions over time. Functional groups likely to undergo hydrolysis are amides (lactams), esters (lactones), carbamates imides, imines, alcohols (epimerization for chiral center) and aryl amines.

To initiate hydrolytic studies, a preliminary solubility screen of the drug substance was performed. Solubility of at least 1 mg/mL in neutral, acidic and basic conditions was recommended for the neutral/acid/base stress testing. However, concentration less than 1 mg/mL can be used, if solubility is an issue. In some cases, a co-solvent may be necessary to achieve the target concentration. Special attention should be given to the drug substance structure when an appropriate co-solvent was chosen.

2.8.2. Oxidative degradation

Oxidative degradation of drug substances in pharmaceutical formulation is well documented. Although exact mechanistic details about what promotes reaction between drug substance and molecular oxygen in pharmaceutical formulations are not fully understood, such reactions are generally thought to be in the category of auto-oxidation process.

There are three major pathways: (i) autoxidation or radical mediated oxidation, (ii) peroxide-mediated oxidation and (iii) photochemically induced oxidation. Traditionally, dilute aqueous peroxide solutions have been used for oxidative stress testing of pharmaceuticals. In addition to the auto-oxidation processes, peroxide-mediated oxidative degradation can occur, which may not be observed using radical indicator. The process of oxidation depends on the amount of oxygen in the air and the nature of the material it touches. True oxidation happens on the molecular level. We can only see the large scale effects as the oxygen causes free radicals on the surface to break away.

2.8.3. Photolytic degradation

For photolytic degradation, the drug was exposed to the direct sunlight. Sufficient amount of the drug was taken in a closed petri-dish and exposed to sunlight. At different time intervals, the drug was taken out, diluted appropriately and injected into HPLC to determine the amount of degradation of the drug.

2.8.4. UV-degradation

The goal of UV-degradation studies is to force the degradation of drug substance via UV and fluorescent condition over time to determine the primary degradation products. A molecule absorbs light when an absorption band overlaps to some extent with the incident light energy and a valence electron in the relevant atmosphere rose to an excited state. A near UV fluorescent lamp has a spectral distribution from 320 nm to 400 nm with a maximum energy emission between 350 nm and 370 nm, and the significant proportion of UV should be in both bands of 320–360 nm and 360–400 nm.

2.8.5. Thermal degradation

To evaluate thermolytic pathways, evaluated temperatures (e.g. 50 °C and −80 °C) in the solid state and/or in solution can be used. Many compounds began to degrade via different mechanisms above 80 °C, giving rise to degradation products. To solid-state stressing, the use of high and low humidity atmosphere of the evaluated temperature is appropriate. To evaluate stability under the temperatures above, stress conditions were selected based on a conservative estimate of the Arrhenius expression, a quantitative relationship of reaction rate and temperature using average activation energy.

\[ K_{obs} = A \exp^{-\frac{E_a}{RT}} \]
3. Results and Discussion

3.1. Optimization of chromatographic conditions

Optimization studies were carried out to develop the HPLC method for estimation of Ticagrelor in the presence of its degradation products. In this case, various parameters were investigated including composition of mobile phase, stationary phase, wavelength, flow rate and injection volume. The optimum parameters based on peak shapes and retention time values. Selection of wavelength depends upon UV spectra of Ticagrelor in which it shows maximum absorbance at 254 nm, so this wavelength was selected as analytical wavelength. Different mobile phases were tested in the development stage of the method. According to literature, ACN in combination with water, phosphate buffer and ammonium acetate buffer were reported for the determination of Ticagrelor. With methanol as organic phase no HPLC method is reported, so we made a trial with methanol as organic phase and water as aqueous phase. With the increase methanol the retention time decreased but increase in methanol causes less number of theoretical plates. To make the method economical we chosen less percentage of methanol in the composition. Mobile phase in the ratio of methanol : water (20:80,v/v) provided good peak shape and retention, so this composition is optimized. Injection volumes of 5, 10, 15 and 20 μL were investigated. Peak area values increased with the increasing injection volume and better peak shapes were obtained. Therefore, 20 μL was found to be the optimum injection volume. To determine the optimum flow rate, flow rates of 0.8, 1.0 and 1.2 mL min−1 were tested. There was no significant difference on the obtained peak area values. The highest peak area values were obtained with the flow rate of 0.8 mL min−1, however, the peak tailing was a little bit more compared to other flow rates. Due to the fact that increase on flow rate caused high column pressure, the lowest possible flow rate was selected. For this purpose, the flow rate of 1 mL min−1 was chosen as the flow rate of the method. Figure 2 illustrates a typical chromatogram obtained at optimum conditions. The optimized chromatographic conditions are shown in Table 1.

Table 1 Optimized chromatographic conditions

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stationary Phase (Column)</td>
<td>C18 (250 × 4.6 mm i.d., 5μ)</td>
</tr>
<tr>
<td>Mobile Phase</td>
<td>Methanol: Water (20:80,v/v)</td>
</tr>
<tr>
<td>Flow rate (ml/min)</td>
<td>1.0 mL/min</td>
</tr>
<tr>
<td>Run time (min)</td>
<td>10 min</td>
</tr>
<tr>
<td>Column temperature (°C)</td>
<td>ambient</td>
</tr>
<tr>
<td>Volume of injection loop (μL)</td>
<td>20</td>
</tr>
<tr>
<td>Detection wavelength (nm)</td>
<td>254 nm</td>
</tr>
<tr>
<td>Retention time (min)</td>
<td>5.786</td>
</tr>
</tbody>
</table>

Figure 1 Chromatogram of Ticagrelor
3.2. Method validation

3.2.1. System Suitability Test

After setting the optimum conditions, system suitability parameters for the developed method were determined and compared with recommended limits. To determine the parameters, the study was performed with standard solution of 50 µg/ml concentration and the results were acquired from six injections. System suitability parameters of the method were demonstrated in Table 2. According to results, all of the system suitability parameters were within the recommended limits and the method was found to be suitable for the analysis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Criteria</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capacity factor ($k'$)</td>
<td>$k' &gt; 2$</td>
<td>4.824</td>
</tr>
<tr>
<td>Tailing factor ($T$)</td>
<td>$T &lt; 2$</td>
<td>1.211</td>
</tr>
<tr>
<td>Theoretical plates ($N$)</td>
<td>$N &gt; 2000$</td>
<td>2421</td>
</tr>
<tr>
<td>% RSD (peak area)</td>
<td>% RSD ≤ 1</td>
<td>0.87</td>
</tr>
</tbody>
</table>

3.2.2. Stability

The sample solution stability was analyzed by injecting the same solution at 0, 12, 24, and 48 h. Identical change was not observed in the developed method. Also, results were found within acceptable limits (RSD <2), which are summarized in Table 3.

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Assay(%)</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>100.08</td>
<td>---</td>
</tr>
<tr>
<td>After 12 hr</td>
<td>100.02</td>
<td>0.05</td>
</tr>
<tr>
<td>After 24 hr</td>
<td>99.87</td>
<td>0.21</td>
</tr>
<tr>
<td>After 36 hr</td>
<td>99.16</td>
<td>0.92</td>
</tr>
<tr>
<td>After 48 hr</td>
<td>98.32</td>
<td>1.76</td>
</tr>
</tbody>
</table>

3.2.3. Linearity and sensitivity

Linearity study was performed with calibration standards with 10, 20, 30, 40, 50, 60, 70, 80 and 90 ppm concentrations. The standards were injected in triplicate. Calibration curves were obtained by plotting the peak areas against the given concentrations. The calibration curve was evaluated by the determination coefficient. The determination coefficient ($R^2$) of the calibration curves was 0.9999. Therefore, the calibration curve for Ticagrelor was found to be linear within the range of 10–90 µg/ml concentrations. The regression equations were calculated from the calibration graphs. The sensitivity of the analytical method was evaluated by determining the limits of detection (LOD) and quantitation (LOQ). The values of LOD and LOQ are given in Table 4. The low values of LOD and LOQ indicates the sensitivity of method.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection wavelength (nm)</td>
<td>254</td>
</tr>
<tr>
<td>Linearity range (µg/ml)</td>
<td>10–90</td>
</tr>
<tr>
<td>Coefficient of determination ($r^2$)</td>
<td>0.9988</td>
</tr>
<tr>
<td>Regression equation ($Y^o$)</td>
<td>$Y = 29989x+34171$</td>
</tr>
<tr>
<td>Slope (m)</td>
<td>29989</td>
</tr>
</tbody>
</table>
3.2.4. Accuracy

To study the reliability, the suitability, and the accuracy of the method, recovery experiments were carried out. Known quantities of the pure drug were added to the placebo to make samples at the levels of 80 %, 100 %, and 120 %, and were assayed by the proposed method. Accuracy was calculated as the percentage of recovery and the results are shown in Table 5.

**Table 5  Accuracy**

<table>
<thead>
<tr>
<th>% Level</th>
<th>Concentration(µg/mL)</th>
<th>Recovery(%)</th>
<th>Statistical Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formulation Pure drug</td>
<td></td>
<td>Mean SD %RSD</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>50 40 98.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>50 40 98.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>50 40 100.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>50 50 100.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>50 50 101.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>50 50 98.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>50 60 99.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>50 60 99.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>50 60 100.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.2.5. Precision

The precision was demonstrated at three levels: repeatability, intermediate precision, and reproducibility (between laboratories’ precision). Each level of precision was investigated by 3 sequential replicate of injections of three concentrations of 40, 50 and 60 µg/mL. The precision was expressed as relative standard deviation (RSD) or coefficient of variation (CV). The results of three levels of precision are shown in Table 6.
Table 6 Precision Data

<table>
<thead>
<tr>
<th>Precision</th>
<th>Results</th>
<th>Concentration(µg/mL)</th>
<th>RSD of Peak area</th>
<th>RSD of Retention Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repeatability</td>
<td></td>
<td>40 0.89 0.021</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 1.21 0.088</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>60 1.11 0.123</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermediate precision</td>
<td></td>
<td>40 1.42 0.087</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 0.75 0.066</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>60 0.67 0.062</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reproducibility</td>
<td></td>
<td>40 1.64 0.111</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 0.78 0.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>60 0.85 0.094</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.2.6. Robustness and ruggedness

Robustness of the method was studied by deliberate variations of the analytical parameters such as flow rate (0.5±0.1 mL/min) and different pH values. The results are given in Figure 3. Ruggedness of the method was carried out by different analysts. The results are displayed in Figure 4.

![Figure 3 Robustness](image1)

![Figure 4 Ruggedness](image2)
3.2.7. Mobile phase stability

The stability of the mobile phase was evaluated, so the mobile phase was stored at 4–8 °C for 1 week. The aged mobile phase was compared using a freshly prepared one. The mobile phase was stable up to 1 week at 4–8 °C.

3.3. Forced degradation studies

3.3.1. Degradation in neutral condition

Neutral degradation of Ticagrelor was performed using distilled water. Ten micrograms of the Ticagrelor bulk was weighed accurately and transferred into a 10 mL clean volumetric flask. The content of the volumetric flask was dissolved in 5 mL of distilled water. Then the volumetric flask was heated on a water bath at 80 °C. Samples were prepared for different time intervals such as 0 min, 30 min, 1 h, 2 h, and 4 h. At different time intervals, different sample solutions were taken out and 5 mL of HPLC grade methanol was added. The sample solution was then sonicated for 5 min and diluted to yield a concentration of 50 μg/mL in the mobile phase. It was then filtered through a 0.22 μm filter and 20 μL was injected into the HPLC for analysis. The obtained chromatogram was observed for any degradation occurred during the time. The results Fig. 5.

![Figure 5 Neutral degradation](image)

3.3.2. Degradation in acidic condition

Acid degradation of Ticagrelor was performed using 0.1 M HCl. Ten micrograms of the Ticagrelor bulk was weighed accurately and transferred into a 10 mL clean volumetric flask. The content of the volumetric flask was dissolved in 5 mL of 0.1 M HCl and then was subjected to heat on a water bath at 80 °C. Samples were prepared for different time intervals such as 0 min, 30 min, 1 h, 2 h, and 4 h. At different time intervals, different sample solutions were taken out and 5 mL of HPLC grade methanol was added. The sample solution was then sonicated for 5 min and diluted to obtain a concentration of 50 μg/mL in the mobile phase. It was then filtered through a 0.22 μm filter and injected into the HPLC. The obtained chromatogram was analyzed for any degradation happened during the time. The results are given in Fig.6.
3.3.3. Degradation in basic condition

Alkaline degradation of Ticagrelor was performed using 0.1 M NaOH. Ten micrograms of the Ticagrelor bulk was weighed accurately and transferred into a 10 mL clean volumetric flask. The content of the volumetric flask was dissolved in 5 mL of 0.1 M NaOH, and then was heated on a water bath at 80 °C. Samples were prepared for different time intervals such as 0 min, 30 min, 1 h, 2 h, and 4 h. At different time intervals, different sample solutions were taken out and 5 mL of HPLC grade methanol was added. The sample solution was then sonicated for 5 min and diluted to obtain a concentration of 50 μg/mL in the mobile phase. It was then filtered through a 0.22 μm filter and injected into the HPLC. The obtained chromatogram was studied for any degradation happened during the time. The results are given in Fig. 7.

3.3.4. Oxidative degradation

For oxidation, the reagent chosen was hydrogen peroxide (3%). Ten micrograms of the Ticagrelor bulk was weighed accurately and transferred into a 10 mL clean volumetric flask. The content of the volumetric flask was dissolved in 5 mL of H₂O₂. Then it was placed at room temperature for degradation. Samples were prepared for different time intervals such as 0 min, 30 min, 1 h, 2 h, and 4 h. At different time intervals, different sample solutions were taken out and 5 mL of HPLC grade methanol was added. The sample solution was then sonicated for 5 min and diluted to yield a concentration of 50 μg/mL in the mobile phase. It was then filtered through a 0.22 μm filter and injected into the HPLC.
The obtained chromatogram was studied for any degradation underwent during the time given. The results are shown in Fig. 8.

![Figure 8 Oxidative degradation](image)

3.3.5. Photolytic degradation

For photolysis, 100 mg of the Ticagrelor bulk was weighed accurately and transferred into a clean petridish. Then the closed petridish was placed under direct sunlight for degradation. At different time intervals, 10 mg of sample was taken out. From it, a stock solution of 1000 μg/mL was prepared. Then it was sonicated for 5 min and diluted to obtain a working solution of 50 μg/mL in the mobile phase. It was then filtered through a 0.22 μm filter and injected into the HPLC. The obtained chromatogram was observed for any degradation occurred during the time. The results are given in Fig. 9.

![Figure 9 Oxidative degradation](image)

3.3.6. UV-degradation

For UV degradation, 100 mg of the Ticagrelor bulk was weighed accurately and transferred into a clean petridish. Then the petridish was placed under a UV chamber 30 cm at distance from the UV lamp. The cover of the petridish was removed for degradation. After 3 h, the UV lamp was switched off and 10 mg of sample was taken out. From it, a stock solution of 1000 μg/mL was prepared with the mobile phase, from which 50 μg/mL of working solution was prepared. It was sonicated and filtered through a 0.22 μm filter. Twenty microlitres of the sample was injected into the HPLC. The degradation of Ticagrelor under UV light is given in Fig. 10.
3.3.7. Thermal degradation

Thermal degradation was performed by placing the Ticagrelor bulk in the hot air oven at 40 °C. Samples were drawn at definite time intervals. The weighed amount of sample was added to 5 mL of HPLC grade methanol and sonicated for 5 min. Volume was made up to mark with methanol, which is the bulk standard stock solution of 1000 μg/mL. From the stock, a working standard solution of 50 μg/mL was prepared with the mobile phase. It was sonicated and filtered through a 0.22 μm filter, and 20 μL of the sample was injected into the HPLC. The obtained chromatogram was analyzed for any degradation underwent during the time, and the results are given in Fig. 11.

When stress conditions were applied to Ticagrelor, the HPLC results showed that there was no interference between the tested drug and the degradation products indicates the specificity and stability indicating nature of the method. It was observed that the drug showed extensive degradation in oxidative condition, alkali hydrolysis, and thermal. Figs. 5–111 show the chromatograms obtained after degradation under different stress conditions.
3.4. Sample Analysis

The developed and validated method was applied for analysis of oral suspension contain Ticagrelor. The sample was analyzed in triplicate. Analysis results were evaluated using a calibration curve. The amount of Ticagrelor in the samples was calculated from calibration curve equation and recovery and RSD values were determined. The results of analysis are given in Table 7. The recoveries were in good agreement with the label claims. It was concluded that the method can be applied successfully for the analysis of Ticagrelor in suspension dosage form.

Table 7 Assay

<table>
<thead>
<tr>
<th>Sample</th>
<th>Labelled amount (mg)</th>
<th>Amount obtained* (mg)</th>
<th>Percentage Recovery*±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ticagrelor oral dispersible tablets (Brilique®)</td>
<td>5</td>
<td>4.92</td>
<td>98.4 ± 0.92</td>
</tr>
</tbody>
</table>

* Average of five determinations

Compliance with ethical standards

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Disclosure of conflict of interest

The authors attest that they have no conflict of interest in this study.

References


