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A Validated Stability-Indicating High performance liquid chromatography Method for Quantification of Related substances and Assay in Ticagrelor

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Abstract

Ticagrelor is used for the treatment of thrombosis, as a coagulation inhibitor. Ticagrelor is the first adenosine diphosphate (ADP) receptor inhibitor to be administered orally that exhibits reversible binding properties. This work focuses on developing and validating an HPLC method for Determination of Assay and quantification impurities in Ticagrelor drug substance. The specificity of the method was attained by employing an analytical column known as Zodiac C18, with dimensions of 150 mm X 4.6 mm and a particle size of 3.5 µm. A suitable mobile phase consisting of 0.1% orthophosphoric acid and methanol in a volumetric ratio of 95:5 v/v has been used as Solvent A. Acetonitrile used as solvent B in the gradient program. The flow rate is recorded as 1.0 millilitres per minute. The injection volume utilized in the experiment is 10µL, with detection occurring at a wavelength of 210 nm. The overall run time of the investigation is 30.0 minutes. The samples were prepared for forced degradation under ambient, thermal, humidity, water, acid, base, photolytic and oxidation conditions. The methodology was assessed and confirmed to meet the criteria of specificity, sensitivity, precision, linearity, ruggedness, robustness, and accuracy by the recommendations set out by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH). The linearity of the method for determining impurities and the ticagrelor was seen throughout a range of concentrations, from the quantification limit (QL) level to 150% concentration level. The correlation coefficient (r2) exceeded 0.990, indicating a robust linear relationship between the measured values and the concentration levels. The accuracy assessment was conducted for the impurities within a concentration range spanning from the Quantitation Limit (QL) to 150% level. The mean recovery for these measurements was determined to be within the scope of 98-102%. The findings from the analytical degradation and verified analysis demonstrate the inherent instability of the substance under oxidative circumstances. Hence, this approach holds potential for use within the realms of pharmaceutical research and development, as well as quality control departments.

Keywords: Ticagrelor, HPLC, impurities, ICH guidelines, Forced degradation, Stability Indicating method.

Full length article **Corresponding Author*, e-mail: vkap@gitam.edu

1. Introduction

Ticagrelor (TGR) is an inhibitor of adenosine absorption, an inhibitor of platelet aggregation, an antagonist of P2Y12 purinoceptors, and an inhibitor of coagulation [1]. This medication manages thrombosis, angina, ischemic heart disorders, and coronary artery diseases [2]. Ticagrelor represents the initial orally administered adenosine diphosphate (ADP) receptor antagonist with reversible binding properties, exhibiting a unique chemical composition from thienopyridine drugs such as Clopidogril [3]. The compound preferentially hinders the activity of P2Y12, a crucial receptor for ADP. The inhibition of ADP receptor blockade impedes the functioning of platelets within the bloodstream, hence decreasing the occurrence of repeated thrombotic events. The medicine has demonstrated a statistically significant

primary efficacy when compared to the commonly given medication Clopidogrel (PLAVIX) in the prevention of cardiovascular (CV) events, such as myocardial infarction (heart attacks), stroke and cardiovascular death, among patients diagnosed with acute coronary syndrome (ACS)[4]. Chemical name of Ticagrelor is (1S,2S,3R,5S)-3-[7-{[(1R,2S)-2-(3,4-Difluorophenyl) cyclopropyl]amino}-5-(propylthio)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl]-5-(2-hydroxyethoxy)cyclopentane-1,2-diol and molecular weight of 522.57 g/mol. It corresponds to the chemical formula C23H28F2N6O4. The substance is observed as a white or off-white to pale pink powder, displaying solubility that is not influenced by pH [2]. According to the Biopharmaceutics Classification System (BCS), it is categorized as having 'poor solubility'. Ticagrelor, a compound of BCS class IV, also exhibits limited permeability [5].

The literature survey indicated no chromatographic methods for determining degradation impurities present in TGR[5–11].

The primary aim of the present study was to develop a sensitive, specific, accurate, linear, precise, robust, and stability-indicating method for detecting impurities found in TRG. A proven quantitative analytical process called the stability-indicating method typically involves forced degradation and validation experiments [8–11]. Figure 1- 4 presents the chemical structures of Ticagrelor (Fig 1) and impurities 1 (Fig 2), impurity 2 (Fig 3), impurity 3 (Fig 4) respectively.

2. Materials and Methods

2.1 Instrumentation

The HPLC system utilized for the initial development of the chromatographic method was the Agilent 1260 Infinity II module, which was equipped with quaternary gradient pumps, an integrated auto-injector, a thermostatic compartment, and a UV detector. The LC solutions program performed the data processing and system suitability calculations. The peak purity calculations were performed using a photodiode array detector[12]. The method development and validation equipment consisted of an Agilent 1260 Infinity II model equipped with a UV detector and a Shimadzu LC-2030C PDA.

2.2 Materials and reagents

Analytical grade reagents such as orthophosphoric acid (H3PO4) (Rankem), milli-Q Water, methanol (Rankem), and acetonitrile (Rankem) were used in the method development, optimization and Validation.

2.3 HPLC Method Development and Optimization

2.3.1 Analytical Method Development

The present study focused on developing an appropriate high-performance liquid chromatography (HPLC) method for accurately quantifying TGR and its related three impurities [13]. Various factors including diluent selection, buffer type, buffer concentration, organic solvent composition for the mobile phase, and other chromatographic conditions were investigated to determine their impact on the method's performance[14].

2.3.2 Selection of UV-absorption

Approximately 2.0 mg of TGR was precisely put into a 100 ml volumetric flask containing 50 ml of methanol. The flask was subjected to sonication for a period of five minutes in order to facilitate the dissolution of the sample. Subsequently, diluted the solution with methanol until it reached the label on the flask and thoroughly mixed to ensure homogeneity. UV-absorption spectra has been recorded at 200 to 400 nm wavelength range. The wavelength of 210 nm was chosen for the quantitative investigation of TGR and its related three impurities due to its UV-absorption spectrum [15]. Figure 5 represents the UV-absorption spectrum of TGR.

2.3.3 Selection of stationary phase

The appropriate choice of method is based on the characteristics of the sample, including whether it is ionic, ionisable, or a neutral molecule, as well as its molecular weight and solubility [16]. The TGR exhibited dissolution in a polar solvent, and as a result, the same solvent was chosen for its quantification of impurities in TGR. Preliminary experiments were conducted using various combinations of buffer and organic phases in the mobile phases, covering a pH range of 2-7[16]. After evaluating multiple options, the Zodiac C18 150 x 4.6 mm, 3.5µm column was the most appropriate choice for conducting quantitative analysis of Ticagrelor in the current research investigation [17].

2.3.4 Selection of mobile phase

The compound TGR shows acidic properties, and its peak retention can be maximized by operating above its pKa in an acidic pH environment. Additionally, further optimization can be performed by utilizing an organic phase. The pH values of aqueous solutions are 2.0 to 7.0, with concentrations spanning from 0.0005 mol/L to 0.5 mol/L. Based on this fact, preliminary experiments were conducted using various compositions using acetonitrile, as well as methanol, in an attempt to attain the needed separation between Ticagrelor and its impurities. In the undertaken testing, separating pollutants from the Ticagrelor peak was inadequate. The buffer solution was prepared by dissolving 1.0 mL of orthophosphoric acid in a 1000 mL water solution. The optimization process involved the selection of Solvent-A as a buffer and methanol in a volumetric ratio of 95:5 v/v and Solvent-B as Acetonitrile. This selection was made considering factors such as peak shape and pump pressure. The wavelength of the detector utilized in the experiment was 210 nm. The volume of the sample injected into the system was 10 µl. The column temperature was maintained at a constant value of 30°C throughout the experiment. The flow rate of the mobile phase was set at 1.0 ml.min-1. The duration of each run was 30 minutes. A diluent consisting of acetonitrile and water in a volumetric ratio 1:1 was employed to fabricate samples.

2.3.5 Preparation of system suitability solution:

The TGR standard and test sample were accurately measured, each equivalent to 25 mg. These amounts were subsequently transferred into separate 25 ml volumetric flasks. Later, 15 ml of diluent was added to each flask, and the contents were sonicated for five minutes to facilitate the dissolution of the sample [18]. Finally, the solutions were diluted to the desired level with diluent. A further 1.0 ml of the above solution was transferred using a pipette into a 10 ml volumetric flask. The flask was then filled to the desired volume using a diluent. The resulting solution was thoroughly mixed on a cyclo mixer for two minutes. Subsequently, the solution was filtered and later injected into the HPLC system [19–23].

2.4 Specificity and Forced Degradation

2.4.1 Specificity

The definition of specificity refers to the ability to accurately determine the analyte without any ambiguity, even when other substances that are likely to be present are also present. Commonly, these might involve contaminants, degradants, matrix components, and other similar entities. The absence of specific information in a particular statistical technique can be mitigated by implementing other complementary statistical techniques. The specificity can be ascertained through the introduction of excipients, contaminants, and degradation products into pure compounds and comparing the obtained test findings with those of the pure substances. In the presence of its impurities, the stability-indicating characteristic and specificity of the suggested RP-HPLC technique for Ticagrelor has been evaluated using forced degradation studies performed by the guidelines ICH. Figure 6 illustrates the HPLC chromatograms of the blank (6A), Ticagrelor, and spiked sample chromatograms (6B). No interferences were detected at impurity-1, impurity-2, impurity-3, and Ticagrelor retention time. The elution order of impurity-1, impurity-2, and impurity-3, as observed in both the individual solution and the test and impurity blend solution, demonstrated a consistent correspondence. The average retention time of Ticagrelor is around 7.38 minutes. The retention periods of impurity-1, impurity-2, and impurity-3 are approximately 3.49, 8.30, and 10.82 minutes, respectively.

2.4.2 Forced degradation

The drug experienced degradation under various environmental and chemical settings to investigate the degradation rate and the drug's stability within these limits. The method of experimentation employed for the examination of forced degradation is outlined in the following section [24–26].

2.4.2.1 Ambient Condition (25±2°C):

About 2.0 g of a TGR sample was transferred into a petri dish and evenly distributed. The Petri container was then placed under laboratory conditions, namely at a temperature of 25±2°C, for one week. After this period, the sample was subjected to analysis for related substances and assay. The stability of the sample was found to be stable when exposed to ambient conditions.

2.4.2.2 Thermal degradation:

Approximately 2.0 g of the TGR sample was evenly distributed into a petri dish. The dish was then placed in an oven set at a temperature of 80°C for one week. After this, the sample was removed from the oven and allowed to cool to room temperature before undergoing analysis. Upon the expiration of the designated time frame, the sample was extracted from the oven and subjected to analysis to determine the presence of related substances and assess the assay in TGR. The results indicated that the sample exhibited stability when subjected to extreme temperature conditions.

2.4.2.3 Photolytic degradation:

Approximately 2.0 g of the TGR sample was evenly distributed into two petri dishes where one covered with aluminum foil as dark sample. The dish was then placed in Photostability chamber to reach the set conditions of 1.2 million Lux hours and 200-Watt hours of UV-Visible light. After attaining the set conditions, the sample was removed from the chamber. The sample was subjected to analysis to determine the presence of related substances and assess the assay in TGR. The results indicated that the sample exhibited stability when subjected to photolytic conditions.

2.4.2.4 Exposure to humidity (90% RH):

Approximately 2.0 g of TGR material were carefully transferred onto a petri dish and distributed evenly. Subsequently, the petri dish was placed within a desiccator containing a saturated ammonium chloride solution, achieving a relative humidity of 90%. This arrangement was maintained for a duration of one week. Upon the conclusion of the specified time frame, the sample was extracted and subjected to analysis to identify and quantify the related substances and Assay of TGR. The results indicate that the sample demonstrated stability when exposed to humid conditions.

2.4.2.5 Hydrolysis (Water degradation):

The TGR sample was precisely weighed and transferred, amounting to 50 mg, into a volumetric flask with a capacity of 100 ml. Subsequently, 10 ml of water was added to the flask, and the resulting solution was heated at a temperature of 80°C for 5 hours. Following completion of the designated duration for exposure, the solution underwent a cooling process. Subsequently, the solution was supplemented with diluent to reach the desired volume. Thorough mixing was then performed, and the solution was later analyzed to identify and quantify the related substances and Assay of TGR. The stability of the material was observed under hydrolysis conditions.

2.4.2.6 Acid hydrolysis (Acid degradation):

A precise amount of 50 mg of the TGR sample was accurately measured and subsequently transferred to a volumetric flask with a capacity of 100 ml. Subsequently, a volume of 10 ml of water was introduced into the flask, and the resultant solution was subjected to heating at a temperature of 80°C for a duration of 5 hours. Following the completion of the designated exposure time period, the solution was subjected to cooling. Subsequently, the solution was adjusted to its original volume by adding a diluent and thoroughly mixed. The resulting mixture was then subjected to analysis to identify and quantify the related substances and Assay of TGR. The stability of the material was noted when exposed to an acidic environment.

2.4.2.7 Base hydrolyses (Base degradation):

The TGR sample was carefully measured and transferred, equivalent to 50 mg, into a volumetric flask with a capacity of 100 ml. Subsequently, a volume of 10 milliliters of water was introduced into the flask, and the resultant solution was subjected to heating at a temperature of 80°C for a duration of 5 hours. After the designated exposure time period was completed, the solution was cooled and adjusted to the desired volume using a diluent. Thorough mixing was then performed, and the solution was subsequently analysed to identify and quantify the related substances and Assay of TGR. The stability of the material was noted when subjected to hydrolysis conditions.

2.4.2.8 Oxidation:

The TGR sample was accurately measured and transferred, equivalent to 50 mg, into a volumetric flask with a capacity of 100 ml. Subsequently, a volume of 10 ml of a 10% H2O2 solution was introduced into the flask, and the resultant solution was subjected to heating at a temperature of 80°C for a duration of 5 hours. After the exposure was completed, the solution was cooled and subsequently adjusted to the desired volume using a diluent. The resulting mixture was well mixed. The solution underwent analysis to identify and quantify the related substances and Assay of TGR. The degradation of TGR was detected in oxidative circumstances. Figure 7 illustrates the degradation chromatograms commonly observed in HPLC where 7A represents 1N HCl, where 7B represents 0.1N NaOH, 7C represents 10% H2O2 and 7D represents Water hydrolysis. The overview of forced degradation studies is presented in Table 1. Upon conducting forced degradation studies, it was ascertained that TGR remained unaffected by acid, base hydrolysis, thermal, humidity, water, photolytic and ambient conditions. An appreciable decrease was observed under the oxidative stress condition. A PDA detector conducted the peak purity test on the TGR sample under various stress conditions. In every state of stress, the threshold for a single point in the TGR was found to be lower than the peak purity index value. The assay studies were conducted on stress samples compared against standard, and the mass balance, including the percentage of the assay, impurities, and degradation products were determined. The assay for the TGR was determined by adding all three contaminants (impurity-1, 2, and 3) at the specified level, corresponding to 0.15% of impurity-1, 2, and 3 relative to the analyte concentration (0.5 mg/ml). The mass balance data related to the forced deterioration samples are presented in Table 2.

2.5 Analytical Method Validation

2.5.1 Preparation of solutions

2.5.1.1 Preparation of Ticagrelor impurities stock solution:

A precise measurement was conducted on a quantity of 15.0 mg of standards for Impurity-1, Impurity-2, and Impurity-3. The standards were transferred into a volumetric flask with a capacity of 100 ml. Dissolve the standards in the flask using a suitable solvent and dilute to the desired volume with a diluent. Thoroughly mix the contents of the flask.

2.5.1.2 Preparation of Ticagrelor Stock solution:

Accurately weighed about 10.0 mg of the Ticagrelor standard and transferred it into a 100 mL volumetric flask. Proceed to dissolve the substance in the flask and subsequently dilute it to the desired amount using a suitable diluent. Finally, thoroughly mix the contents of the flask.

2.5.1.3 Preparation of Ticagrelor Standard solution (0.10%):

Diluted 0.25 mL of Ticagrelor stock solution to 50 mL with diluent.

2.5.1.4 Preparation of system suitability solution:

Accurately weigh 25.0 mg of the test sample and transfer it into a 50 mL volumetric flask. Added 0.5 mL of the Impurity-2 stock solution into the mixture, ensuring complete dissolution. Subsequently, dilute the solution to the desired amount using the appropriate diluent.

2.5.1.5 Preparation of test solution:

The sample was accurately weighed about 25.0 mg and then put into a 50 ml volumetric flask. The sample was dissolved and diluted with a suitable diluent until the flask reached its specified volume.

2.5.1.6 Preparation of assay standard solution (100 µg/ml):

The TGR standard was accurately measured 10 mg and subsequently put into a 100 ml volumetric flask. The standard was then dissolved and diluted with an appropriate diluent until the flask reached its total volume.

2.5.1.7 Preparation of assay test solution (100 µg/ml):

The TGR test sample was prepared to get a concentration of 100 µg/ml for all degradation samples. The

resolution between TGR and impurity-2 was evaluated. The resolution between closely eluting substances of TGR was demonstrated in Table 3, representing the system suitability properties.

2.5.2 Detection limit (DL) & Quantitation limit (QL)

The detection limit (DL) and quantification limit (QL) for Ticagrelor, impurity-1, impurity-2, and impurity-3 have been determined using a signal-to-noise ratio of 3:1 and 10:1, correspondingly. This was achieved through the administration of several diluted solutions possessing established concentrations, as reported in reference. The DL and QL chromatograms are presented in Figure 8. A study was conducted at the QL level to assess precision. In this study, a total of six replicates of Ticagrelor were injected after being spiked with a blend of impurity-1, impurity-2, and impurity-3. The % RSD (relative standard deviation) for the peak areas was calculated. The S/N ratio was derived from the DL solution, QL solution was prepared, and the S/N ratio was calculated. Table 4 presents the values for the DL and QL of Ticagrelor, impurity-1, impurity-2, and impurity-3, which were determined based on the signal-to-noise ratio values. The %RSD was calculated for the area of each impurity and Ticagrelor. Table 5 displays the precision data for the QL of Ticagrelor and its three impurities.

2.5.3.1 Preparation of linearity stock solutions:

In order to ascertain the linearity of solutions containing Ticagrelor, impurity-1, impurity-2, and impurity-3, diverse aliquots of impurity stock and reference solutions were prepared in the following proportions: 0.25, 0.40, 0.45, 0.50, 0.60, and 0.75 ml. The aliquots were subsequently diluted with diluent to the final volume in separate 20 ml volumetric flasks. The resulting solutions were thoroughly mixed on a cyclomixer for five minutes. The chromatographic column was loaded with the concentrations specified above, which varied from the quantification limit (QL) to 150% of the specification level (0.15%). The surface area of each peak was determined, and a calibration curve was generated through the plotting of the impurity area against the percentage concentration. The values of the residual sum, slope, correlation coefficient, and y-intercept derived from the calibration curve are shown in Table 6. These calculations provide evidence of a strong linear relationship between peak regions and concentration.

2.5.4 Accuracy

The evaluation of impurities accuracy was carried out in triplicate at three different concentrations: 0.075% (0.375 µg/ml), 0.15% (0.75 µg/ml), and 0.225% (1.125 µg/ml), corresponding to 50%, 100%, and 150% of the TGR concentration (0.5 mg/ml or 500 µg/ml). The study determined that the mean recoveries of all impurities at 50%, 100%, 150%, and QL levels across three replicates ranged from 90.9% to 106.4%. The mean results of recovery are presented in Table 7.

2.5.6 Robustness

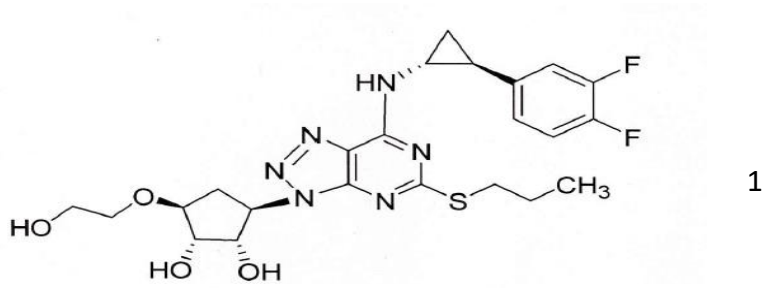
In the robustness study, the chromatograms reflecting the intentional modification of chromatographic conditions were documented. These conditions included varying flow rates of 1.0 ml/min, 0.8 ml/min, and 1.2 ml/min, as well as column temperatures of 30°C, 28°C, and 32°C. As demonstrated, the procedure exhibited robustness across a satisfactory range of its operational parameters, as shown in Table 9.

3. Results and Discussions

The potential existence of impurities in the vast majority of drug substances can have a significant impact on the pharmaceutical product's quality and safety. Hence, an examination of the impurities profile of the active pharmaceutical ingredient (API) intended for utilization in producing a pharmaceutical formulation. Three contaminants were identified during the investigation of laboratory batches of Ticagrelor. The structures of the impurities above were successfully identified and assigned the nomenclature of impurities-1, 2 and 3 with chemical names given as (1S,2S,3R,5S)-3-(7-(((1R,2S)-2-(3,4-difluorophenyl)cyclopropyl)amino)-5-(propylsulfonyl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-5-(2 hydroxyethoxy)cyclopentane-1,2-diol, (1S,2S,3S,5S)-3-(7-((1R,2S)-2-(3,4-difluorophenyl)cyclopropylamino)-5-(propylthio)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-5-(2-hydroxyethoxy)cyclopentane-1,2-diol and 2-((1S,2S,3S,4R)-4-(7-((1R,2S)-2-(3,4-difluorophenyl)cyclopropylamino)-5-(propylthio)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-2,3-dihydroxycyclopentyloxy) ethyl acetate. A straight forward and accurate statistical approach using RP-HPLC gradient was devised to quantify related impurities and to determine the assay of TGR samples. No blank interferences were found in the developed HPLC procedures due to diluents, solvents, or reagents. The chromatographic separation was performed by administering a volume of 10 µl using a Zodiac C18 column with dimensions of 150 x 4.6 mm and a particle size of 3.5 µm. The separation was carried out in gradient mode at an oven temperature of 30°C. The components were monitored at a wavelength of 210 nm, and the flow rate was set at 1.0 ml/min for 30 minutes. The gradient protocol for analyzing related impurities and assay involved time and % mobile phase-B values. These values were as follows: 0.01/50%, 5/50%, 10/75%, 15/75%, 20/80%, 25/50%, and 30/50%. The retention durations observed for Ticagrelor, impurity-1, impurity-2, and impurity-3 were around 7.38, 3.50, 8.30, and 10.81 minutes, respectively. The new analytical method demonstrated specificity towards Ticagrelor and its three impurities.

Utilizing the conventional RP-HPLC technique, the specificity and forced degradation of TGR was determined in the presence of its impurities. At the retention periods for impurity-1, impurity-2, impurity-3, and Ticagrelor, no interferences from the blank were detected. The elution order of impurity-1, impurity-2, impurity-3, and Ticagrelor, as observed in individual and impurity blend solutions, remains consistent, with identical retention periods. Throughout the experimental forced degradation investigations, it was found that the Ticagrelor sample did not undergo substantial degradation when exposed to various conditions, including ambient temperature (25+2°C), thermal stress (105°C for one week), acid

hydrolysis (0.1N HCl, 5 hours at 80°C), and base hydrolysis (0.1N NaOH, 5 hours at 80°C), water (80°C for 5 hours), photolytic (1.2MLux Hours/200 Watt Hours) and humidity (90%RH). A significant decline in oxidative stress status was noticed.



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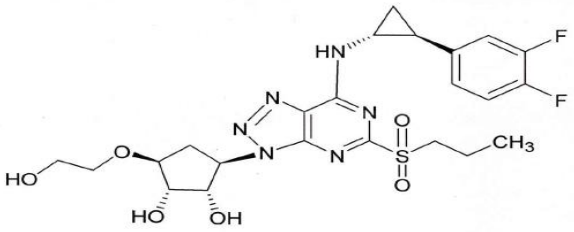

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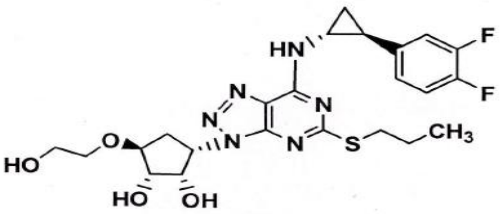

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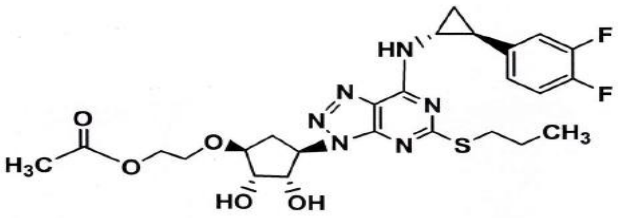

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Figure 1- 4: presents the chemical structures of Ticagrelor (Fig 1) and impurities 1 (Fig 2), impurity 2 (Fig 3), impurity 3 (Fig 4) respectively.

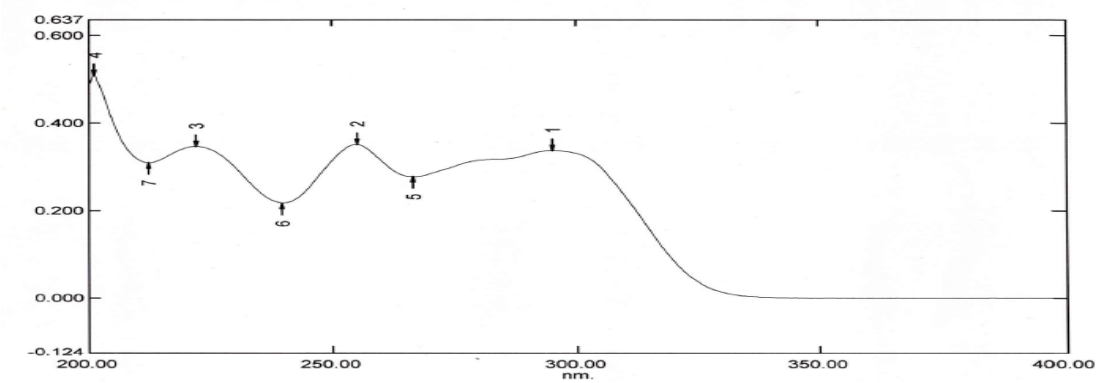

Figure 5: UV-absorption spectrum of TGR.

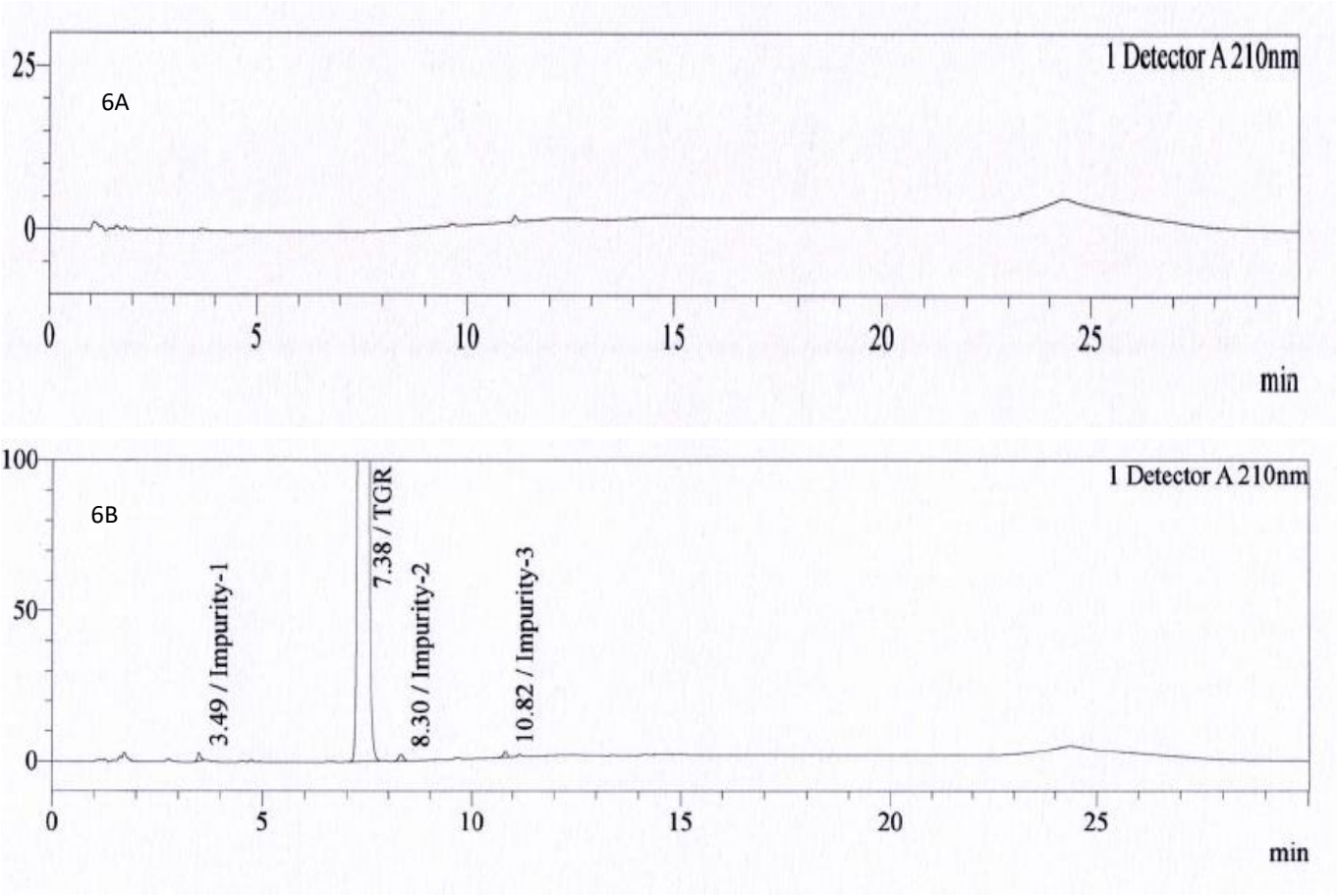

Figure 6: Typical HPLC chromatogram of Blank (6A) and TGR spiked with 0.15% of Impurity 1, 2 and 3 (6B)

Table 1: Forced degradation data (Impurities) of Ticagrelor

Name of the sample	Stressed conditions and period of exposure	% of imp-1	% of imp-2	% of imp-3	% of MSUI	% of TI
Mother sample (As such)	----	ND	ND	ND	0.09	0.22
Ambient sample	Weekdays	ND	0.01	ND	0.09	0.23
Thermal sample	Weekdays	ND	0.01	ND	0.09	1.74
Humidity sample	Weekdays	ND	0.01	ND	0.09	0.22
Water hydrolysis	Heated at 80°C in H_2O for 4hrs	ND	ND	ND	0.04	0.04
Acid hydrolysis	Heated at 80°C in 0.1N HCl for 5hrs	ND	0.57	ND	1.16	1.45
Base hydrolysis	Heated at 80°C in 1N NaOH for 5hrs	ND	0.81	ND	0.32	1.36
Oxidation	Heated at 80°C in 10% H_2O_2 for 5hrs.	4.5	ND	ND	25.44	58.96
Photolytic	1.2M Lux/200 W Hours	ND	ND	ND	0.12	0.61

ND-Not Detected

Table 2: Mass balance (Impurities &Assay) data of Ticagrelor

Type of sample	Stressed conditions and period of exposure	Assay % w/w	%Total impurities	Mass balance	Observations
Mother sample (as such)	-	101.4	0.22	100.6	-
Ambient	25°C± 2°C, 10 days	100.0	0.23	100.1	No significant Degradation
Thermal	Heated at 105°C for 10 days	100.2	1.74	102.0	No significant Degradation
Humidity	90%RH, 10 days	99.9	0.23	100.1	No significant Degradation
Water hydrolysis	Heated at 80°C in H_2O for 5 hrs	96.1	2.88	99.0	No Significant Degradation
Acid hydrolysis	Heated at 80°C in 0.1N HCl for 5 hrs	101.6	2.02	103.6	No significant Degradation
Base hydrolysis	Heated at 80°C in 0.1N NaOH for 5 hrs	97.6	1.36	99.0	No significant Degradation
Oxidation	Heated at 80°C in 10% H_2O_2 for 5 hrs	48.7	58.96	107.7	Significant Degradation
Photolytic	1.2M Lux/200 W Hours	98.6	0.61	99.3	No significant Degradation

Table 3: System suitability data of Ticagrelor

System suitability parameters for related compounds		**Acceptance criteria**
Resolution criteria	**USP resolution**	
Between Ticag	3.90	Not Less than 2.0
System suitability parameters for assay compounds		
Retention time of Ticagrelor	7.43 minutes	
Asymmetry	1.16	Not more than 2.0

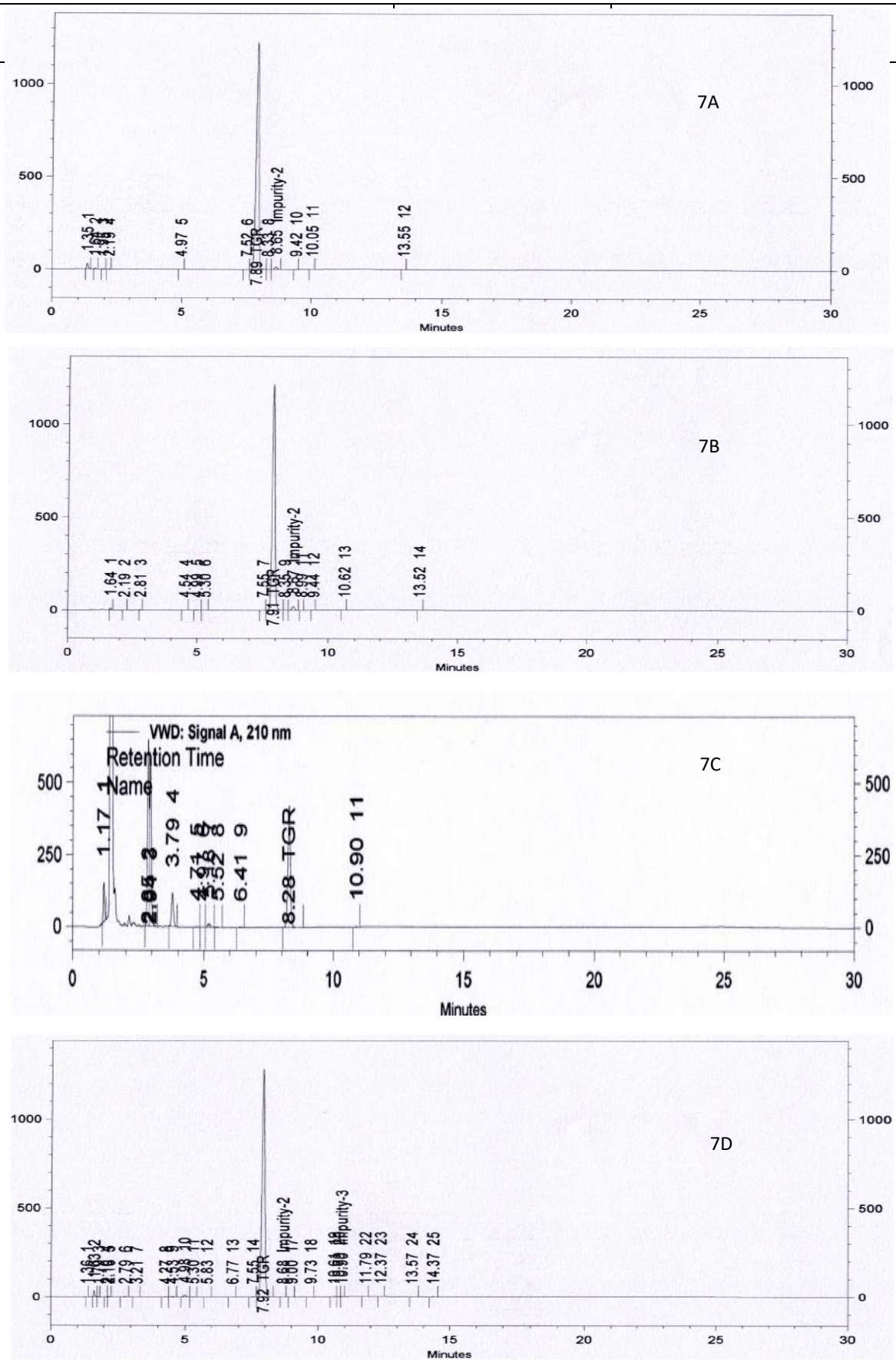

Figure 7: Typical HPLC chromatogram of acid (0.1N HCl) stressed solution (7A), base (0.1N NaOH) (7B), oxidative (10%H2O2) (7C), water hydrolysis solution (7D)

Table 4: DL & QL S/N ratio data of Ticagrelor

Component	Concentration of DL solution (%)	S/N Ratio	Concentration of QL solution (%)	S/N Ratio
Impurity-1	0.01	3.06	0.03	20.56
Impurity-2	0.01	2.65	0.03	15.77
Impurity-3	0.01	5.80	0.03	21.79
Ticagerlor	0.01	3.23	0.03	12.49

Table 5: QL precision data of Ticagrelor

Replicates	Area of Ticagrelor	Area of impurity-1	Area of impurity-2	Area of impurity-3
1	2701	2558	2967	3103
2	2759	2572	2869	3044
3	2639	2501	2884	3050
4	2686	2527	2980	3098
5	2679	2500	2903	3072
6	2672	2511	2918	3082
Average	**2689**	**2528**	**2920**	**3075**
% RSD	**1.48**	**1.20**	**1.53**	**0.79**

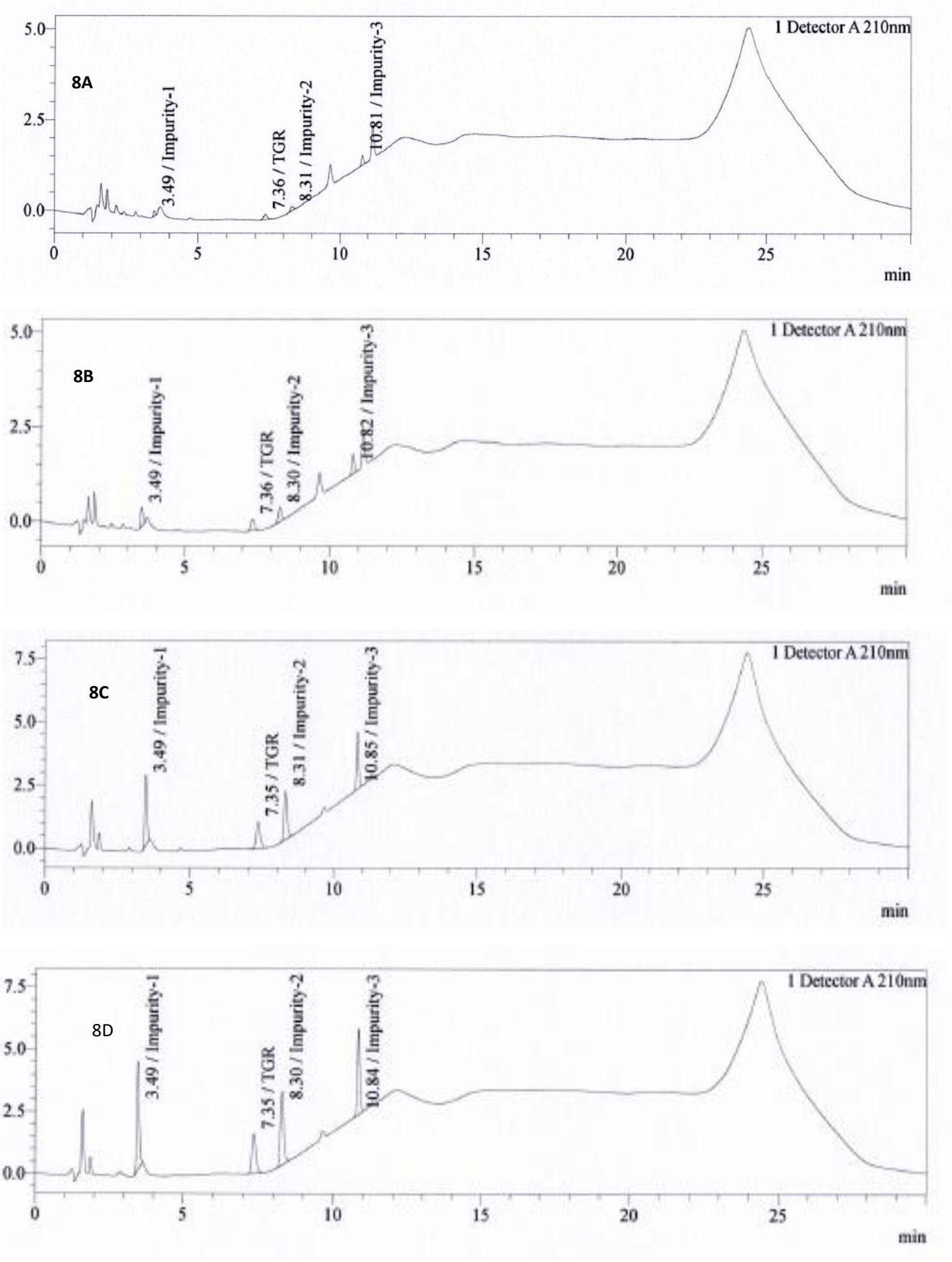

Figure 8: Typical HPLC chromatogram of DL (8A), QL (8B), 100% (8C), 150% (8D) solution

Table 6: Linearity for Impurities and Ticagrelor

	Impurity-1		Impurity-2		Impurity-3		Ticagrelor	
Level	**Conc. (%)**	**Mean Area**	**Conc. (%)**	**Mean Area**	**Conc. (%)**	**Mean Area**	**Conc. (%)**	**Mean Area**
Level-1	0.0270	2643	0.0280	3049	0.0270	3049	0.0300	3089
Level-2	0.0670	7832	0.0710	8050	0.0660	6836	0.0440	5011
Level-3	0.1070	13167	0.1130	12788	0.1130	12788	0.0710	7904
Level-4	0.1330	15907	0.1420	15410	0.1420	15410	0.0890	10125
Level-5	0.1600	20428	0.1700	19953	0.1700	19953	0.1070	12578
Level-6	0.2200	25749	0.2130	23640	0.2130	23640	0.1330	15250
Correlation coefficient		**0.9966**		**0.998**		**0.9946**		**0.9993**
Y Intercept (c)		**-224**		**-30**		**409**		**-362**
% of Y Intercept		**-1.41**		**-0.2**		**3.25**		**-3.58**
Slope(m)		**121950**		**112714**		**97853**		**118416**

Table 7: Accuracy of impurity-1, impurity-2 and impurity-3

% of Recovery of impurity-1					
Level	**Preparation-01**	**Preparation-02**	**Preparation-03**	**Mean**	**95% confidence interval of mean**
Level-1	113.7	95.3	90.9	100.0	86.3-113.7
Level-2	98.60	98.9	98.6	98.7	98.5-98.9
Level-3	107.2	105.0	106.9	106.4	105.0-107.7
Level-4	93.5	95.6	95.2	94.8	93.5-96.0
% of Recovery of impurity-2					
Level-1	105.5	108.3	93.6	102.5	93.6-111.3
Level-2	93.10	91.8	92.9	92.6	91.8-93.4
Level-3	102.30	101.8	101.4	101.8	101.3-102.3
Level-4	91.4	93.9	91.9	92.4	90.9-93.9

% of Recovery of impurity-3					
Level-1	103.1	102.0	98.3	101.1	98.3-104.0
Level-2	96.5	96.1	97.4	96.7	95.9-97.4
Level-3	105.6	102.9	104.1	104.2	102.7-105.7
Level-4	84.50	87.1	86.3	86	84.5-87.5

Table 8: Method precision data of Ticagrelor

Preparations	% of impurity-1	% of impurity-2	% of impurity-3	% of MSUI	% of TI
Prep'n-01	0.14	0.16	0.14	0.07	0.80
Prep'n-02	0.14	0.15	0.14	0.08	0.81
Prep'n-03	0.14	0.15	0.14	0.06	0.79
Prep'n-04	0.15	0.16	0.16	0.06	0.78
Prep'n-05	0.14	0.15	0.14	0.06	0.82
Prep'n-06	0.14	0.16	0.14	0.07	0.87
Average	0.14	0.16	0.14	0.07	0.77
STDEV	0.00	0.01	0.01	0.01	0.03
% RSD	**2.88**	**3.53**	**5.70**	**12.25**	**4.14**
Confidence interval of mean from	**0.14**	**0.15**	**0.14**	**0.06**	**0.74**
Confidence interval of mean to	**0.14**	**0.16**	**0.15**	**0.07**	**0.80**

Table 9: Robustness data of Ticagrelor

Component	Condition	Mean	STDEV	% RSD
% of impurity-1	Flow rate 0.8 mL. min^{-1}	0.14	0.01	7.02
	Flow rate 1.2 mL. min^{-1}	0.15	0.01	7.87
	Column temperature at 28°C	0.15	0.01	6.67
	Column temperature at 32°C	0.15	0.02	11.55
% of impurity-2	Flow rate 0.8 mL. min^{-1}	0.16	0.01	6.38
	Flow rate 1.2 mL. min^{-1}	0.17	0.01	6.93
	Column temperature at 28°C	0.16	0.01	6.25
	Column temperature at 32°C	0.16	0.02	10.83
% of impurity-3	Flow rate 0.8 mL. min^{-1}	0.14	0.01	6.98
	Flow rate 1.2 mL. min^{-1}	0.14	0.01	7.14
	Column temperature at 28°C	0.15	0.01	7.87
	Column temperature at 32°C	0.14	0.02	10.66
% of MSUI	Flow rate 0.8 mL. min^{-1}	0.07	0.01	11.35
	Flow rate 1.2 mL. min^{-1}	0.07	0.01	7.87
	Column temperature at 28°C	0.07	0.01	7.87
	Column temperature at 32°C	0.07	0.01	7.87
% of TI	Flow rate 0.8 mL. min^{-1}	0.77	0.06	7.22
	Flow rate 1.2 mL. min^{-1}	0.82	0.1	11.8
	Column temperature at 28°C	0.77	0.06	8.25
	Column temperature at 32°C	0.8	0.1	12.31

The mass balance results obtained from the analysis of ticagrelor-forced degradation samples ranged from 100.1% to 101.7%. Assay experiments were performed to evaluate stress samples compared to a qualified reference standard. Subsequently, the mass balance was determined by summing the percentages of the assay, impurities, and degradation products. The assay for the bulk sample was determined by introducing all three contaminants at the specified level (0.15% of impurity-1, impurity-2, and impurity-3 relative to the analyte concentration of 0.5 mg/ml). The investigation also included the determination of the detection limit (DL), quantitation limit (QL), and precision at the QL level (%RSD, 0.79-1.5) for Ticagrelor spiked with impurity-1, impurity-2, and impurity-3. The % RSD for the peak areas was calculated. The detection limits for impurity-1, impurity-2, and impurity-3 are 0.01%, 0.01%, and 0.01%, respectively. The quantitative thresholds for the three contaminants are 0.03%, 0.03%, and 0.03% respectively. The approach that was created demonstrated high precision, with a precision value of less than 5.0%. Additionally, the accuracy of the approach was found to range from 93.6% to 113.7% at the quantification limit (QL) level. A calibration curve was constructed by graphing the peak area of impurities against their corresponding concentrations, which were reported as percentages (specifically, at QL, 50, 80, 90, 100, 120, and 150% levels). This calibration curve determined many parameters like the correlation coefficient, slope, y-intercept, and residual sum. The determined correlation coefficient ranged from 0.995 to 0.999. The mean recoveries of all contaminants in three replicates at 50%, 100%, and 150% were within the 84.5-108.3% range. The percentage relative standard deviation (% RSD) of the area for each impurity was calculated to assess system precision, method accuracy, and intermediate precision. The % RSD values were determined to be within acceptable bounds. The demonstrated approach exhibited robustness within a satisfactory range of operating parameters.

4. Conclusions

A simple, gradient HPLC approach was developed to quantify TGR and its related substances and assay is precise, accurate and specific. Ticagrelor is susceptible to deterioration under peroxide (oxidation) and mild degradation under acidic and hydrolysis environments while no degradation observed demonstrating stability when exposed to base, thermal, ambient, photolytic and humidity conditions. The observed degradation and validation results indicate that the approach exhibits specific, linear, accurate, robust, and stability-indicating properties. The utilization of this method can provide an estimation of Ticagrelor inside the dosage form of the pharmaceutical product. The method that has been developed demonstrates qualities that indicate stability and may be readily employed by the quality control department for the quantification of associated components and the analysis of regular TGR and stability samples.

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Conflict of Interest
The author has no conflict of interest to declare.

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