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Original Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION STUDIES OF TICAGRELOR TABLETS BY RP-HPLC

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ABSTRACT

Objective: The present study was conducted to develop a simple and precise analytical method for the estimation of ticagrelor in tablet formulation.

Methods: Reverse Phase HPLC was used for method development and validation studies of ticagrelor**.** The optimum chromatographic conditions comprised of C18 column (Kromasil, 250×4.6 mm, 5 µ) as the stationary phase and aqueous buffer (containing 0.5 ml formic acid and triethylamine each in water) and acetonitrile in the ratio of 50:50 v/v as the mobile phase. The flow rate was 1.3 ml/min with detection at 256 nm and a run time of 6 min. Forced degradation studies were conducted and the isocratic mode was modified to a gradient mode to make the method stability indicating in nature.

Results: The retention time of ticagrelor was 3.372 min. The linearity studies indicated that the range of the developed method was 20-90 ppm with a correlation coefficient of 0.9956. The method was specific with a percent mean recovery was found to be 99.93%.. The % RSD in the precision studies was 0.069. The validated method was applied to conduct the assay of ticagrelor in tablets and the with a percent mean recovery of 99.82%. The modified method was capable of resolving 13 related substances from the ticagrelor peak in the forced degradation studies.

Conclusion: The developed and validated RP-HPLC isocratic method was simple, accurate and precise as per the ICH guidelines. It was suitable for the analysis of ticagrelor in bulk and tablet formulation. The modified gradient method can be used to resolve the in-process impurities and related substances and can be directly applied to liquid chromatography hyphenated with mass spectroscopy (LC/MS) studies with minor modifications for identification of related substances.

Keywords: RP-HPLC, Agilent 1260 UV/PDA, Ticagrelor, Kromasil C18, Related substances, Forced degradation

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INTRODUCTION

Chromatography is a powerful separation method that finds application in all branches of science which is used for method development. High Performance Liquid Chromatography or High Pressure Liquid Chromatography (HPLC) is mainly a chromatography technique that can separate a mixture of compounds and is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of a mixture of analytes by distributing between the mobile phase (a flowing liquid) and a stationary phase (sorbents packed inside a column). Ticagrelor is newly introduced into the market, hence the data on the qualitative and quantitative estimation of this drug is limited. It is marketed as a tablet for the treatment of thrombosis. It reduces the rate of thrombotic cardiovascular events in patients with the acute coronary syndrome. It belongs to the class of triazolo pyrimidines which are polycyclic aromatic compounds containing triazole ring fused to a pyrimidine ring. Ticagrelor and its major metabolite reversibly interact with the platelet P_2Y_{12} 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 [1]. The marketed formulation of ticagrelor replaced clopidogrel-containing 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. This antiplatelet drug formulation was developed and marketed by AstraZeneca, the patent was granted on 10th June 2008 and patent expire on 3rd December 2018. The clinical evidence for the effectiveness of ticagrelor was derived from Plato, a randomized double-blind study comparing ticagrelor with clopidogrel. This product is used to treat people who have had a recent heart attack or severe chest pain that happened because their heart was not getting enough oxygen or are treated with a procedure to open blocked arteries in the heart [2-4]. A literature search revealed very few papers on analytical methods of this drug. One research paper has reported a UV method of analysis and another one has reported a HPLC method of analysis for ticagrelor using UV detector [5-6]. Hence the aim of this study was to develop a simple, accurate and precise RP-HPLC analytical method for the estimation of ticagrelor in tablet formulation.

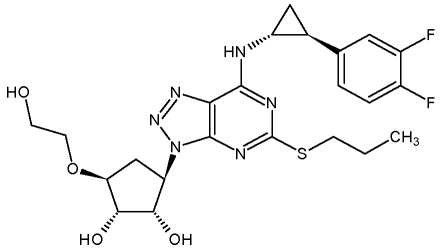

Fig. 1: Chemical structure of ticagrelor

MATERIALS AND METHODS [7-9]

Chemicals and reagents

Ticagrelor reference standard was obtained as a gift sample from Watson® Pharmaceuticals. Methanol and acetonitrile (HPLC grade), MilliQ water. hydrochloric acid (AR) 37%, sodium hydroxide (AR grade), orthophosphoric acid (HPLC grade) 85%, ammonium acetate (GR), potassium dihydrogen phosphate (AR), hydrogen peroxide (ACS) 50%, formic acid (AR), triethylamine (HPLC grade), sodium

lauryl sulphate, polysorbate 80 (GR) were procured from Merck® and Fischer Scientific®.

Instruments used

The HPLC system used was an Agilent 1260 infinity equipped with a quaternary solvent pump, a thermostated autosampler, degasser, UV/VWD detector and a column oven. A double beam UV-Visible spectrophotometer (Shimadzu 1800), USP Type II-Paddle apparatus (Labindia DS2000 with Ismatec high precision multichannel pump as autosampler), photo stability chamber (Atlas Suntest XLS+), HPLC water purifier (Millipore–Merck Millipore), orbital shaker (Thermo scientific) and a centrifuge (REMI research centrifuge) were used for this analysis.

Preparation of solutions

UV studies

Preparation of standard stock solution (10 ppm)

1 mg of ticagrelor reference standard was weighed accurately and transferred into a 100 ml volumetric flask. 30 ml diluent (acetonitrile: water 90:10 v/v) was added and the mixture was sonicated. The solution was diluted up to the mark with the diluent to give the standard stock solution. The λ_{max} was determined using UV-VIS spectrophotometer. A working standard range from 2 to 7 ppm was prepared from the stock solution of 10 ppm and used for linearity studies.

Preparation of standard stock solution (90 ppm)

25.0 mg of ticagrelor reference standard was weighed and transferred into a 25 ml volumetric flask to obtain a solution of 1000 ppm in duplicate. 18 ml of methanol was added and sonicated. This solution was diluted to volume with methanol and mixed well. 9.0 ml of standard stock solution was taken and diluted to 100 ml with diluent to give a standard stock solution of 90 ppm.

Preparation of sample solution

Five tablets of ticagrelor were weighed and transferred to 250 ml volumetric flask. 100 ml of water was added and sonicated for 1h to disperse the contents. Further 50 ml of water was added and the flask was kept in an orbital shaker at 50 rpm for 2h at room temperature (RT). The flasks were made up to the mark with water. The solution was then filtered using a 0.45µ Whatman Teflon filter. The filter was saturated by discarding the first 10 ml of filtrate. 5.0 ml of the above filtrate was further diluted to 100 ml with diluent and mixed well to get the desired concentration.

Preparation of buffers

Preparation of buffered mobile phase

0.5 ml and 0.5 ml of concentrated triethylamine solution were added using 1 ml pipette to 500 ml water, filtered through 0.45µ Nylon 6, 6 membrane filter under suction and sonicated to degas.

Preparation of other buffers

6.8 g of potassium dihydrogen orthophosphate (KH_2PO_4) was added, filtered through 0.45µ Nylon 6, 6 membrane filter under suction and sonicated to degas. The pH was adjusted and maintained using concentrated orthophosphoric acid (OPA) to get pH 3.0, pH 4.5, pH 6.8 and pH 7.5 respectively.

0.1% and 0.2% of sodium lauryl sulphate (SLS) media: 10g and 20 g of SLS was dissolved in 10 L of water and sonicated. Similarly for preparing 0.1% and 0.2% of polysorbate (tween) media: 10 g and 20 g of tween 80 were dissolved in 10 L of water and sonicated.

Filter compatibility studies

Filter compatibility tests were conducted to get a particle-free solution, which also should reproduce 100% response as per the respective content. The filtrate from each filter was compared with the centrifuged sample. The filter complying with all the parameters and showing same concentration as that of centrifuged sample was selected for further studies.

Five intact tablets were weighed and transferred to 250 ml volumetric flask. 25 ml of miliQ water was added and sonicated for 10 min. 150 ml of acetonitrile was added and sonicated for 20 min with intermittent swirling. This was further diluted with acetonitrile mixed and filtered using 0.45µ Whatman Teflon filter. 10 ml of filtrate was discarded and further 5 ml was diluted to 100 ml with diluent. A 20 ml aliquot was subjected to centrifuge at 5000 rpm for 20 min and another 20 ml per filter was passed through the filters listed in fig. 2.

Fig. 2: Filter study-Whatman 0.45 µm Nyl w/GMF, Whatman 0.7 µm GF/F w/GMF, Millipore millex-HV Hydrophilic PVDF 0.45 µm respectively

The four solutions were chromatographed and were compared for peak shape and peak area. The filter that matched the centrifuged sample data was selected for further studies.

Selection of diluent (HPLC compatible solvent) and solution stability studies

The stability of the solution under study was established by keeping the solution at room temperature for 24 h. A standard solution was prepared using acetonitrile: water (90: 10 v/v, 50: 50 v/v, and 10: 90 v/v), methanol: water (90: 10 v/v, 50: 50 v/v and 10: 90 v/v). The samples were injected at different time intervals of 5 min, 1 d and 1 w.

Selection of flow rate and load volume

Flow rates (0.8 ml/min. and 1.3 ml/min.) and loading volumes (1, 4, 5, 6, 10, 20, 50 µl) were analyzed to get a precise and reproducible peak of ticagrelor with minimum tailing or fronting.

Selection and optimization of mobile phase

Various mobile phase compositions like acetonitrile: water (50: 50 v/v, 90: 10 v/v, 60: 40 v/v, 40: 60 v/v and 10: 90 v/v), acetonitrile: methanol: water (80: 10: 10 v/v, 50: 25: 25 v/v, 30: 40: 30 v/v, 40: 30: 30 v/v and 10: 45: 45 v/v), acetonitrile: TEA buffer in water (50: 50 v/v ratio with 0.25 ml and 0.5 ml concentration of TEA per 500 ml.), acetonitrile: TEA and FA buffer in water (50: 50 v/v ratio with 0.25 ml: 0.25 ml and 0.5 ml: 0.5 ml concentration of TEA and FA per 500 ml.), acetonitrile: buffer [pH range 3 to 7.5] (50: 50 v/v, 80: 20 v/v and 20: 80 v/v) were evaluated in an effort to get symmetrical, sharp and reproducible peaks of ticagrelor.

Selection and optimization of stationary phase

Using the temporary developed chromatographic method a variety of columns with different attributes like polarity, particle size (3.5 µ and 5 µ), carbon loading (C8 and C18) and dimensions were screened in an effort to arrive at the best column for the given analysis. Some of the columns studied were; Symmetry©C18 50 × 4.6 mm 3.5µ, Akzonobel Kromasil© C18 150 × 4.6 mm 5µ, Waters Xterra© C18 150 × 4.6 mm 3.5µ, BDS Hypersil thermo© C8 50 × 4.6 mm 5µ, Fortis©C8 50 × 4.6 mm 5µ and Agilent zorbax©RX C18 150 × 4.6 mm, etc.

Selection of dissolution media by Biopharmaceutical Classification System (BCS)

A ticagrelor standard solution was prepared and the final dilution was done using water, 0.1 N HCl, pH 4.5 buffer, pH 6.8 buffer, SLS media and tween media respectively.

Five tablets were weighed and transferred to 250 ml volumetric flask.100 ml of water was added and sonicated for 1h to disperse the contents. Further 50 ml of water was added and the flask was kept in an orbital shaker at 50 rpm for 2h at RT. The solutions were made up to the mark with water. The solutions were mixed well and filtered using 0.45µ whatman teflon filter. The filter was saturated by discarding 10 ml of filtrate and 5.0 ml of above solution was diluted to 100 ml with diluent and mixed well.

The % content of the ticagrelor into the respective media was calculated using formula;

$$\frac{A_T}{A_S} \times \frac{W_S}{25} \times \frac{9}{100} \times \frac{250}{5} \times \frac{100}{5} \times \frac{P}{100} \times \frac{100}{LC}$$

Where, A_T = Peak area response of ticagrelor in the chromatogram obtained from the test solution. A_S = Average peak area response of ticagrelor in the chromatograms obtained from replicate injections of standard solution. W_S = Weight of ticagrelor standard taken in mg in standard stock solution. P = % Purity of ticagrelor standard. LC = Label claim of ticagrelor in mg per tablet.

Dissolution studies of ticagrelor tablets

Table 1: Dissolution testing conditions of ticagrelor tablets

Apparatus	USP type II (Paddle)
Disso. volume	900 ml
Speed	75 rpm
Temperature	37 °C
Time (min.)	5, 10, 20, 30, 45 and 60.

Six tablets were weighed and transferred to 6 individual jars containing 900 ml distilled water as dissolution media previously maintained at 37 °C and the dissolution apparatus was set as per dissolution testing conditions. At specified intervals of time, 10 ml of sample aliquots were withdrawn and replenished with the same volume of fresh media. The samples were filtered with 0.45µ Whatman Teflon filter. Around 8 ml of the sample was discarded to saturate the filter.

Similarly, the procedure was repeated using 0.1 N HCl, pH 4.5 buffer, pH 6.8 buffer, 0.1% SLS media, 0.2% SLS media and 0.1% Tween 80 media, 0.2% Tween 80 media.

The release of the ticagrelor into the respective media was calculated using formula;

Where, A_T = Peak area response of ticagrelor in the chromatogram obtained from test solution, A_S = Average peak area response of ticagrelor in the chromatograms obtained from replicate injections of standard solution, W_S = Weight of ticagrelor standard taken in mg in standard stock solution, P = % Purity of ticagrelor standard, LC = Label claim of ticagrelor in mg per tablet.

Assay of ticagrelor tablets

Ticagrelor standard solution and ticagrelor sample solution were prepared as discussed in 2.5.1 and 2.5.2. The samples were analysed using the developed chromatographic method and the % content of the drug in the tablets was determined.

Table 2: Gradient sequence for related substances studies

Time (min)	% acetonitrile	% FA: TEA 1:1 v/v in water
0.00	45	55
10.0	20	80
20.0	50	50
30.0	80	20
40.0	45	55

0.1 mg of known impurity (Acetal impurity, RS-13) was weighed accurately and transferred to 10 ml volumetric flask. 5 ml of diluent was added, sonicated and diluted to volume with diluent to get 10 ppm of impurity solution. The chromatograms of ticagrelor reference standard, drug sample and known impurity solutions were compared using the above-mentioned gradient run.

Related substances studies

The isocratic mode of the RP-HPLC method was modified to a gradient mode as given below with a run time of 40 min.

Forced degradation studies

With slight modifications, the developed chromatographic method was used to get accurate results. The optimized chromatographic method was kept same and run time was 12.0 min. Forced degradation studies of ticagrelor were conducted using solution stressors like distilled water, 0.1 N HCl, 0.1 N NaOH, hydrogen peroxide 50%. Photostability studies were conducted at a flux of 4.6 × 1014 photons for 24 h and thermostability studies were conducted at a temperature of 80 °C for 24 h. The results were compared with the untreated standards.

Five tablets were weighed accurately and transferred to 250 ml volumetric flask. 25 ml miliQ water was added and sonicated for 10 min. 150 ml of acetonitrile was added and sonicated for 20 min. with intermittent swirling. 25 ml of respective stressor was added and heated at 80 °C for 24 h on a water bath previously maintained at 80 °C. The sample was cooled to RT and neutralized. It was filtered using 0.45µ Whatman Teflon filter. 10 ml of filtrate was used for filter saturation and remaining 7 ml was used. 5 ml of filtrate was diluted to100 ml with diluent. The results were compared with the untreated sample. For the solid degradation analysis, the tablets were treated with the respective stressor and the results were compared with the untreated sample.

Validation studies [10-15]

Accuracy

The accuracy of the method was determined by calculating % recovery. A known amount of ticagrelor was added to a placebo and the amounts were estimated by measuring the peak area. These studies were carried out in triplicate over the specified concentration range and the amount of ticagrelor was estimated by measuring the peak area ratios. The percentage recovery and standard deviation of percentage recovery were calculated.

Precision

The precision of the method was determined in terms of Intra-day and inter-day precision. For intra-day precision studies, a standard solution of 90 ppm was injected at various time intervals and percent related standard deviation (%RSD) was estimated.

The inter-day precision was studied by injecting the same concentration of standard solution on consecutive days and the % RSD of the signal was calculated. The repeatability, intermediate precision and reproducibility of the developed method were determined.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components etc. The blank (diluent), placebo, standard (90 ppm), sample (90 ppm) were prepared and injected to prove that the method developed was specific to ticagrelor.

Linearity and range

The linearity of the method was determined at six concentration levels ranging from 1-7 ppm of ticagrelor. A regression line was plotted of peak area v/s concentration. The correlation coefficient and equation of the regression line were calculated. The interval of lowest assessed concentration to the highest is the linearity range of the procedure.

LOD and LOQ

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

Where, = the standard deviation of the response, Slope = slope of the calibration curve.

Robustness

Robustness of the developed method was studied by changing the flow rate and column temperature. The effect of flow rate was studied by keeping all chromatographic conditions same except the flow rate, i.e. 1.2 ml/min and in the next run 1.4 ml/min respectively.

Similarly, the effect of temperature was studied by keeping all chromatographic conditions same except the temperature, i.e. 35 °C and in the next run with 25 °C respectively.

System suitability

The system suitability parameters like retention time, the number of USP theoretical plates, USP tailing, peak area and peak height were evaluated.

RESULTS AND DISCUSSION

UV studies

The UV spectrum of ticagrelor in acetonitrile: water (90: 10 v/v) indicated the λmax to be 256 nm (fig. 3a). The response was linear and the regression equation was found to be y = 0.1331x+0.0858. The coefficient of correlation (R^2) was found to be 0.9971(fig. 3b).

Filter compatibility studies

A temporary chromatographic method was used for selection of the best filter. Whatman Nylon w/GMF membrane filter pore size of 0.45µ was selected after comparing the peak area and concentration (table 3) of centrifuged drug solution to other drug samples (fig. 4a and 4b). Based on these observations acetonitrile: water; 90: 10 v/v was selected as the diluent for further studies. The stability of drug sample was determined by keeping the sample in contact with the diluent for 5 min, 24 h and 1 w which indicated no change in the concentration (fig. 5a-5c and table 4).

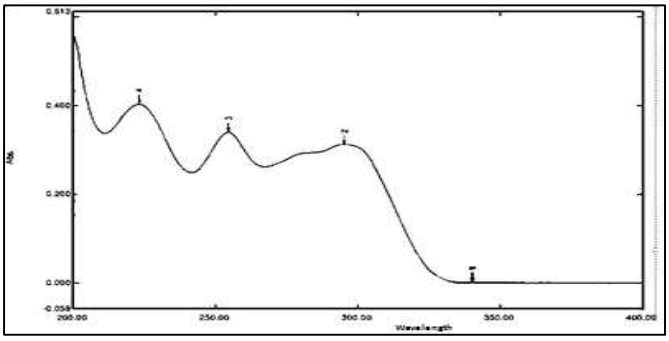

Fig. 3a: UV spectrum scan of ticagrelor in ACN: water (90: 10 v/v)

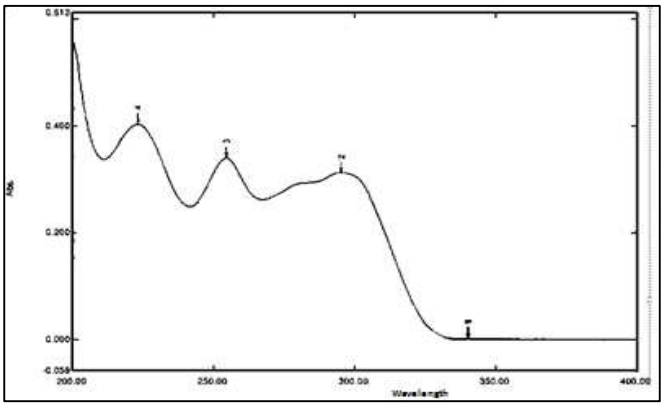

Fig. 3b: Calibration curve and R^2 of ticagrelor

Table 3: Filter compatibility studies

S. No.	Sample analyzed from	Observation	
		Concentration (ppm)	peak area
1	Centrifuge	89.63	1134583
2	0.45µ WhtNyl w/GMF	89.62	1134437

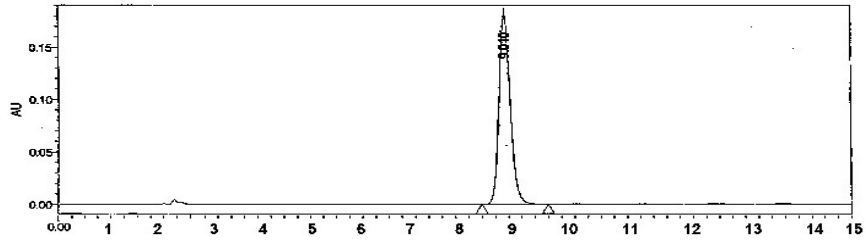

Fig. 4a: Chromatogram of the ticagrelor in the centrifuge solution

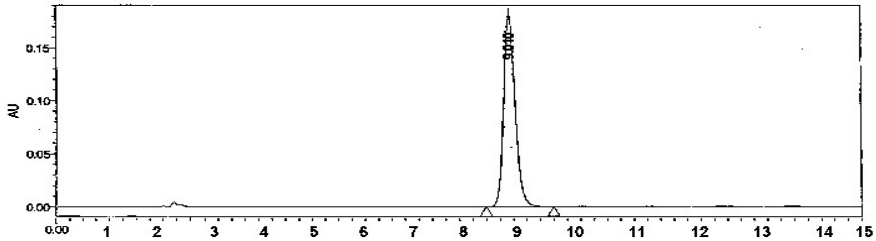

Fig. 4b: Chromatogram of the ticagrelor filtered through 0.45µ Whatman Nylon w/GMF membrane filter

Table 4: Effect of diluent on chromatogram after specified time intervals

S. No.	Analysis after	Observation	
		Retention time (min)	peak area
1	5 min	9.010	1134437
2	24 h.	9.010	1134430
3	1 w	9.010	1134432

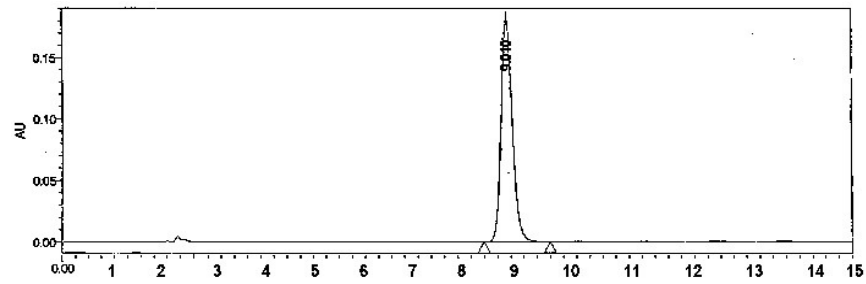

Fig. 5a: Chromatogram of ticagrelor standard solution after 5 min

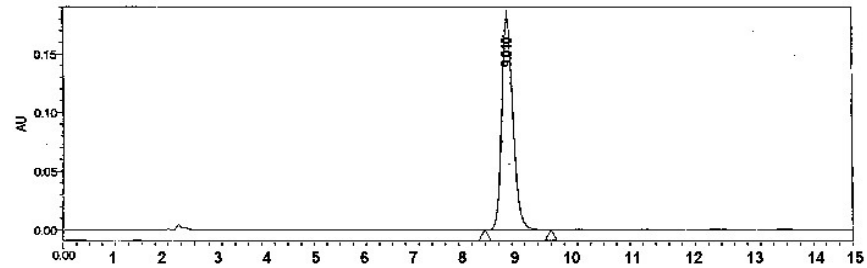

Fig. 5b: Chromatogram of ticagrelor standard solution after 24 h

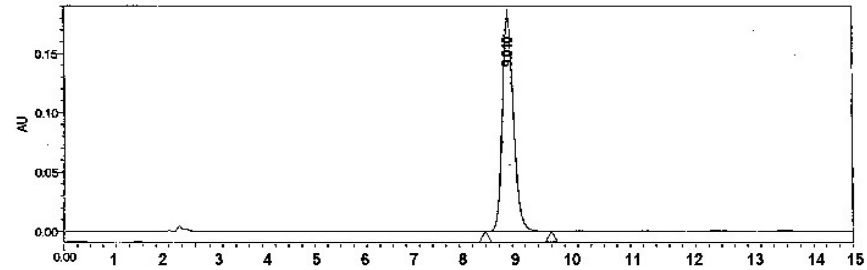

Fig. 5c: Chromatogram of ticagrelor standard solution after 1 w

Selection of mobile phase and stationary phase

According to the observations of peak shape and retention time (fig. 6), a flow rate of 1.3 ml and loading volume 10 µl was selected. Based on the data table 5, acetonitrile: formic acid: triethylamine in water 50: 50 v/v (0.5 ml: 0.5 ml) was selected as the mobile phase for the analysis (fig. 7). A variety of columns with different attributes like polarity, particle size, carbon loading and dimensions were screened in an effort to arrive at the best column for developing the analytical method of ticagrelor in API and tablet formulation (table 6) Akzonobel Kromasil© C18 150 × 4.6 mm 5µ was selected as the column for the analysis of ticagrelor (fig. 8a-8b).

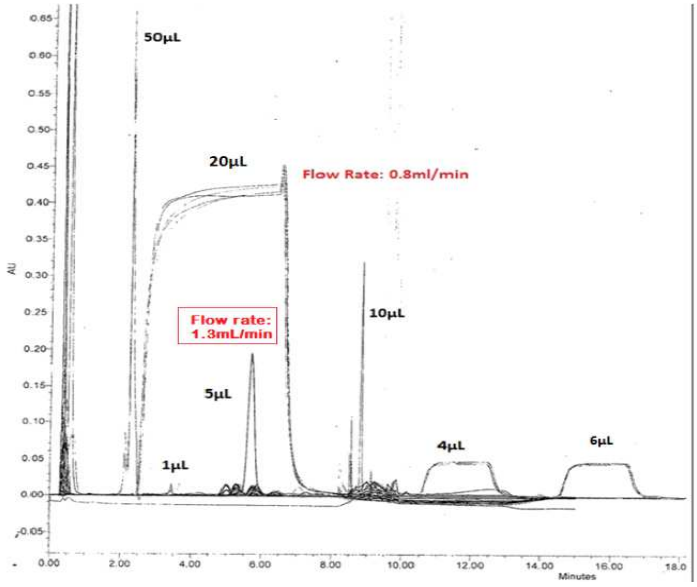

Fig. 6: Effect of flow rate and loading volume on peak shape

Table 5: Effect of mobile phase composition on chromatogram

S. No	Mobile phase composition	Observation	
		Retention time (min)	peak shape
1	Acetonitrile: water in 50: 50 v/v.	8.913	Tailing
2	Acetonitrile: water 90: 10 v/v.	6.341	Sharp
3	Acetonitrile: water in 60: 40 v/v.	9.009	Tailing
4	Acetonitrile: water in 40: 60v/v	12.252	Sharp
5	Acetonitrile: water 10: 90 v/v.	11.472	Shoulder
6	Acetonitrile: methanol: water 80: 10: 10 v/v.	9.001	Sharp
7	Acetonitrile: methanol: water 50: 25: 25 v/v.	7.882	Sharp
8	Acetonitrile: methanol: water 30: 40: 30 v/v.	9.624	Sharp
9	Acetonitrile: methanol: water 40: 30: 30 v/v.	12.113	Sharp
10	Acetonitrile: methanol: water 10: 45: 45 v/v.	8.624	Sharp
11	Acetonitrile: triethyl amine in water (TEA buffer) 50: 50 v/v 0.25 ml	9.991	Tailing
12	Acetonitrile: triethyl amine in water (TEA buffer) 50: 50 v/v 0.5 ml.	9.100	Tailing
13	Acetonitrile: formic acid: triethyl amine in water 50: 50 v/v 0.25 ml: 0.25 ml.	9.931	Sharp
14	Acetonitrile: formic acid: triethyl amine in water 50: 50 v/v 0.5 ml: 0.5 ml	9.434	sharp
15	Acetonitrile: buffer pH 3.0 in 50: 50 v/v	7.021	Merged
16	Acetonitrile: buffer pH 4.5 in 50: 50 v/v	9.982	Merged
17	Acetonitrile: buffer pH 6.8 in 50: 50 v/v.	3.718	Tailing
18	Acetonitrile: buffer pH 7.5 in 50: 50 v/v.	-	-

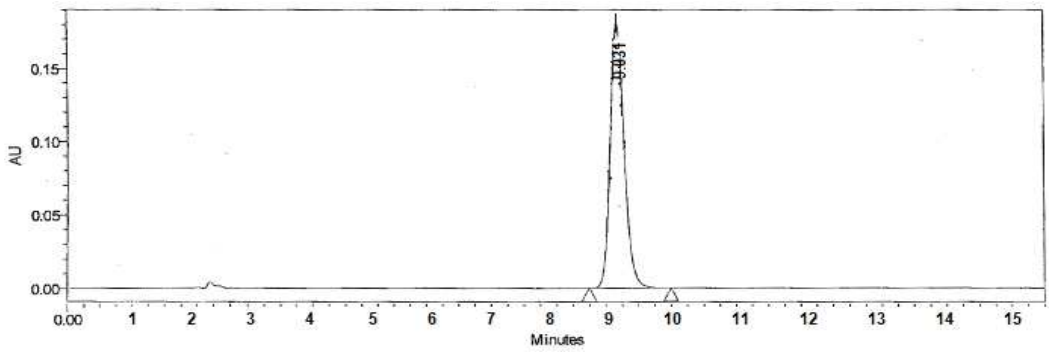

Fig. 7: Chromatogram of ticagrelor with acetonitrile: formic acid: triethylamine in water 50: 50 v/v (0.5 ml: 0.5 ml) as mobile phase

Table 6: Effect of stationary phase on chromatogram

S. No.	Stationary phase	Observation	
		Retention time (min)	peak shape
1	Symmetry©C18 50 × 4.6 mm 3.5µ	9.850	Sharp
2	Waters Xterra© C18 150 × 4.6 mm 3.5µ	8.989	Broad peak
3	Fortis©C8 50 × 4.6 mm 5µ	8.989	Symmetric
4	Inertsil ODS© C18 250 × 4.6 mm 5µ	9.434	Sharp
5	AkzonobelKromasil© C18 150 × 4.6 mm 5µ	9.001	Sharp
6	BDS Hypersil thermo© C8 50 × 4.6 mm 5µ	13.001	Fronting
7	YMC© C18 150 × 4.6 mm 5µ	14.101	Broad peak
8	SupelcosilSupelco©C18 300 × 3 mm 3µ	9.130	Sharp peak
9	Hypersil Thermo© C18 300 × 3 mm 5µ	8.022	Sharp peak
10	Agilent ZORBAX©*SB* C18 150 × 4.6 mm 5µ	9.031	Sharp
11	Agilent zorbax©*RX* C18 150 × 4.6 mm 5µ	7.812	Sharp
12	Synergy Hydro Phenomenex© C18 50 × mm 5µ	7.992	Broad peak

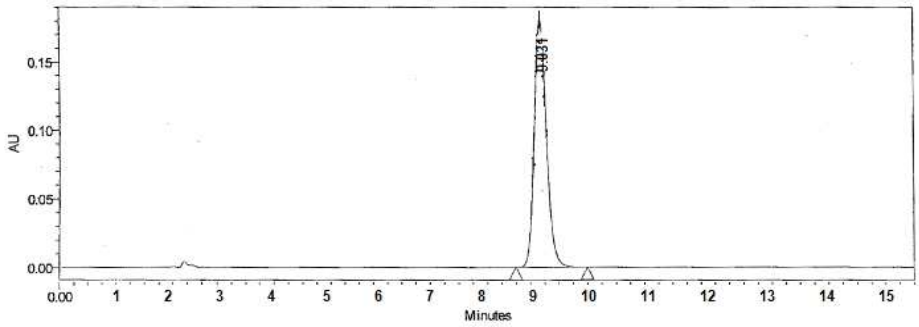

Fig. 8a: Chromatogram of ticagrelor using Inertsil ODS© C18 250 × 4.6 mm, 5µ

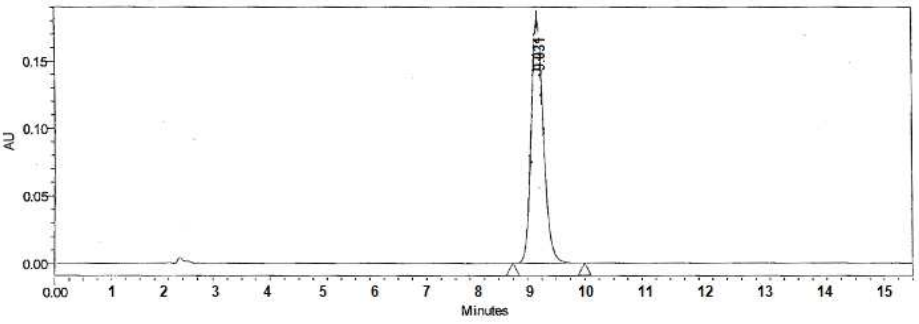

Fig. 8b: Chromatogram of ticagrelor using akzonobel Kromasil© C18 150 × 4.6 mm, 5µ

Selection of dissolution media by biopharmaceutical classification system (BCS)

Ticagrelor is a class BCS IV drug hence the selection of the solvent for dissolution studies of ticagrelor tablets was crucial. After a series of trials using different solvents (table 7) 0.2% tween 80 was selected as the solvent (fig. 9).

Dissolution studies and assay of ticagrelor tablets

The results of dissolution studies indicated that the developed RP-HPLC method was suitable for this purpose (table 8). The dissolution results indicated a release of 99.4% in 60 min. at 75 rpm (fig. 10a). The assay of ticagrelor tablets was conducted using the developed and validated RP-HPLC method and the drug content was found to be 99.9% (fig. 10b).

Table 7: Effect of various media on the stability of ticagrelor

S. No.	Solvent	Peak area		% content
		Standard	Sample	
1	Distilled water	-	-	No peak
2	0.1 N HCl	1921	1850	101.23
3	pH 4.5 buffer	10421	10393	99.73
4	pH 6.8 buffer	120105	121021	100.283
5	pH 7.5 buffer	-	-	No peak
6	0.1 % SLS	1042301	1042498	99.63
7	0.2% SLS	1042598	1042502	10.00
8	0.1 % Tween 80	1075400	1075412	99.61
9	0.2 % Tween 80	1134682	1132817	99.48

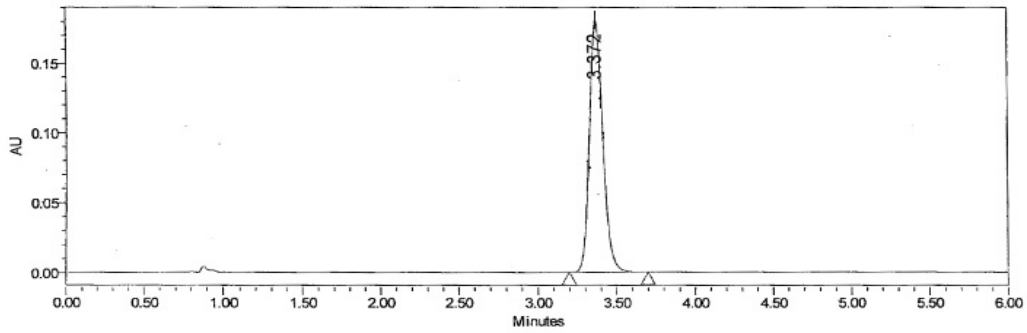

Fig. 9: Chromatogram of ticagrelor in 0.2% tween 80 in water as solvent

Table 8: Release studies of ticagrelor in 0.2% tween 80

S. No.	Time (min)	Peak area		% release
		Standard	Sample	
1	5	1134680	481342	42.26
2	10	1134680	501212	44.02
3	15	1134680	541822	47.58
4	30	1134680	755817	66.38
5	45	1134680	1084912	95.27
6	60	1134680	1132817	99.48

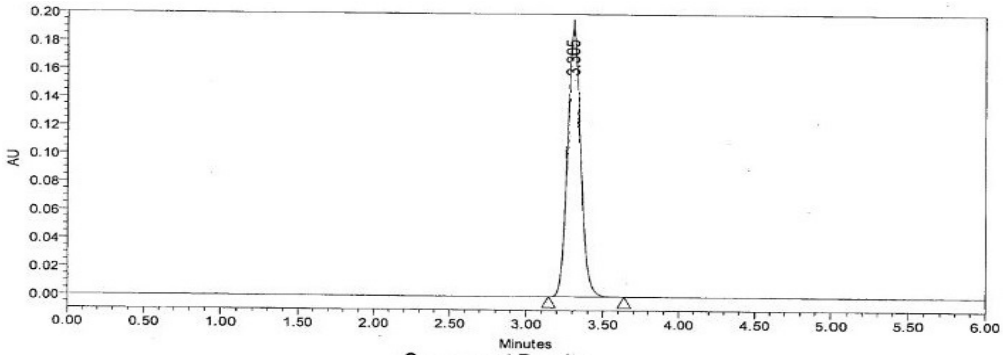

Fig. 10a: Chromatogram of ticagrelor showing release in 60 min. (Dissolution test)

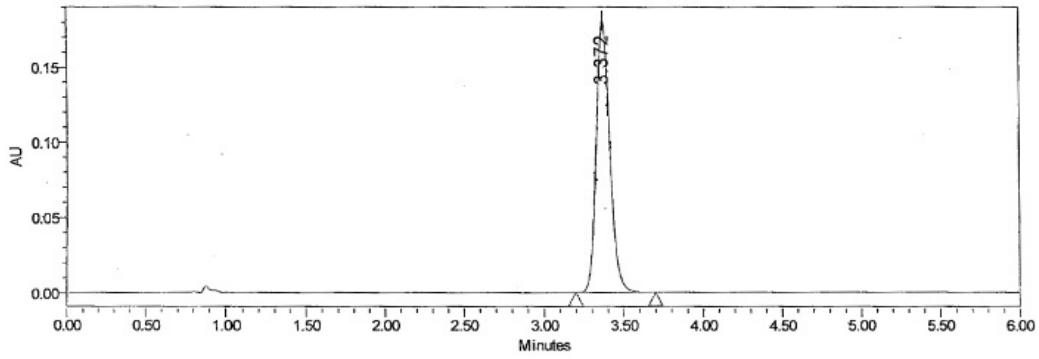

Fig. 10b: Chromatogram of ticagrelor sample (assay)

Related substances (RS) and forced degradation studies (FD)

The modified gradient RP-HPLC method with a run time of 40 min run was used for the determination of related substances in ticagrelor tablets after conducting the forced degradation studies. This method was found to be capable of resolving ticagrelor from thirteen related substances (fig. 11).

The modified analytical method with a run time of 12.0 min was used for the determination of impurities generated in the ticagrelor API sample after conducting forced degradation using various stressors. The results obtained (table 9) indicated that the developed method was able to resolve all the degradation peaks from the ticagrelor (fig 12a-12f).

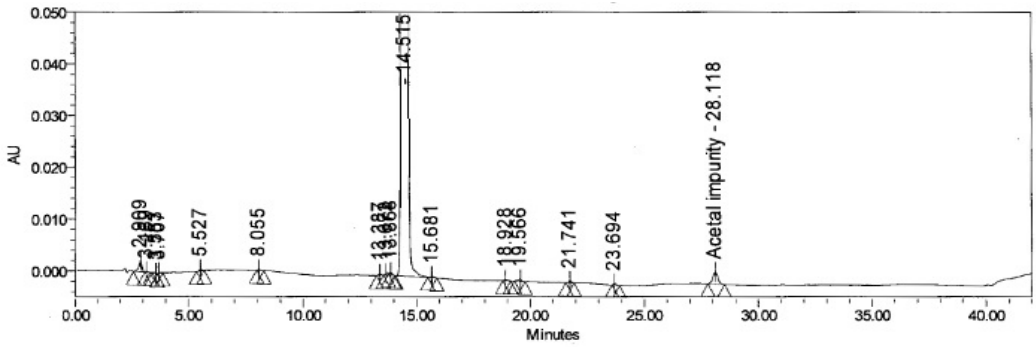

Fig. 11: Chromatogram of ticagrelorat 14.515 min with 13 related substances (RS)

Table 9: Forced degradation studies of ticagrelor

S. No.	Stressor condition	Retention time of API (min)	Impurities generated
1.	Thermostability	6.071	3
2	Photostability	6.076	3
3	Hydrolytic degradation	6.102	10
4	Oxidative degradation	6.100	11
5	Acid hydrolysis	6.089	13 and 1 Major impurity at 6.730 min
6	Alkaline hydrolysis	6.085	19

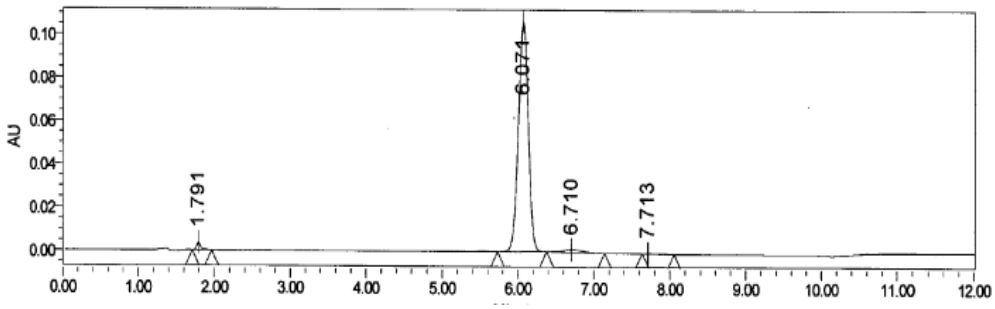

Fig. 12a: Chromatogram of ticagrelor (thermal stability)

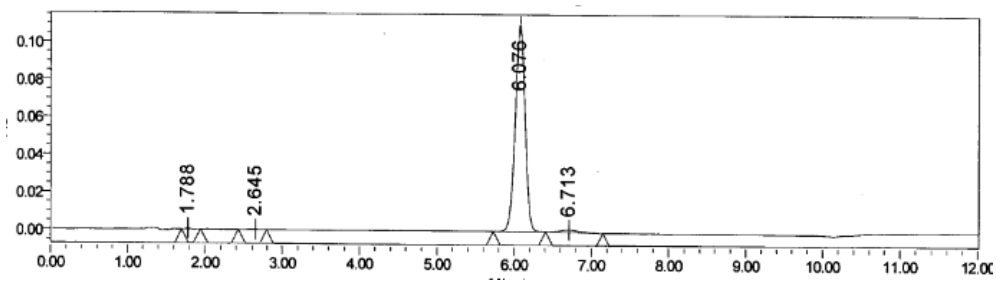

Fig. 12b: Chromatogram of ticagrelor (photostability)

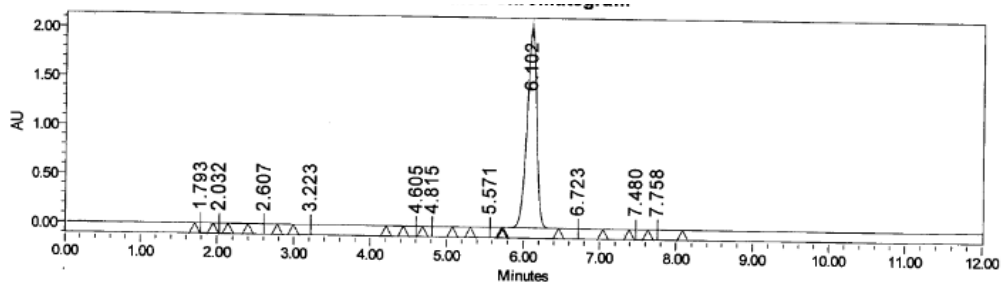

Fig. 12c: Chromatogram of ticagrelor (hydrolytic degradation)

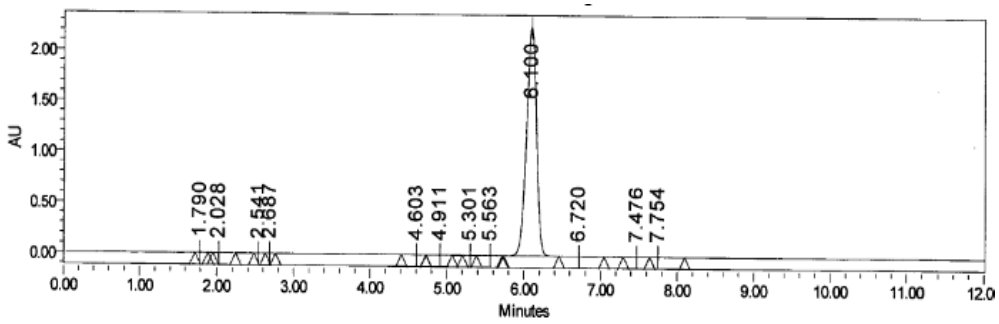

Fig. 12d: Chromatogram of ticagrelor (oxidative degradation)

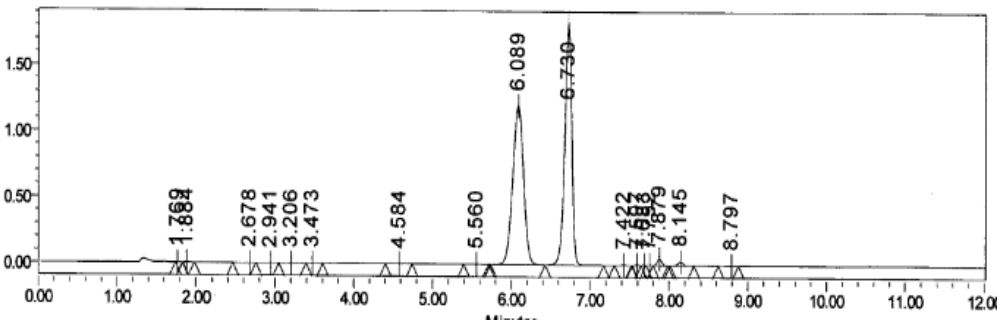

Fig. 12e: Chromatogram of ticagrelor (acid hydrolysis)

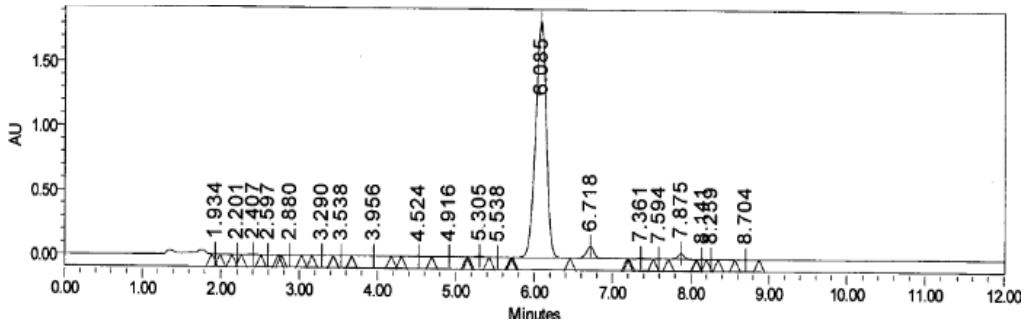

Fig. 12f: Chromatogram of ticagrelor (alkaline hydrolysis)

Validation studies

The developed RP-HPLC method was validated according to the International Conference on Harmonization (ICH) Q2 guidelines for various parameters like accuracy, precision, specificity, linearity and range, LOD and LOQ, robustness and system suitability.

Accuracy

The accuracy of the developed method was evaluated by conducting recovery studies. The accuracy was calculated by assay method at concentrations of 50%, 100% and 150%. The calculated amount of ticagrelor stock solutions were spiked to get the concentrations. The % mean recovery of spiked samples was found to be 99.82% (table 10).

Precision

The intra-day precision studies were performed using ticagrelor reference standard solution of 90 ppm. The solution was injected at various time intervals and percent related standard deviation (% RSD) was determined. The inter-day precision was studied by injecting the same concentration of standard solution on consecutive days and the % RSD was calculated. The relative standard deviation obtained from 12 assay results was found to be 0.069% (table 11).

Table 10: Accuracy studies of ticagrelor

Level	Conc. added (µg/ml)	Conc. found (µg/ml)	% recovery	% mean recovery
50%	45.051	44.51	99.80	
	45.042	44.96	99.82	99.79%
	45.064	44.96	99.76	
100%	90.01	90.01	100.0	
	89.92	89.84	99.91	99.93%
	90.04	90.01	99.90	
150%	135.012	134.674	99.74	
	135.003	134.74	99.80	99.75%
	135.014	134.62	99.71	

Table 11: Precision studies of ticagrelor

Precision parameter	Peak area				SD	% RSD
	Run 1	Run 2	Run 3	Mean		
Reproducibility	1389984	1389953	1388300	1389412	963.433	0.069
Intermediate precision	1389986	1389958	1388303	1389416	963.699	0.069
repeatability	1389983	1389956	1388309	1389416	958.785	0.069

Table 12: Specificity studies of ticagrelor

S. No.	Standard (Area AU)	Sample (Area AU)
1	138668	138650
2	138764	138758
3	138599	138596
Mean	138677	138668
SD	82.86736	82.48636
%RSD	0.059756	0.059485

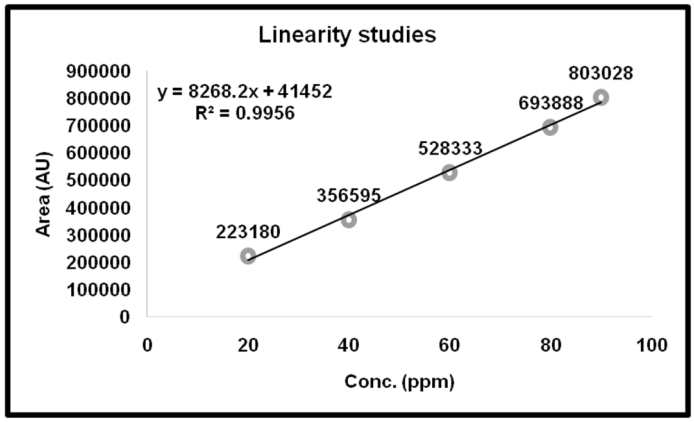

Fig. 13: Linearity and range studies of ticagrelor

Specificity

No interferences were observed in the chromatogram of ticagrelor standard due to the presence of excipients and blank (table 12).

Linearity and range

A graph of peak area versus concentration (ppm) were plotted for ticagrelor at concentration range between 20-90 ppm. The linear regression equation and correlation coefficient (r^2) were y= 8268.2x+41452 and 0.995 respectively (fig. 13).

LOD and LOQ

The results of signal to noise ratio was compared with the response of ticagrelor standard. The LOD and LOQ were found to be 0.2887 ppm and 0.8749 ppm respectively.

System suitability

System suitability tests were performed using ticagrelor standard and test solutions to check for compliance with specified parameters (table 13).

Table 13: System suitability studies of ticagrelor

S. No.	Parameter	Ticagrelor
1	Retention time	3.305
2	Number of theoretical plates	5538
3	Tailing factor	0.85
4	Peak area	1134680
5	Peak height	190457

Table 14: Robustness studies of ticagrelor

Factor	Level	T_R	Tailing factor
Parameter: Flow rate			
1.2 ml/min	-1	3.53	0.86
1.3 ml/min	0	3.31	0.92
1.4 ml/min	+1	3.20	0.74
Mean		**3.34**	**0.84**
Parameter: Column temperature			
25 °C	-5	3.48	0.75
30 °C	0	3.32	0.88
35 °C	+5	3.14	0.81
Mean		**3.31**	**0.79**

Robustness studies

The changes were applied and system suitability parameters were checked, found to be within the acceptable limits. It was noted that trivial changes in temperature and flow rate does not affect the method and produces results which passes system suitability. Hence the method was robust (table 14).

CONCLUSION

A new RP-HPLC method for the determination of ticagrelor was developed and validated. The developed method was accurate, precise, specific and sensitive for the quantitative analysis of ticagrelor both in bulk drug and tablets. Validation studies indicated the method to be robust on minor variations in the chromatographic parameters. No attempt was made to quantify the degradation products in this research project. This method can be used for routine quality monitoring of ticagrelor and its tablets. This method can be extended for application in LC-MS for quantitative estimation of known and unknown impurities generated during forced degradation and related substance studies both for API and finished products of ticagrelor.

CONFLICT OF INTERESTS

Declared none

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