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Research Article

Development and validation of stability indicating UPLC method for the estimation of ticagrelor in bulk and its tablet dosage form

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ABSTRACT

The objective of the method was to develop a simple, rapid, sensitive, precise, accurate and validated Ultra Performance Liquid Chromatographic (UPLC) method for the estimation of Ticagrelor in tablet dosage form. Chromatographic separation was achieved on an acquity UPLC BDS C8 (150 x 4.6 mm, 5µ) column with a mobile phase composed of Buffer 0.1% OPA (2.2 pH) and Acetonitrile in the ratio of 60:40 at a flow rate of 1.0 ml/min and 1 µl injection volume. The effluents were detected at a wavelength of 240 nm using TUV detector. The retention time of Ticagrelor was found to be at 0.942 min. %RSD of the Ticagrelor was found to be 0.7 The method was validated with respect to specificity, accuracy, linearity, precision, robustness. The correlation coefficient for Ticagrelor was found to be 0.999. Recovery of Ticagrelor in formulation was found to be 99.51% . LOD, LOQ values obtained from regression equations of Ticagrelor were 0.45, 1.35 respectively. Due to simplicity, high precision and rapidness the method can be successfully applied for estimation of Ticagrelor in tablet dosage form.

Keywords: Ultra Performance Liquid Chromatographic, Ticagrelor, Tablet dosage form.

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 is a platelet aggregation inhibitor . Like the thienopyridines prasugrel, clopidogrel and ticlopidine, ticagrelor blocks adenosine diphosphate (ADP) receptors of subtype P2Y12. In contrast to the other antiplatelet drugs, ticagrelor has a binding site different from ADP, making it an allosteric antagonist, and the blockage is reversible. Moreover, the drug does not need hepatic activation, which might work better for patients with genetic variants regarding the enzyme CYP2C19 (although it is not certain whether clopidogrel is significantly influenced by such variants). The structure of Ticagrelor was shown in **Fig. 1**.

The literature survey revealed that there are few RP-HPLC[1-4] and UV[5-7] methods are available for the estimation of Ticagrelor . However, a stability indicating UPLC method was not available. Hence, present work focused on the development and validation of a simple, rapid, robust and economical stability indicating UPLC method. To the best of our knowledge the anticipated method is the first UPLC method to allow estimation of Ticagrelor in tablet dosage form.
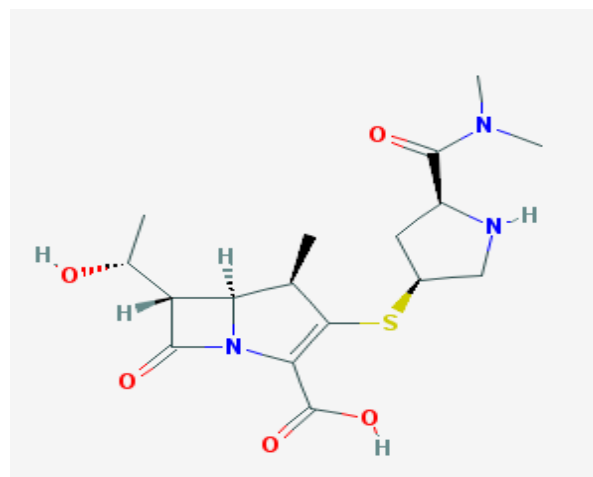

Figure 1: Chemical Structure Of Ticagrelor

2. MATERIALS AND METHODS

2.1 RP-HPLC method

2.1.1 Apparatus: The separation was carried on Waters Acquity UPLC 2996 with Empower 2 software that consisted of a binary solvent manager equipped with automatic sampler. An acquity UPLC Hibra C18 2.1 ×100 mm, 1.8 μ column was used for separation of active ingredients. Analytes were monitored with TUV detector at a wavelength 222 nm. Ultrasonicator was used to remove dissolved gases and air bubbles in the mobile phase.

2.1.2 Materials: Ticagrelor standard sample was obtained as gift samples from Spectrum Labs, Hyderabad. HPLC grade

water and methanol were purchased from Merck Ltd., Mumbai. Analytical grade acetonitrile and orthophosphoric acid were obtained from Rankem, Avantor Performance Material India Ltd. Marketed formulation of combination was purchased from local market.

2.1.3 Chromatographic Conditions: Separation of analytes was achieved with a mobile phase consisting of 0.1% OPA and acetonitrile at a ratio of 50:40 delivered at a flow rate of 0.3ml/min through column kept at 25 ºC. The volume of injection was 1 μl and runtime was 2min. The eluents were detected at a wavelength 260 nm. Chromatograms of optimized method and standard were shown **Fig. 2 and Fig. 3.**
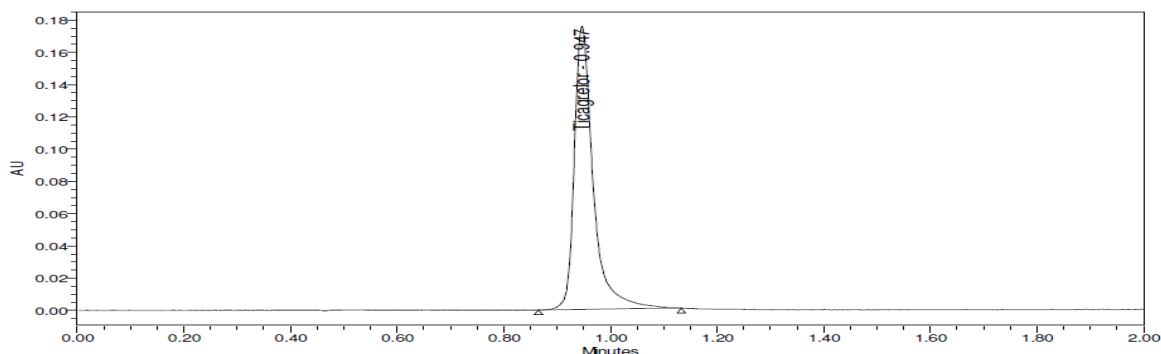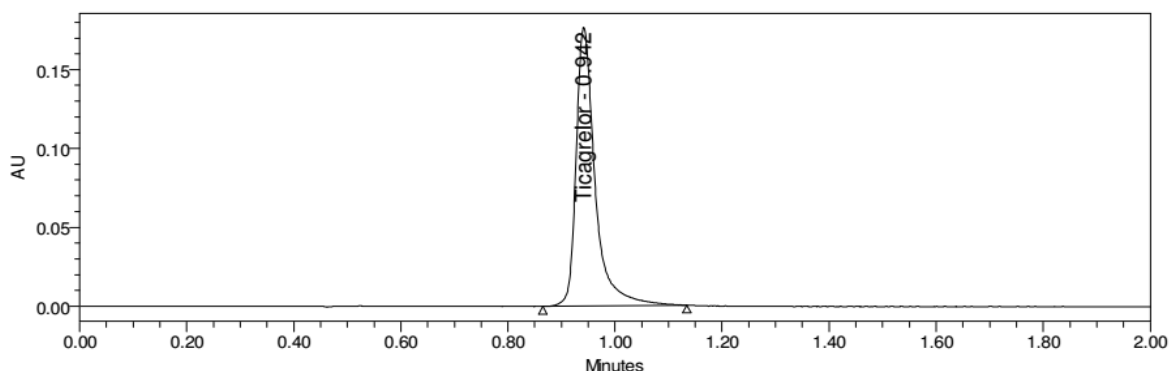

Figure 2: Chromatogram of optimized method

Figure 3: Chromatogram of standard preparation

2.1.4 Preparation of Standard stock solutions: Accurately weighed 15mg of Ticagrelor transferred to individual 25 ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution. (600μg/ml of Ticagrelor) . 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (60μg/ml Ticagrelor)

2.1.5 Preparation of Sample stock solutions: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (600μg/ml of Ticagrelor). 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (60μg/ml of Ticagrelor).

2.2 Validation of the HPLC method [8-11]

2.2.1. System suitability

The developed method was validated according to ICH guidelines[8]. To check the system performance, the system

suitability parameters were measured. System precision was determined on six replicate injections of standard preparations. Number of theoretical plates and asymmetry were measured[9-10]

The system suitability parameters were determined by preparing standard solutions of Ticagrelor (60ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections results should not be more than 2%.

2.2.2 Linearity

Accurately weighed 15mg of Ticagrelor transferred to individual 25 ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution. (600μg/ml of Ticagrelor). The calibration graphs were plotted over 5 different linear concentrations in the range of 15-150 μ g/mL for ticagrelor.

2.2.3 Accuracy

Accuracy is the percent of analyte recovered by assay from a known added amount. For the measurement of accuracy data

from nine determinations over three concentration levels covering the specified range were determined.

2.2.4 Precision

Precision is the degree of repeatability of an analytical method under normal operational conditions. The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day) and reported as % R.S.D. for a statistically significant number of replicate measurements. The intermediate precision was studied by comparing the assays on 3 different days and the results documented as standard deviation and %R.S.D[11].

2.2.5 LOD and LOQ

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with acceptable accuracy, precision and variability (ICH guideline Q2B, 2005).

2.2.6 Robustness

The robustness of the method was evaluated by assaying the test solutions after slight but deliberate changes in the analytical condition ns like flow rate (+0.1 mL min−1), and mobile phase composition (2%).

3. RESULT AND DISCUSSION

3.1 System suitability

Ticagrelor was eluted at 0.947 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

Table 1: System suitability

S: no	Ticagrelor		
Inj	RT(min)	USP Plate Count	Tailing
1	0.940	4206	1.6
2	0.941	4222	1.6
3	0.941	4204	1.6
4	0.941	4200	1.6
5	0.941	4209	1.6
6	0.942	4176	1.6

According to the USP, the HPLC method is considered suitable when the ticagrelor of peak area <1%, tailing factor <2 and the theoretical plates >2000.

All the system suitability parameters were within the range and satisfactory as per ICH guidelines. The results of system suitability are shown in Table 1.

3.2 Linearity

Six linear concentrations of Ticagrelor (15-90μg/ml) was injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Ticagrelor was y = 7237.7.x + 1401.1. Correlation coefficient obtained was 0.999 for the drug. The regression analysis is shown in table 2.

Table 2: Regression analysis

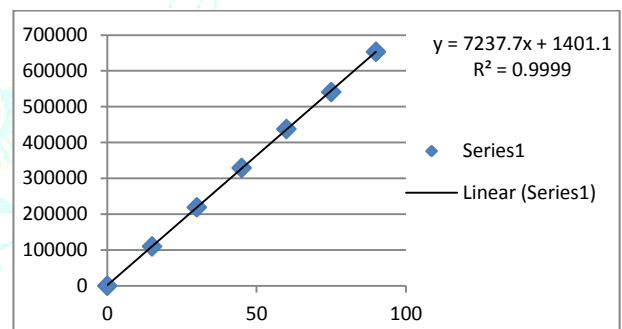| Ticagrelor	
Conc (μg/mL)	Peak area
0	0
15	109870
30	219188
45	329037
60	437619
75	540777
90	653198

Figure 2: Calibration Graph of Ticagrelor

3.3 Accuracy

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.51% for Ticagrelor respectively.

Table 3: Recovery studies of Ticagrelor

% Level	Amount Spiked(μg/mL)	Amount recovered (μg/mL)	% Recovery	Mean % Recovery
50%	30	30.25	100.84	99.51%
	30	29.42	98.06	
	30	29.92	99.73	
100%	60	60.12	100.20	
	60	59.47	99.12	
	60	59.51	99.19	
150%	90	88.67	98.52	
	90	89.31	99.24	
	90	90.62	100.69	

3.4 Precision

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations

were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD

were calculated for drug and obtained as 0.7% respectively for Ticagrelor. As the limit of Precision was less than "2" the system precision was passed in this method.

Table 4: Repeatability table of Ticagrelor

S. No	Area of Ticagrelor
1.	439649
2.	434070
3.	438841
4.	431807
5.	433398
6.	436850
Mean	435769
S.D	3158.0
%RSD	0.7

Limit of Detection and Limit of Quantification (LOD and LOQ): The limit of detection is the point at which a measured value is larger than the uncertainty associated with it. It is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified.

Table 5: LOD and LOQ results of ticagrelor

Molecule	Ticagrelor
LOD	0.45
LOQ	1.33

The limit of quantitation is the lowest injected amount that produces quantitative measurements in the target matrix with acceptable precision in chromatography. The quantitative limit is particularly used for the determination of impurities and degradation products. The results were shown in Table 5.

3.5 Robustness

Robustness conditions like Flow minus (0.27ml/min), Flow plus (0.33ml/min), mobile phase minus (55B:45A), mobile phase plus (65B:35A), temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Table 6: Robustness data for Ticagrelor

S.no	Condition	%RSD of Ticagrelor
1	Flow rate (-) 0.27ml/min	0.4
2	Flow rate (+) 0.33ml/min	1.1
3	Mobile phase (-) 55B:45A	0.5
4	Mobile phase (+) 65B:35A	0.8
5	Temperature (-) 25°C	0.4
6	Temperature (+) 35°C	0.7

Assay: (Brilinta) bearing the label claim Ticagrelor 60mg, Assay was performed with the above formulation. Average % Assay for Ticagrelor obtained was 99.9% and shown in table 7.

Table 7: Assay Data of Ticagrelor

S.no	Standard Area	Sample area	% Assay
1	433305	439649	100.76
2	428777	434070	99.48
3	434092	438841	100.57
4	438930	431807	98.96
5	441475	433398	99.32
6	436287	436850	100.11
Avg	435478	435769	99.9
Stdev	4473.9	3158.0	0.7
%RSD	1.0	0.7	0.7

Forced Degradation Studies: Forced degradation studies were conducted to know the stability of the method. The degradation studies were carried out by applying various stress conditions for the product like acid stress, base stress, UV stress, humidity stress, thermal stress and oxide stress.

Degradation peaks were observed only in acid stress and peroxide stress and all degradation peaks were well resolved from analyte peaks. The results of forced degradation studies were shown in **Table 8**.

Table 8: results of forced degradation studies

Type of degradation	Ticagrelor		
	AREA	%RECOVERED	% DEGRADED
Acid	415118	95.13	4.87
Base	409117	93.76	6.24
Peroxide	419117	96.05	3.95
Thermal	428139	98.12	1.88
Uv	429780	98.49	1.51
Water	434865	99.66	0.34

CONCLUSION

The developed UPLC analytical method provides an ecofriendly, reliable, reproducible, simple, rapid, sensitive, accurate, precise and specific assay method for the simultaneous estimation of Ticagrelor in pharmaceutical formulations. Degradation studies reveal that the developed method was stability indicating. Hence the proposed method can be conveniently used for the routine analysis of Ticagrelor in pure and pharmaceutical dosage forms.

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