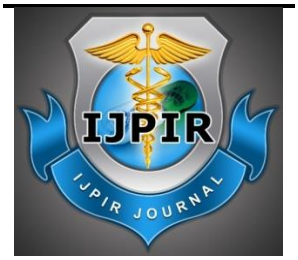
Research Article



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Method development and validation of ticagrelor in bulk and dosage form

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ABSTRACT

A simple, precise, rapid, specific and accurate reverse phase high performance liquid chromatography method was developed for estimation of Ticagrelor in pharmaceutical dosage form. The method was developed in reverse phase high Performance liquid chromatography using INERTSIL (Length 250mm x Dimension 4.6mm, Particle Size 5µm) column with mobile phase comprising of mixture of Methanol: Phosphate buffer (pH 2.5 adjusted with ortho phosphoric acid) in ratio of 80:20 v/v, at flow rate of 1ml/min, with detection of 240 nm. The retention time of Ticagrelor was found to be 4.8. Linearity of Ticagrelor was found in the range of 75-150 µg/mL with correlation coefficient for Ticagrelor was 0.999. The LOD and LOQ values for Ticagrelor were 1.07 and 3.02 µg/mL respectively. The method so developed was validated in compliance with the regulatory guidelines by using well developed analytical method validation parameters like linearity, accuracy, precision, limit of detection and limit of quantitation, robustness and ruggedness are in acceptable criteria.

Keywords: Ticagrelor, RP-HPLC, Method Development and Validation

INTRODUCTION

High Performance Liquid Chromatography is the most widely used of all the analytical separation techniques. The reasons for its popularity are its sensitivity, ready adaptability to quantitative determination, suitable for non-volatile and thermally fragile species.

Ticagrelor

Ticagrelor (1S,2S,3R,5S)-3-(7-{[(1R,2S)-2-(3,4difluorophenyl)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 produced by AstraZeneca. Unlike clopidogrel, ticagrelor is not a prodrug. These are polycyclic aromatic compounds

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containing triazole ring fused to a pyrimidine ring. Triazole is a five-membered ring consisting of two carbon atoms and three nitrogen atoms. Pyrimidine is a 6-membered ring consisting of four carbon atoms and two nitrogen centers at the 1- and 3- ring positions.

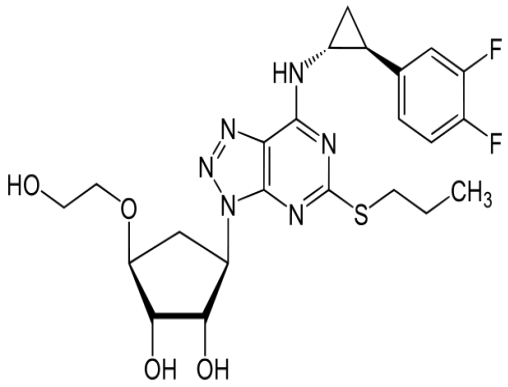

Figure-1 Chemical Structure of Ticagrelor

CAS Number	274693-27-5
Molecular weight	522.568
Molecular formula	$C_{23}H_{28}F_2N_6O_4S$
Physical state	Solid
Solubility	Soluble in Acetonitril, Methanol, Ethanol
Melting point	138-140°C

MATERIALS AND METHODS

Ticagrelor tablet dosage form, Ortho phosphoric acid, Potassium di hydrogen orthophosphate, Acetonitrile, Methanol, Distilled water. All the above chemicals and solvents are from Merck and Rankem.

Instruments

UV-Visible Spectrophotometer- Nicolet evolution 100 model YJ-5200 DT, HPLC- Shimadzu LC 20 AT VP, HPLC Column- INERTSILcolumn,C18(150x4.6 ID) 5µm C18 Ultra sonicator- Citizen, Digital Ultrasonic Cleaner YJ-5200 DT, pH meter- Global digital Digital pH meter, Electronic balance- Shimadzu Hpd-500.

Diluents

Based up on the solubility of the compound, diluents was selected, Phosphate Buffer (pH -2.5): Acetonitrile (20: 80).

Chromatographic Conditions

Mobile phase used are Phosphate Buffer (pH -2.5): Acetonitrile (20: 80). flow rate 1ml/min, column used are INERTSIL, C18(150x4.6 ID) 5µm, Detector wave length was 240nm, column temperature Ambient, Injection volume was 20µl, Run time 6min, Diluent used was Phosphate Buffer (pH -2.5) : Acetonitrile (20 : 80)

Assay

Procedure

20 µL of the standard and sample solutions were injected into the chromatographic system and area for the Ticagrelor peak was measured.

Calculation

The amount of Ticagrelor present in the formulation by using the formula given below

Assay %

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.\ Wt}{LC} \times 100$$

Where:

AT = Average area counts of sample preparation.

AS = Average area counts of standard preparation.

WS = Weight of working standard taken in mg.

P = Percentage purity of working standard

LC = LABEL CLAIM mg/ml.

RESULTS AND DISCUSSION

Optimized method

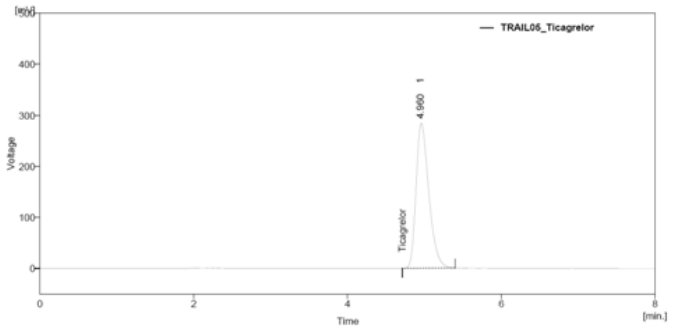

Figure 2- Optimized chromatogram of Ticagrelor

ASSAY

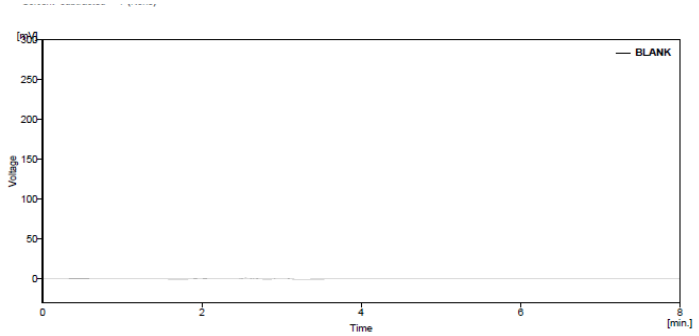

Figure 3- Blank Assay chromatogram

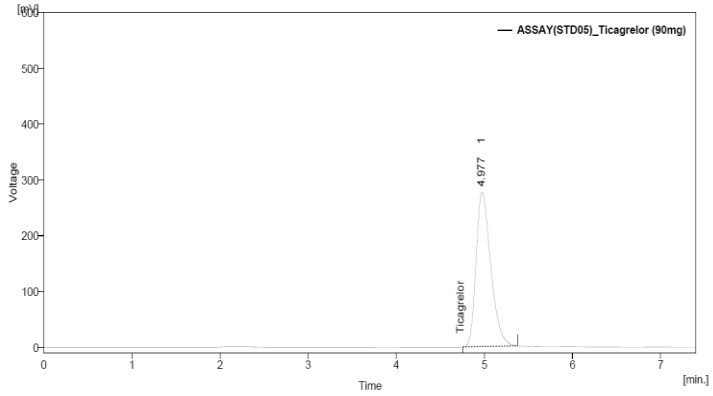

Figure 4- Standard Assay chromatograms

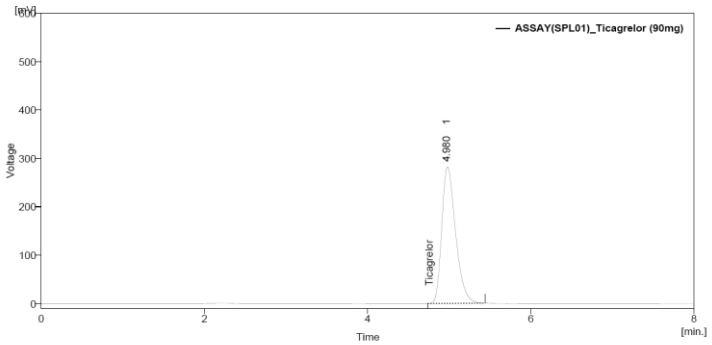

Figure 5- Sample chromatogram

Assay Results: (Ticagrelor)

3281.31 10 100 9 5 . 9 6 305.6

---------- x ----------x --------- x ----------x ---------- x 100 = 98.40 %.

3247.516 100 33.95 100 90

Table 1:

Formulation	Label Claim (mg)	% of Assay
BRILLINTA90mg	Ticagrelor90mg	98.40

System Suitability Parameters

Table 2: System suitability studies ticagrelor

S. No	Parameter	Ticagrelor
1	Retention time	4.937
2	Theoretical plates	4390
3	Tailing factor	1.64
4	Area	3099.478

Linearity

Table 3: Calibration data of ticagrelor

Linearity level	Concentration (ppm)
1	50
2	75
3	100
4	125
5	150

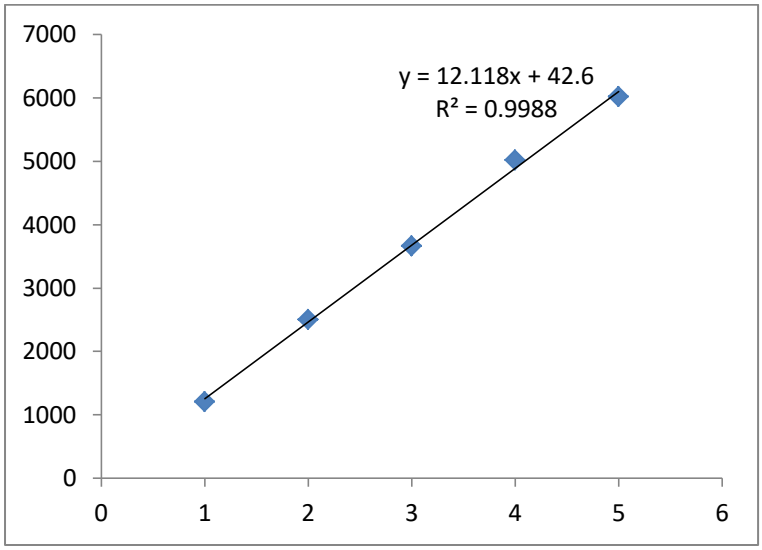

Figure 6- Calibration curve of ticagrelor

Accuracy

Three concentrations 75%, 100%, 125%, were injected and amount Recovered and % Recovery were of following.

Table 4: Table for Accuracy

S. No.	% spike level	Area	Amount recovered (mg)	% Recovery	% Mean recovery
1.	75	3404.393	66.3	98.22	
2.		3069.834	66.2	98.07	98.2
3.		3296.104	66.5	98.5	
1.	100	3838.430	89.6	99.6	
2.		3285.170	88.4	98.22	98.84
3.		3327.673	88.9	98.7	
1.	125	4838.317	111.3	98.9	
2.		4781.051	110.53	98.2	98.36
3.		4663.219	110.2	98.0	

Precision

Precision was performed and % RSD for Ticagrelor were found to be 0.205%

Table 5: Table for Precision

Injection	Ticagrelor Area	%Recovery
Injection 1	3303.21	98.70
Injection 2	3304.2	98.76
Injection 3	3299.52	98.32
Injection 4	3309.3	98.84

Injection 5	3301.9	98.51
Injection 6	3306.8	98.81
Mean	3304.11	98.65
Standard Deviation	3.56	0.202
% RSD	0.10	0.205

LOD: Limit of detection was calculated by standard deviation method for ticagrelor and LOD for ticagrelor were found to be 1.07µg/ml.

$$LOD = \frac{3.3 * S.D}{slope}$$

Table 6: Table for LOD

Drug	Amount (µg/mL)
Ticagrelor	1.07

LOQ: Limit of Quantification was calculated by standard deviation method for ticagrelor and LOQ for ticagrelor were found to be 3.2µg/ml

$$LOQ = \frac{10 * S.D}{Slope}$$

Table 7: Table for LOQ

Drug	Amount (µg/mL)
Ticagrelor	3.2

Robustness: Small deliberate changes in method like Flow rate and wavelength are made but there were no recognized change in the result. Hence it indicates that the method is robust even by change in the flow rate ±10 % and also wavelength.

Table 8: Flow Rate of Ticagrelor

Flow Rate (ml/min)		System Suitability Results		
		USP Plate Count	USP Tailing	Retention time(min)
Low	0.8	4106	1.783	6.080
Actual*	1	4241	1.625	4.980
High	1.2	4233	1.588	4.233

Table 9: Wavelength (nm) variations of Ticagrelor

System suitability parameters	Wavelength (nm) Variation		
	238nm	240nm	242nm
Retention time of main peak	4.97	4.96	4.96
Tailing factor for Ticagrelor peak.	1.6	1.6	1.6
Theoretical plates for Ticagrelor peak.	4390	4212	4373

CONCLUSION

The proposed HPLC method was found to be precise, specific, accurate, rapid and economical for estimation of Ticagrelor in tablet dosage form. The sample recoveries in all formulations were in good agreement with their respective Label Claims and this method can be used for routine analysis. It can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials, formulations, dissolution studies and can be employed for bioequivalence studies for the same formulation.

Acknowledgement

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