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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF TICAGRELOR IN DRUG SUBSTANCE BY RP-HPLC METHOD

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ABSTRACT
Analytical method was developed for the estimation of Ticagrelor drug substance by liquid chromatography. The chromatographic separation was achieved on C18 column (Inertsil ODS 3V 150*4.6, 5um) at ambient temperature .the separation achieved employing a mobile phase consists of 0.1%v/v Formic acid in water : Methanol (10:90). The flow rate was 1.0 ml/ minute and ultra violet detector at 220nm. The average retention time for Ticagrelor found to be 2.71 min the proposed method was validated for selectivity, precision, linearity and accuracy. All validation parameters were within the acceptable range. The assay methods were found to be linear from 100-300µg/ml for Ticagrelor.

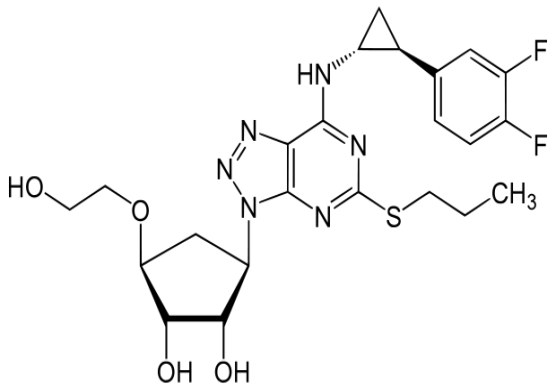
KEY WORDS: Ticagrelor, Isocratic, HPLC, C18, Formic acid, methanol and validation.

Ticagrelor (trade name **Brilinta**, **Brilique**, and **Possia**) is a platelet aggregation inhibitor produced by AstraZeneca.

Ticagrelor is an antagonist of the $P2Y_{12}$ receptor.[1]

Ticagrelor is a prodrug that is activated to Ticagrelorat (LBQ657) by de-ethylation via esterases. Ticagrelor at inhibits the enzyme neprilysin,which is responsible for the degradation of atrial and brain natriuretic peptide, two blood pressure-lowering peptides that work mainly by reducing blood volume. In addition, neprilysin degrades a variety of peptides including bradykinin, an inflammatory mediator exerting potent vasodilatory action.

Ticagrelor is chemically designated as (1*S*,2*S*,3*R*,5*S*)-3-[7-[(1*R*,2*S*)-2-(3,4-Difluorophenyl)cyclopropylamino]-5-(propylthio)- 3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl]-5-(2-hydroxyethoxy)cyclopentane-1,2-diol. Its molecular formula is $C_{24}H_{29}NO_5$, and its molecular weight is 522.567 g/mol. Ticagrelor is a white-to-off white powder. It is freely soluble in methanol and practically insoluble in water.



Structure of Ticagrelor

EXPERIMENTAL
Equipments: The chromatographic technique performed on a waters 2695 with 2487 detector and Empower2 software, reversed phase C18 column (Inertsil 5µ, 150 mm × 4.6 mm) as stationary phase, Ultrasonic cleaner, Scaletech analytical balance, Vaccum micro filtration unit with 0.45µ membrane filter was used in the study.

Materials: Pharmaceutically pure sample of Ticagrelor were obtained as gift samples from Fortune pharma training institute, sri sai nagar, KPHB and Hyderabad, India.

HPLC-grade Methanol was from qualigens reagents pvt ltd. Formic acid (AR grade) was from sd fine chem.

Chromatographic conditions The sample separation was achieved on a C18 (5 µ, 15 cm X 4.6 mm i.d.) INERTSIL column, aided by mobile phase mixture of 0.1%v/v Formic acid in water : Methanol (10:90). The flow rate was 1.0 ml/ minute and ultra violet detector at 220nm, that was filtered and degassed prior to use, Injection volume is 10 µl and ambient temperatures.

Preparation of mobile phase
Buffer Preparation: Take accurately 1ml of formic acid in 1000mL of water.

Mobile phase: Then add 10 volumes of buffer and 90 volumes of Methanol mixed well and sonicated for 5 min.

Preparation of standard stock solution: A 50mg of pure Ticagrelor were weighed and transferred to 50 ml of volumetric flask and dissolved in methanol. The flask was shaken and volume was made up to mark with methanol: water(50:50) to give a primary stock solution containing 1000µg/ml. From the above solution 2ml of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with methanol to give a solution containing 200µg/ml of Ticagrelor.

Preparation of sample solution: A 50mg of Ticagrelor sample were weighed and transferred to 50 ml of volumetric flask and dissolved in water: methanol. The flask was shaken and volume was made up to mark with water: methanol to give a primary stock solution containing 1000µg/ml. From the above solution 12l of solution is pipette out into a 10 ml volumetric flask and volume was made up to mark with water: methanol to give a solution containing 200µg/ml of Ticagrelor.

RESULTS AND DISCUSSIONS

Determination Of Working Wavelength (λ max): 10 mg of the Ticagrelor standard drug is taken in a 10 ml volumetric flask and dissolved in water:methanol and volume made up to the mark, from this solution 0.1ml is pipetted into 10 ml volumetric flask and made upto the mark with the water:methanol to give a concentration of 10 µg/ml. The above prepared solution is scanned in uv between 200-400 nm using water: methanol as blank. The λmax was found to be 220nm.

After several initial trails with mixtures of methanol, water, ACN and buffer in various combinations and proportions, a trail with a mobile phase mixture of 0.1%v/v Formic acid in water: Methanol (10:90). The flow rate was 1.0 ml/ minute brought sharp peaks. The chromatogram was shown in Figure-1**.**

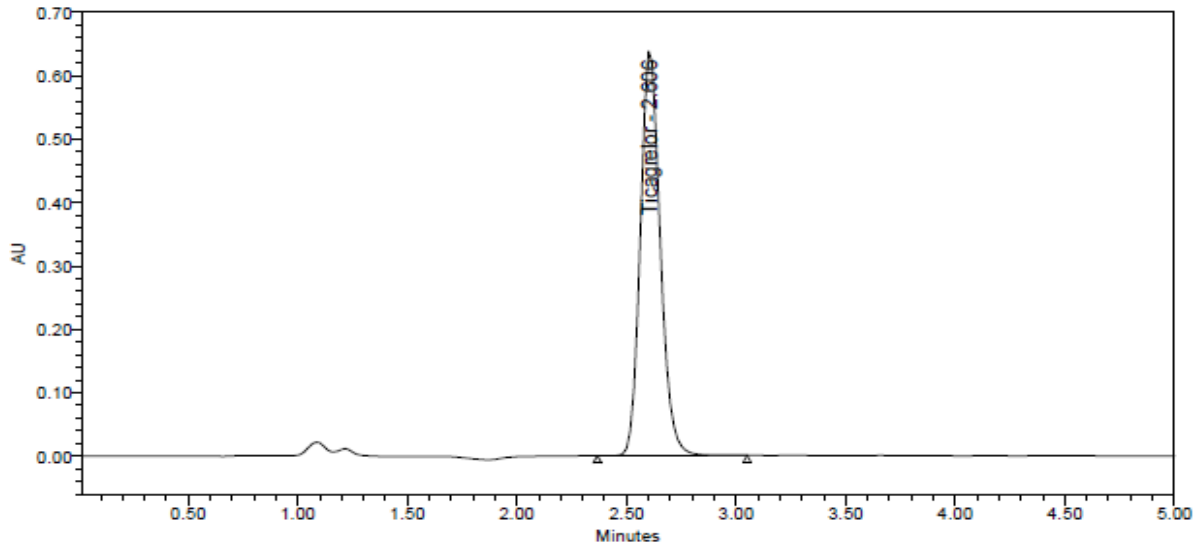


Figure: 1 Chromatogram of Ticagrelor

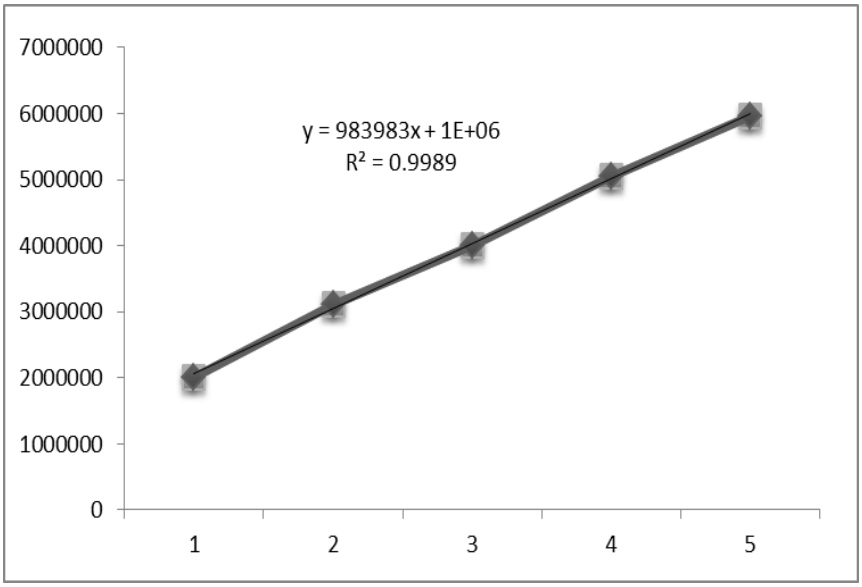
METHOD VALIDATION

Linearity

Linearity was studied by analyzing five standard solutions covering the range of 100-300 µg/ml of Ticagrelor. From the primary stock solution 1.0ml,1.5ml,2.0ml,2.5ml,3.0 ml of aliquots are pipette into 10 ml volumetric flasks and made up to the mark with the mobile phase to give a concentrations of 100µg/mL , 150µg/mL ,200µg/mL ,250µg/mL and 300 µg/mL of Ticagrelor. Calibration curve with concentration verses peak areas was plotted by injecting the above prepared solutions and the obtained data were subjected to regression analysis using the least squares method.

Table No: 1

Level	Concentration (mg/mL)	Peak area
50%	0.1	2013479
75%	0.15	3115751
100%	0.20	4006266
125%	0.25	5057922
150%	0.30	5962307



FigureNo.1: Linearity (calibration) curve of Ticagrelor

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (1) and (2), respectively.

LOD = 3.3 δ/S ……. (1)

LOQ =10 δ/S …….. (2)

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Table no.2: LOD and LOQ values Calculated from calibration curve

	mg
LOD	0.01
LOQ	0.031

Method precision (repeatability)

The precision of the instrument was checked by repeated injections and measurement of peak areas and retention times of solutions (n = 6) for, 100 µg/ml of TICAGRELOR without changing the parameter of the proposed chromatographic method.

Table.3: Summary of peak areas for method precision

Sample No	Retention time	Peak area	% Assay
1	2.601	4028147	101.0
2	2.599	4034076	101.1
3	2.60	4030917	101.1
4	2.60	4026345	100.9
5	2.599	4047111	100.8
6	2.596	3986943	100.4
Mean	2.599	4025590	100.9
%RSD	0.07	0.50	0.27

Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of Ticagrelor by analyzing solutions containing approximately 50%, 100% and 150% of the working strength of Ticagrelor. The percentage recovery results obtained are listed in Table 4.

Table No.4: Recovery data

LEVEL	S.No	%Recovery of TICAGRELOR	Average
50	1	99.8	99.9%
	2	100.4	
	3	99.2	
100	1	101.0	101.1%
	2	101.1	
	3	101.1	
150	1	100.2	100.5%
	2	100.2	
	3	101.3	

Robustness: Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied ±2nm and flow rate was varied ±0.1 ml/min. The results were shown in (Table no.5)

Ruggedness: The ruggedness of the method was studied by analyzing the sample and standard preparations by two analysts. The %RSD assay values between two analysts was calculated i.e.,(limit <2%).

This indicates the method was rugged. The results were shown in Table no.6.

Table No.5: Results of Robustness study

parameter	Rt of TICAGRELOR	Theoretical plates	Asymmetry
Decreased flow rate (0.9ml/min)	2.880	4286	1.17
Increased flow rate (1.1ml/min)	2.369	3760	1.14
Wave Length 218nm	2.606	3867	1.16
222	2.606	3903	1.14

Table No.6: Results of Ruggedness

		%Assay	%RSD
Analyst-1	**TICAGRELOR**	101.0	0.07%
Analyst-2		101.1	

Table No.7: Validation parameters of evaluated method:

S. No	Parameter	Limit	Value Obtained
1	**Linearity concentrations Range (mg/mL)**	NLT 0.990	0.1 to 0.3 mg\ml
	Correlation coefficient		0.9994
2	**Method precision (Repeatability) (%RSD, n = 6)**	98.0 to 102.0 %	100.4 to 101.1 %
3	**ACCURACY(%Recovery)**	98-102%	99.9 to 101.1%
4.	**Robustness**	It should be meet System suitability criteria	Complies
	Flow Variation(0.7mL to 0.9 mL/min)		
	Wavelength Variation (313nm to 317nm)		
5.	**Ruggedness (Intermediate Precision) (%RSD analyst to analyst variation)**	NMT2%	0.07%

*RSD = Relative standard deviation

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of TICAGRELOR was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories.

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