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RP-HPLC AND UV SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF TICAGRELOR IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

An accurate, precise, sensitive and reproducible High-performance liquid chromatographic and UV spectrophotometric method were developed and validated for the quantitative determination of ticagrelor, a novel antiplatelet agent used in acute coronary syndrome in bulk drug and pharmaceutical formulation. Chromatography was carried out by isocratic technique on a reversed-phase Thermo C18 (250 × 4.6 mm, 5µm) column with mobile phase consisting of 20 mM KH_2PO_4: acetonitrile (pH 3.0 with OPA) in the ratio of 20:80 v/v at flow rate of 1.0 ml/min. The retention time for ticagrelor was 8.102± 0.3 min. The UV spectrophotometric determinations were performed at 282 nm using water as a solvent. The proposed methods were validated according to International Conference on Harmonization ICH Q2 (R1) guidelines. The linearity range for ticagrelor was 5-25 µg/ml for HPLC and UV method. The linearity of the calibration curves for each analyte in the desired concentration range was good (r2 >0.999) by both the HPLC and UV methods. Both the methods were accurate and precise with recoveries in the range of 98 and 99 % and relative standard deviation <2 %. The developed methods were successfully applied for determination of ticagrelor in tablets.

K**eywords:** Pharmaceutical formulation, Ticagrelor, RP-HPLC, UV

INTRODUCTION

Ticagrelor (Fig.1) is a new drug intended for the prevention of acute coronary syndromes (ACS) characterized by the formation of atherosclerotic plaques that rupture inside the arteries. It belongs to the cyclopentyltriazolopyrimidine class and it is the first agent that reversibly binds to P2Y12 ADP-receptor [1-4]. This receptor has a central role in platelet activation and drugs that selectively target it had been widely used as antiplatelet agents [5-6]. Chemically, it 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-hydroxyethoxycyclo pentane1 ,2-diol) [7]. AstraZeneca produces it as coated tablets containing 90 mg of ticagrelor (Brilinta®). European Commission approved the drug product in 2010 and FDA and Agência Nacional de Vigilância Sanitária (ANVISA/BRAZIL) in 2011. The wide-ranging systematic literature review for ticagrelor revealed very few methods based on varied techniques, viz, UV-spectroscopic [8], LC-MS [9,10] and HPLC [11,12] for the estimation of ticagrelor either in pharmaceutical formulation or in biological fluid. However there is no combine method available for the determination of ticagrelor drugs.

Therefore, an attempt was made to develop a new, rapid and sensitive method for the determination of ticagrelor in bulk drug and pharmaceutical formulation by UV and RP-HPLC method. To assess the reproducibility and wide applicability of the developed method, it was validated as per ICH guidelines [13, 14].

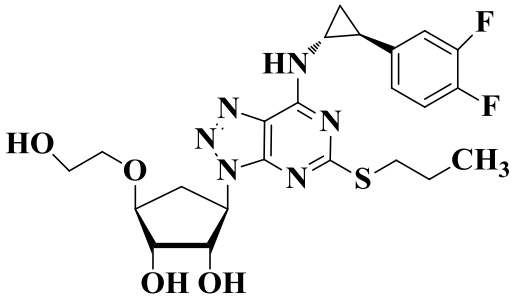


Fig. 1 Chemical structure of ticagrelor

EXPERIMENTAL

Reagents and chemicals

The working standard of Ticagrelor was provided as gift sample from Spectrum Labs, Hyderabad, India. The market formulation Brilinta tablets (Ticagrelor 90 mg) were procured from local market. Acetonitrile (HPLC Grade), methanol (HPLC Grade), supplied by Merck Ltd, New Delhi, India. Triple distilled water was generated in house. All solvents and reagents were of analytical grade. All the solutions were protected for light and were analyzed on the day of preparations. Triple distilled water was generated in house.

Instrument

In UV-spectrophotometric method, Labindia model-3000+ series were used, which is a wavelength accuracy ±1 nm, with 1cm quartz cells.

Liquid chromatographic system from Waters model no 784 comprising of manual injector, water 515 binary pump for constant flow and constant pressure delivery and UV-Visible detector connected to software Data Ace for controlling the instrumentation as well as processing the generated data.

UV spectrophotometric method

Determination of wavelength of maximum absorbance (λ_{max}) of ticagrelor

Wavelength of maximum absorption was determined by scanning 10µg/ml solution of ticagrelor using UV spectrophotometer from 200 to 400 nm. This showed maximum absorbance at 282 nm (Fig. 2).

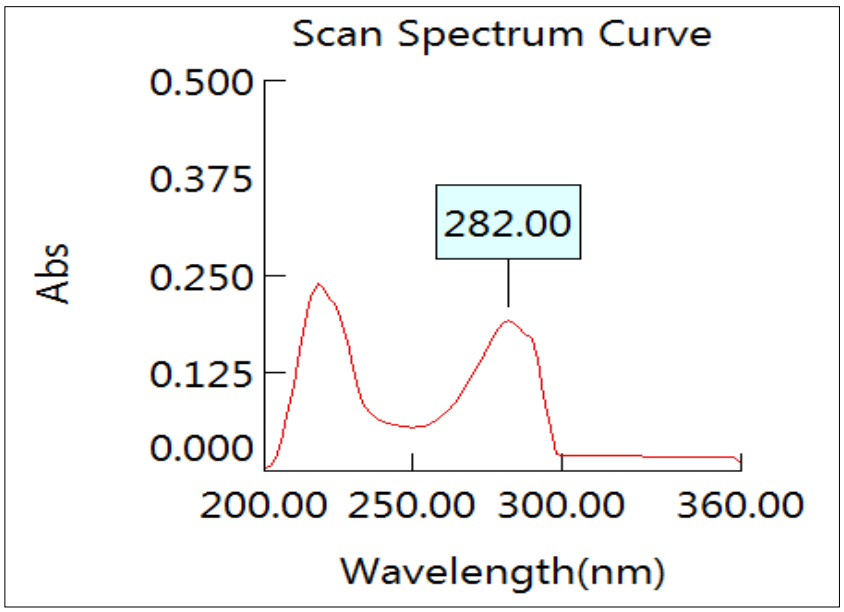


Fig. 2 Determination of λmax of ticagrelor

Preparation of standard stock solution (Stock-A)

Standard stock solutions were prepared by dissolving 100 mg of drug in 50 ml of water and the flask was sonicated for about 10 min to solubilize the drug and the volume was made up to the mark with water to get a concentration of 1000 µg/ml (Stock-A) for drug.

Preparation of sub stock solution (Stock-B)

Aliquots of 2.5 ml withdrawn with help of pipette from standard stock solution A of ticagrelor and transferred into 25 ml volumetric flask separately and diluted up to 25 ml with RO Water that gave concentration of 100 µg/ml (Stock-B).

Preparation of working standard solution

0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml and 2.5 ml from sub stock solution (Stock-B) were taken separately in 10 ml volumetric flask and volume was made up to 10 ml with RO Water. This gave the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20 µg/ml and 25 µg/ml respectively for ticagrelor.

Preparation of the calibration curves of the drug

The calibration curve was prepared by scanning test samples ranging from 5-25µg/ ml at 282 nm for ticagrelor. The calibration curve was tested by validating it with inter-day and intra-day measurements. Mean of n =5 determinations was plotted as the standard curve (Fig.3).

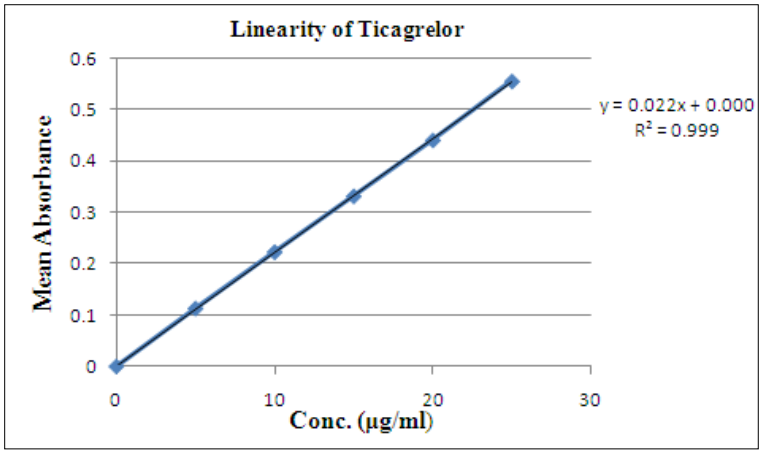


Fig. 3 Calibration curve of ticagrelor

Validation of calibration curve method

Linearity

Linearity of drug was established by response ratios of drug. Response ratio of drug calculated by dividing the absorbance with respective concentration. Then a graph was plotted between concentration and response ratio table 1.

Table 1 Response ratio of Ticagrelor

S. No.	Ticagrelor		
	Conc. (µg/ml)	ABS	Response Ratio
1.	0	0	0
2.	5	0.113	0.0226
3.	10	0.2234	0.0223
4.	15	0.3326	0.0221
5.	20	0.4422	0.0221
6.	25	0.5566	0.0222

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution of ticagrelor to preanalysed tablet solutions. The resulting solutions were then re-analysed by proposed methods. Whole analysis procedure was repeated to find out the recovery of the added drug sample. This recovery analysis was repeated at 3 replicate of 5 concentrations levels table 2.

Table 2 Results of recovery studies

Recovery Level %	% Recovery (Mean±SD)*
	Ticagrelor
80	99.046±0.284
100	99.055±0.400
120	99.828±0.308

Precision

Precision of the methods was studied at three level as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility. Repeatability was performed by analyzing same concentration of drugs for five times. Day to Day was performed by analyzing 5 different concentration of the drug for three days in a week. The results are shown in table 3.

Table 3 Results of precision (%R.S.D.)

PARAMETER		Calibration curve Method
		Ticagrelor
Precision (%R.S.D.)*	Repeatability	0.571
	Day to Day	1.071
	Analyst-to-Analyst	0.656
	Reproducibility	0.984

*Average of five determination

Analysis of tablet sample

Twenty marketed tablets of ticagrelor were weighed and ground to a fine powder; amount equal to 50 mg of ticagrelor was taken in 10 ml volumetric flask and sonicated for about 10 min to solubilize the drug present in tablet powder and the volume was made up to the mark with water. After sonication filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with RO Water to get the final concentrations of drug in the working range. The absorbances of final dilutions were observed at selected wavelengths and the concentrations were obtained from calibration curve method. The procedure was repeated for five times table 4.

Table 4 Analysis of tablet formulation

Conc. Present (µg/ml)	Replicate	
	Conc. Found (µg/ml)	% Conc. Found
Ticagrelor	Ticagrelor	Ticagrelor
5	4.98	99.00
10	9.95	98.75
15	14.96	99.33
20	19.99	99.88
25	24.94	99.40

RP-HPLC method

Chromatographic condition

The isocratic mobile phase consisted of 20 mM KH_2PO_4: acetonitrile (pH 3.0 with OPA) in the ratio of 20:80 v/v, flowing through the column at a constant flow rate of 1.0 ml/ min. The mobile phase was filtered through nylon 0.22 µm membrane filters and was degassed before use (30 min). A Thermo (C-18) Column (5 µm, 250mm x 4.60mm) was used as the stationary phase. By considering the chromatographic parameter, sensitivity and selectivity of method for drugs, 275 nm was selected as the detection wavelength for UV-Visible detector.

Preparation of standard stock solution

Accurately weighed 10 mg of ticagrelor was transferred into 50 ml volumetric flasks and dissolved in 10 ml of acetonitrile, then volume was made up to 50 ml with acetonitrile and vortex it to get complete dissolution of drug. Stand it aside for few minute, concentration of ticagrelor was 200 µg/ml. (Stock- A)

Preparation of sub stock solution

5 ml of solution was taken from stock-A of ticagrelor transferred into 10 ml volumetric flask and diluted up to 10 ml with diluent (Acetonitrile) to give concentration of 100 µg/ml (Stock-B).

Preparation of Different Solution

0.5ml, 1.0 ml, 1.5ml, 2.0ml and 2.5ml of stock-B was taken in 10 ml volumetric flask and volume was made up to 10ml with (Acetonitrile). This gives the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, 25µg/ml for drug.

Preparation of calibration curve

The calibration curve was prepared by injecting concentration of 5-25 µg/ml for ticagrelor solutions manually in triplicate to the HPLC system at detection wavelength of 275 nm. Mean of n =5 determinations was plotted as the standard curve (Fig.4). The calibration curve was tested by validating it with inter-day and intra-day measurements. Linearity, accuracy and precision were determined for both inter day and intra-day measurements.

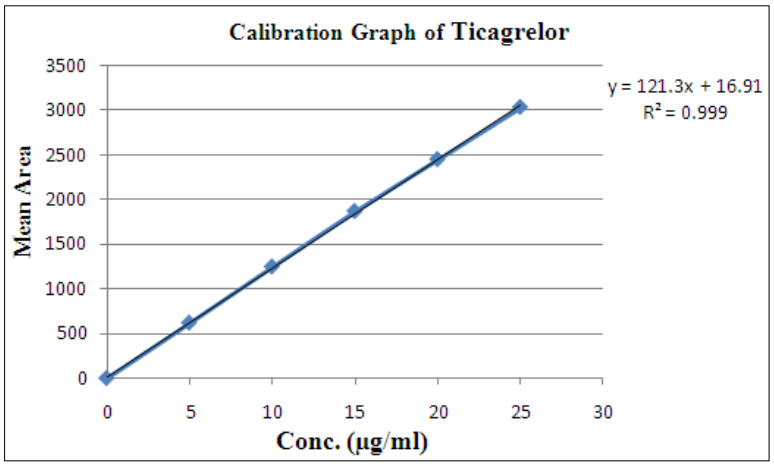


Fig.4 Calibration curve of ticagrelor

System Suitability

The system suitability parameter was carried out to verify that the analytical system was working properly and could give accurate and precise result. The six replicates of reference standard, 10 µg/ml of ticagrelor were injected separately and chromatogram was recorded. The result of system suitability parameter is reported in table 5.

Table 5 Results of system suitability parameters

Parameters	Ticagrelor
No. of Theoretical Plates	3232.833
AUC	1246.274
Tailing Factor	1.163
Retention time	8.104±0.001344

Linearity

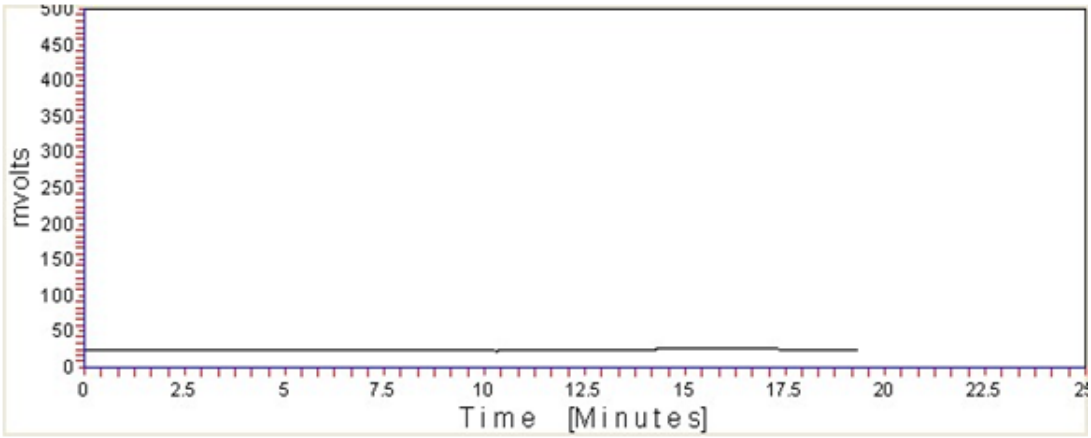
Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different (from 5 to 25 µg/ ml) concentrations and areas for each concentration were recorded three times and mean area was calculated. The regression equation and correlation coefficient of curve are given and the standard calibration curve of the drug is shown in fig 4. From the mean of AUC observed and respective concentration value, the response ratio (response factor) was found by dividing the AUC with respective concentration table 6.

Table 6 Response ration data for linearity of ticagrelor

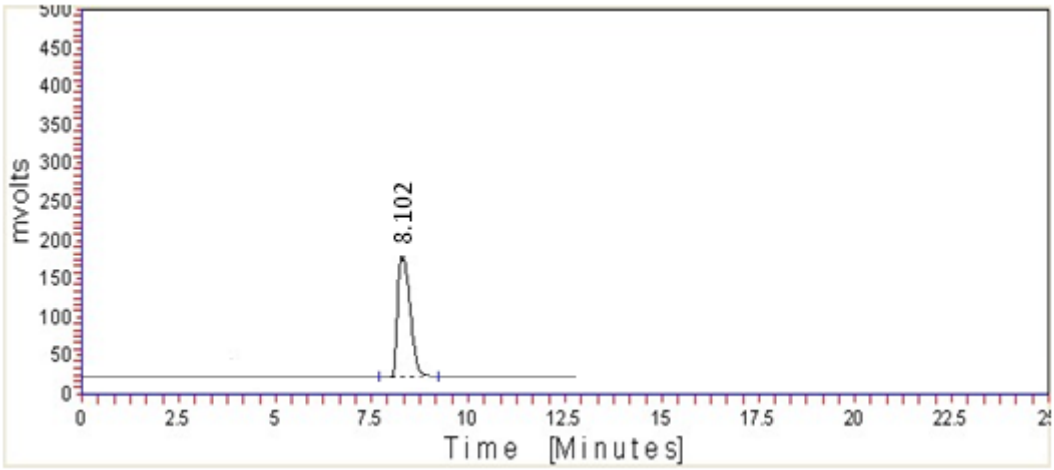
Replicates	Concentration (µg/ml)	Mean AUC	Response Ratio
Rep-1	5	619.035	123.807
Rep-2	10	1246.274	124.6274
Rep-3	15	1865.908	124.3939
Rep-4	20	2445.883	122.2942
Rep-5	25	3028.579	121.1432
SD			1.331
%RSD			1.080

Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present, such as impurities, degradation products and matrix components fig 5.



(A)



(B)

Fig. 5 Chromatogram of (A) mobile phase (B) ticagrelor

Accuracy

The validity and reliability of proposed methods were assessed by recovery studies. The recovery of added standards (80%, 100% and 120%) was found at three replicate and three concentrations level. The value of % means just close to 100, SD and % RSD are less than 2 indicate the accuracy of method. Result of recovery study shown in table 7.

Table 7 Results of recovery study

%LEVEL	% MEAN±SD*
	Ticagrelor
80%	99.48±0.215
100%	99.14±0.440
120%	98.54±0.553

***** Value of three replicate and three concentrations.

Precision

Precision was determined by repeatability and Intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and %RSD are less than 2 indicate the precision of method. Result of precision shown in table 8.

Table 8 Results of precision

Parameter	% MEAN±SD* Ticagrelor
Repeatability	98.790±0.162
Intermediate precision	
Day to day	99.179±0.085
Analyst to Analyst	99.125±0.215
Robustness	99.415±0.065

Robustness

As per ICH norms, small, but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, 20mM Phosphate Buffer: acetonitrile (20:80 % v/v), to (15:85 % v/v). Results of robustness are reported in table 8.

Detection limit and quantitation limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve was found to be 0.35 and 0.95 μg/ml.

Analysis of tablet sample

Twenty tablets were taken and their average weight was determined. They are crushed to fine powder; amount equal to 50 mg of ticagrelor was taken in 100-ml volumetric flask. The volume is made up to the mark by ACN and filtered by whatman filter paper (no.41) and the filtrate was used to prepare samples of different concentration. Results of tablet analysis are reported in table 9.

Table 9 Analysis of tablet sample

S. No.	Ticagrelor % Found
1	99.26
2	0.125
3	0.256

Result and discussion

RP-HPLC and UV-Spectrophotometric methods were developed for ticagrelor which can be conveniently employed for routine analysis in pharmaceutical dosage forms and will eliminate unnecessary tedious sample preparations. The chromatographic conditions were optimized in order to provide a good performance of the assay. The retention times (Rt) of ticagrelor was 8.102± 0.3 min. The chromatograms have been shown in Fig. 5. A five point calibration curve was constructed with working standards and was found linear (r2 = 0.999) for each of the analytes over their calibration ranges. The slopes were calculated using the plot of drug concentration versus area of the chromatogram. The developed UV and HPLC method was accurate, precise, reproducible and very sensitive.

For UV Method: Y = 0.022 x +0.000 (r2= 0.999)

For RP-HPLC: Y = 121.3 x +16.91 (r2= 0.999)

All the method validation parameters are well within the limits as specified in the ICH Q2B guidelines. Table 2&7 lists the percent recovery (content uniformity) of ticagrelor in the commercial formulations by UV and HPLC methods. The intra- and inter-day precision (%R.S.D.) at different concentration levels was found to be less than 2% (Table 3 & 8). Moreover the %R.S.D. (less variation) shows good precision of both developed methods. The calculated LOQ and LOD concentrations confirmed that the methods were sufficiently sensitive. The methods were specific as none of the excipients interfered with the analytes of interest. Hence, the methods were suitably employed for assaying ticagrelor in commercial marketed formulation (Table 4 & 9).

Conclusion

The HPLC method and the UV spectrophotometric method for the determination of ticagrelor in pharmaceutical formulations were found to be simple, rapid, precise, accurate and sensitive. Moreover, the UV method offers a cost effective and time saving alternative to HPLC method of analysis for ticagrelor from formulations. The HPLC method enables faster quantification of ticagrelor within run time of eight minutes without interference of excipients. In summary, the proposed methods can be used for routine quality control of pharmaceutical formulation containing ticagrelor.

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