UV-Vis spectrophotometric assay determination of oral antiplatelet ticagrelor drug in pharmaceutical formulation: Application to content uniformity

Darshana Pandya, Madhavi Patel, Ravi Ghediya, Anamik Shah and Ranjan Khunt*

Centre of Excellence in Drug Discovery, NFDD Complex, Department of Chemistry, Saurashtra University, Rajkot, Gujarat, India

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ABSTRACT

The purpose of this study is to develop and validate a spectroscopic method for the antiplatelet drug, Ticagrelor. The method was performed on Pharmaco-model of UV–Spectrophotometer Shimadzu1700. The method was validated as per the ICH guidelines and results of this method were superior over the other reported method.

Keywords: UV-Vis Spectrophotometer, Method Development, and Validation, Ticagrelor, Formulation, Content Uniformity.

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INTRODUCTION

Analytical chemistry is a branch of chemistry which deals with methods for determination of chemical composition and their purity. The drugs or dosage form for human use must have excellent quality and purity because they are directly affecting the life, hence, their analysis is necessary. The Ultraviolet-Visible Spectroscopy is a common analytical technique for quantitative and qualitative analysis of a sample which worked on Lambert- Beer's law. This method is develop for the estimation of Ticagrelor (figure -1), Chemical name is (1S,2S,3R,5S)-3-[7-{[(1R,2S)-2-(3,4difluorophenyl) cyclopropyl]amino}-5-(propylthio)-3H-[1,2,3]-triazolo [4,5-*d*]pyrimidin-3-yl]-5-(2-hydroxyethoxy)cyclopentane-1,2-diol [1]and is used as a non-thienopyridine, reversible inhibitor of adenosine diphosphate(ADP) receptors P2Y12 on platelets and is used to decrease the risk of thrombotic cardiovascular events in patients with acute coronary syndrome[2] ,[3]. Some detailed mechanistic studies pointed out that Ticagrelor and its major metabolite reversibly interact with the platelet P2Y12 ADP-receptor to prevent signal transduction and platelet activation, which inhibits platelet aggregation and thrombus formation in atherosclerotic disease [4-6]. Literature survey revealed that few HPLC, LC-MS and UV spectrophotometric methods have been developed and reported for the estimation of Ticagrelor[7-11].To avoid the obvious disadvantages of several sophisticated and expensive methods and to help the analysts to choose the most applicable method for a given laboratory routine. The method was generally developed and validated as per Table-1…..
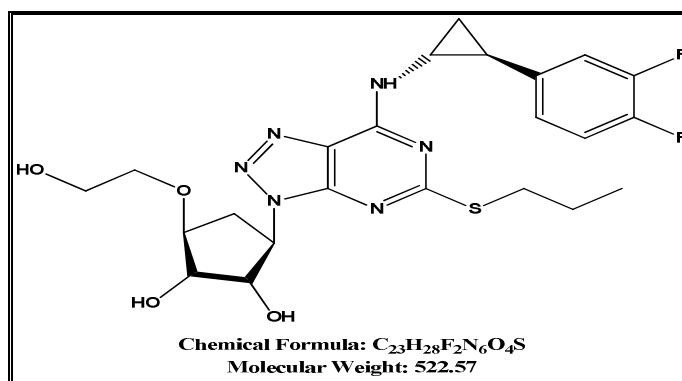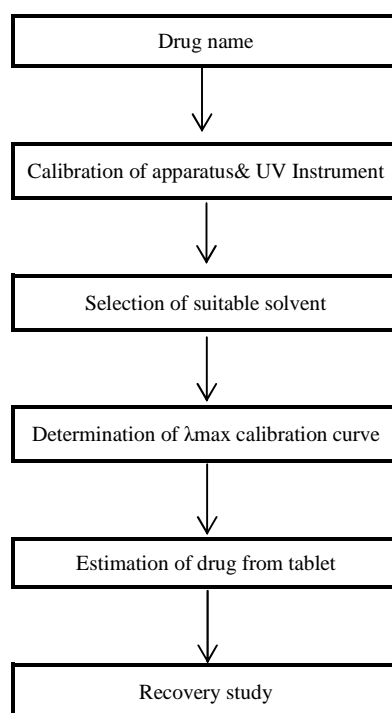
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Chemical Formula: C₂₃H₂₈F₂N₆O₄S
Molecular Weight: 522.57

Figure -1: Structure of Ticagrelor

Table-1: Developed and Validated Protocol

EXPERIMENTAL SECTION

Ticagrelor (Potency 99.87% pure) was procured from ANLON CRO, Rajkot (Gujarat) India, as a gift sample. Ticagrelor tablet (Brilinta-90mg) and Pharmaceutical excipients like magnesium stearate, SDS, Croscarmellose and Povidone were procured from commercial vendors. Chromatographic grade solvents such as ethanol, methanol and dichloromethane were purchased from Merck India Limited, Mumbai, India. Analytical grade hydrochloric acid was obtained from Ranbaxy Fine Chemicals (New Delhi, India) and 0.22 µm nylon syringe filter was obtained from Millipore (Mumbai, India). Double distilled water was generated using Milli-Q purification system of Millipore (Milford, MA, USA).

Instrumentation
The spectroscopic analysis was done by SHIMADZU UV1700 system in the range of 190-500 nm with 1cm matched quartz cells.

Development & Optimization of the Spectrophotometric Method
The method was optimized in order to get simple, easy and reproducible method. Final method was selected after testing under the different parameter which may affect spectrophotometric analysis like scanning wavelength, solvents, the concentration ofsample, temperature, etc. Selection of analytical wavelength was done by diluting the stock solution at a concentration of 20µg/ml with water: methanol (50:50) at ambient temperature. Below figure -2 represent the UV-spectrum of standard preparation scanned in the wavelength range of 190 to 500 nm.

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Preparation of stock and standard solutions
Standard stock solution (100µg/ml) of Ticagrelor was prepared by transferring 10 mg, accurately weighed, into a 100ml volumetric flask then 50 ml of diluent was added. The solution was sonicated for 30 min to dissolve Ticagrelor with intermittent shaking then diluted up to the mark with diluent. The standard was filtered through 0.22µm nylon syringe filter. The working standard was prepared and further diluted to obtain desired concentration (20µg/ml).

Preparation of test solutions
Twenty tablets were weighed and finely powdered. An aliquot of powder equivalent to 10mg of Ticagrelor was transferred into a 100 ml volumetric flask then 50 ml diluent was added. The solution was sonicated for 30 min to dissolve Ticagrelor with intermittent shaking then diluted up to mark with diluent. The standard was filtered through 0.22µm nylon syringe filter. The concentration obtained was 100µg/ml of Ticagrelor. The final solution was prepared and further diluted to obtain 20µg/ml concentration.
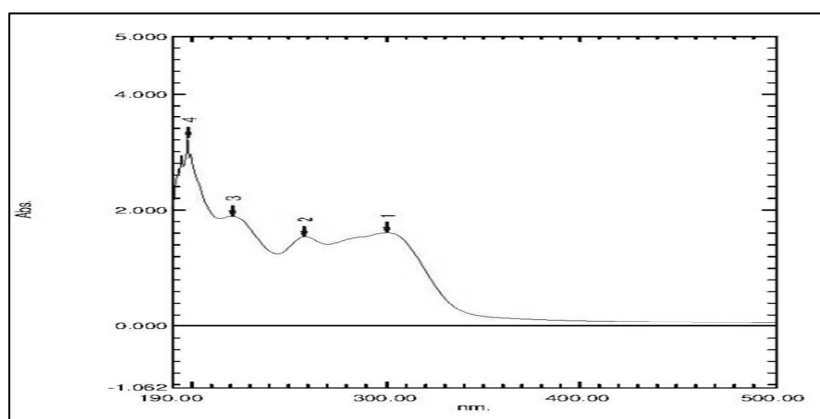

Figure-2: UV spectrum of Ticagrelor standard

Method validation
As per ICH guideline specificity, Linearity, LOD& LOQ, accuracy, precision, robustness and solution stability was determined practically as well as statistically. For linear response measurement, the least squares method was applied.

RESULTS AND DISCUSSION

Proposed UV spectrophotometric method was established for determination of Ticagrelor in bulk form as well as dosage form, which was developed and completely validated. Ticagrelor is a UV-absorbing molecule with specific chromophores in the structure that absorb at a particular wavelength and this fact was successfully employed for their quantitative determinations using the UV spectrophotometric method. The λ_{max} of the drug for analysis was determined by taking scans of the drug sample solutions to the entire UV region. The λ_{max} was found at the wavelength of 222 nm.

The development of spectrophotometry methods for the determination of drugs has increased considerably in recent years because of their importance in pharmaceutical analysis. Due to greater stability in methanol:water (1:1, %v/v); it was selected for further study. The range of wavelength was selected from 190 to 500nm after scanning the standard solution over the range 190-800 nm by use of the UV detector. After the development of the analytical method, it was completely validated in accordance with ICH[12]. This furnished evidence the method was suitable for its intended purpose.

The percentage recovery range of 99% to 100% was indicating the accuracy of the method which is shown in table-2.

From the proposed method, it was found that Ticagrelor obeys linearity within the concentration range of 8-32 µg/ml (figure-3) shown in table-3.
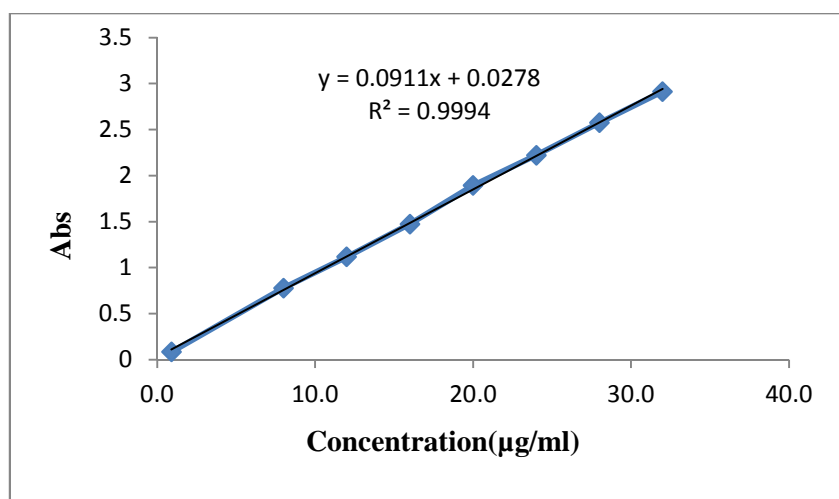
y = 0.0911x + 0.0278
R² = 0.9994

Figure -3: Calibration curve of Ticagrelor

Table-2: Recovery study of Ticagrelor

Level	Set No.	Value Added [a]Con µg/ml	Value Found [a]Con µg/ml	% [b]R	Mean % [b]R	[c]Std Dev	% [d]RSD
70%	1	14.1	14	99.56			
	2	14.2	14.1	99.47	99.45	0.13	0.13
	3	14.3	14.2	99.32			
100%	1	20.3	20.1	99.16			
	2	20.2	20	99.06	99.21	0.18	0.18
	3	20.1	20	99.4			
130%	1	26.2	26.1	99.9			
	2	26	26	99.96	100.1	0.27	0.27
		26.3	26.4	100.39			
[a]Concentration,[b]Recovery,[c]Standard Deviation, [d]Relative standard deviation							

In the all tests including content uniformity (table-4),% RSD of Ticagrelor was less than 2 which is the nominal value, as recommended by the United States Pharmacopoeia [13].The solution is stable till 36 h at room temperature and more than 48 h at 5°C. The good results for accuracy, LOD, LOQ, robustness and precision indicate that our method is efficient (table-5, 6 & 7).

Table-3: Linearity study of Ticagrelor

Concentration leval (%)	Final concentration (µg/ml)	Mean Absorbance
40	8	0.778
60	12	1.117
80	16	1.473
100	20	1.893
120	24	2.221
140	28	2.575
160	32	2.914

Table-4: Content Uniformity study of Ticagrelor

Description	% Assay
Set 1	101.06
Set 2	100.51
Set 3	100.72
Set 4	101.04
Set 5	100.20
Set 6	100.04
Set 7	100.20
Set 8	100.04
Set 9	100.20
Set 10	100.04
Mean	100.41
Stdev.	0.40
% RSD	0.40

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Table-5: Precision study of Ticagrelor

Study	Precision Set No.	%Assay	Mean %Assay	Std. Dev.	% RSD
Method Precision	1	100.96	100.82	0.13	0.13
	2	100.65			
	3	100.80			
	4	100.86			
	5	100.95			
	6	100.68			
Intermediate Precision	1	99.80	99.98	0.31	0.31
	2	100.36			
	3	99.94			
	4	99.90			
	5	100.33			
	6	99.57			
Overall	Mean	100.40			
	Std. dev.	0.20			
	%RSD	0.20			

Table-6: overview of validation

Parameter	Result
Absorption maxima(nm)	222nm
Beer's Range (µg/ml)	8 - 32
Standard Regression Equation	$y = 0.0911x + 0.0278$
Correlation Coefficient(r2)	0.9994
Accuracy (% recovery ± SD)	99.97±0.34
Precision(%RSD)	100.4 ±0.2
LOD(µg/ml)	0.30
LOQ(µg/ml)	0.90
% Drug found in tablet formulation	99.99

Table-7: Assay result of Ticagrelor

Formulation	Label Claim (mg)	Value Found (mg)	% Recovery	%Assay
BRILINTA	90.00	89.97	99.97	99.99

CONCLUSION

There is no any reported UV spectrometric method with complete validation for determination of Ticagrelor in pharmaceutical formulations, which could be directlyimplemented tothe quality control laboratories. The proposed method was found to be accurate, selective, robust, and sensitive. The validation data shows that the excipients present in pharmaceutical dosage form did not interfere in the analysis. Thus, the proposed method is specific for unequivocal determination of Ticagrelor in the presence of matrix compounds (excipients). The results obtained from validation parameters were within the acceptable limits. There was the use of inexpensive reagents, solvents and instruments that are available in laboratories. Hence, these methods can be conveniently adopted for the routine analysis in quality control laboratories.

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