Research Article

Development and Validation of UV Spectrophotometric Method for the Estimation of Ticagrelor (Oral Antiplatelet (OAP) in Pharmaceutical Dosage Form

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ABSTRACT

To develop and validate a precise, accurate, simple, cost-effective UV Spectrophotometric method for the determination of Ticagrelor marketed tablet dosage form. UV- Spectrophotometric method was performed by using UV/Vis double beam spectrophotometer with spectral band width of 1 nm and 1.0 cm matched quartz cells and glass cells were used for UV regions respectively. Ticagrelor shows the highest λmax at 255 nm. The linear calibration range was found to be 2 µg/mL to 10 µg/mL in the UV region. The correlation coefficient (R^2) is 0.9999, and the regression equation is y=0.0411x+0.0007 in the UV region. The % recovery was found to be in the range lies between 99.26-100.25 %. The percentage assay of Ticagrelor obtained was 98.66. The Limit of detection and Limit of quantification was found to be 0.18962 and 0.57462, respectively. The method was validated in terms of Linearity, Precision, Accuracy, Robustness, LOD, and LOQ as per ICH Q2 (R_1) guidelines.

Keywords: Ticagrelor, Validation, Ultraviolet spectroscopy, Method development.

INTRODUCTION

The ticagrelor (Brilinta) is a P2Y12 receptor inhibitor that belongs to the CPTP (Cyclo pentyl triazolo pyrimidine) class. Ticagrelor reversibly binds to the P2Y12 receptor and prevents ADP from binding. This prevents signal transduction and platelet activation that can lead to pathologic thrombus formation. Ticagrelor is indicated to reduce the rate of cardiovascular death, myocardial infarction (MI), and stroke in patients with an acute coronary syndrome or history of myocardial infarction. Brilinta is superior to clopidogrel. It also decreases the rate of stent thrombosis in patients who have been stented for treatment of acute coronary syndrome (ACS).

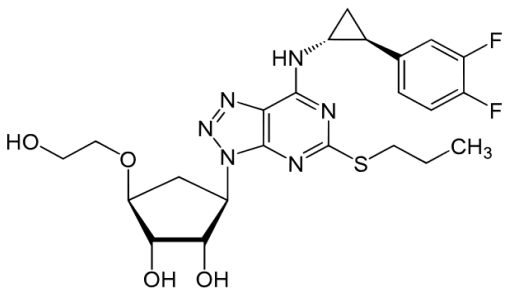

Figure 1: Chemical Structure of Ticagrelor

As per the Literature Survey, it is revealed that the drug has been estimated by LC-MS[1], UPLC electrospray ionization-tandem mass spectrometry[2], HPLC[3-11]. But only a few UV – Spectroscopic methods have been reported for the estimation in bulk and pharmaceutical dosage forms. Methanol and acetonitrile are used as a solvent. Hence an attempt has been made to develop and establish a novel, simple, rapid, and sensitive UV spectrometric method in accordance with ICH guidelines for the estimation of Ticagrelor in the bulk formulation. Usually HPLC has proven to be useful in diagnostic purposes and the pharmaceutical industry[12-13]. Chemical Structure of ticagrelor is shown in figure 1.

MATERIALS AND METHODS

Instrumentation

A double beam ELICO SL 210 UV spectrophotometer containing two matched quartz cells with a one cm light path was taken for measuring of absorbance of Ticagrelor. Essaevibra AJ (0.1 mg sensitivity) balance was used for weighing. Ultra Sonicator bath Model no - 91250, PCI Ltd., Mumbai were used in this present study.

Chemicals and reagents

Ticagrelor was procured from Hetero Drugs Ltd., Hyderabad, and Telangana, India. The tablets containing 90 mg labeled claim of Ticagrelor (Brilinta) tablets were used for this study. CH_3OH were procured from E. Merck specialties, private Ltd., Mumbai, India.

Selection of the solvent

Plentiful trials were executed to find out the suitable solvent system for dissolving the Ticagrelor. The solvents such as acetonitrile, DMSO, hexane, ethanol, methanol, and triple distilled water were tried based on the solubility of the drug. Ticagrelor is soluble in solvents such as ACN, methanol, and DMSO, ticagrelor showed a maximum in methanol, so this absorbance was selected all the way through the experiment.

Selection of detection wavelength

To determine the optimum λmax of Ticagrelor, 10 µg /ml of the Ticagrelor solution was prepared in CH_3OH and

scanned over the range of 200 - 400 nm. When methanol and acetonitrile are used as a solvent, it was observed that the drug showed maximum absorbance at 255 nm. So, this wavelength was used for further study.

Preparation of standard stock solution

Accurately weighed 100 mg of drug in 100 ml of diluent (methanol and acetonitrile-50:50) to get a 1000 µg/ml stock solution. Working standard solutions were further diluted to get a concentration range of 2-10 µg/ml.

Preparation of Calibration curve

From the above prepared Ticagrelor stock solution, appropriate dilutions were prepared to get the final concentration of 2, 4, 6, 8, and 10 µg/ ml, and absorbance was taken at λ_{max} 255 nm. Average of such five sets of values were taken for standard calibration plot, and the calibration curve was plotted. The aliquots of concentration ranging from 2-10 µg/ ml concentrations were used. The linearity was calculated by the least square regression method Calibration data of Ticagrelor is depicted in Table 1, and Linear regression data is tabulated in table 1a. The summary output of Ticagrelor by ANOVA is shown in table 1b. Figure 2 shows the calibration curve of Ticagrelor, and Figure 2a shows the ultraviolet overlain spectra of Ticagrelor.

Method development and validation[14-16]

Different types of solvents were tested for solubility for Ticagrelor solvents such as Methanol, ethanol, DMSO, acetonitrile, and distilled water at 10 µg/ml concentrations. Nevertheless, Ticagrelor was soluble and stable in methanol and acetonitrile for a minimum of 48 hours at room temperature. Therefore diluent (methanol and acetonitrile-50:50) was used for the detection of wavelength and preparation of standard and working concentration. To ensure the planned method of the pharmaceutical formulation, an assay of Ticagrelor 90 mg tablets was utilized at working concentration. Assay for working concentration of the sample at 255 nm was analyzed. A UV spectrophotometric method is validated according to ICH Q2 (R1) guidelines[17] for validation of analytical procedures. The process was validated for parameters such as Linearity, specificity, accuracy, precision, robustness, LOD, and LOQ.

Table 1: Calibration data of Ticagrelor

S.No	Concentration (µg/ml)	Absorbance
1	2	0.0838
2	4	0.1658
3	6	0.2458
4	8	0.3312
5	10	0.4112

Table 1a: Linear regression data

Parameter	Results
Detection wavelength (λ_{max})	255 nm
Beer's law limits (µg/ml)	2-10
Molar absorptivity (L. mole^{-1} cm^{-1})	21407.82
Sandell's sensitivity (µg /cm^2/0.001 absorbance unit)	0.02441
Regression equation (Y = mx+ c)	0.0411x+0.0007
Slope (m)	0.0007
Intercept (c)	0.04111
The standard error of slope (S_m)	0.000159223
The standard error of intercept (S_c)	0.000964203
Standard error of estimate (S_e)	0.00133238
Correlation coefficient (r^2)	0.9999

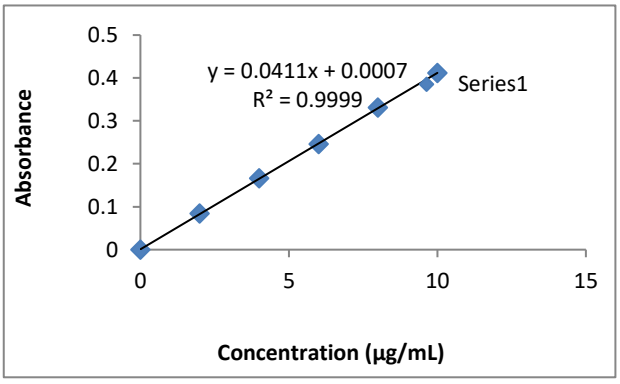

Figure 2: Calibration curve of ticagrelor.

Table 1b: The summary output of Ticagrelor (ANOVA):

	A	B	C	D	E	F	G	H	I
1	SUMMARY OUTPUT								
2									
3	*Regression Statistics*								
4	Multiple R	0.999970006							
5	R Square	0.999940014							
6	Adjusted R Square	0.999925017							
7	Standard Error	0.001332238							
8	Observations	6							
9									
10	ANOVA								
11		*df*	*SS*	*MS*	*F*	*Significance F*			
12	Regression	1	0.118343361	0.118343	66677.68223	1.34942E-09			
13	Residual	4	7.09943E-06	1.77E-06					
14	Total	5	0.11835046						
15									
16		*Coefficients*	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
17	Intercept	0.000714286	0.000964203	0.740804	0.499941621	-0.00196277	0.003391342	-0.00196277	0.003391342
18	X Variable 1	0.041117143	0.000159233	258.2202	1.34942E-09	0.040675042	0.041559244	0.040675042	0.041559244

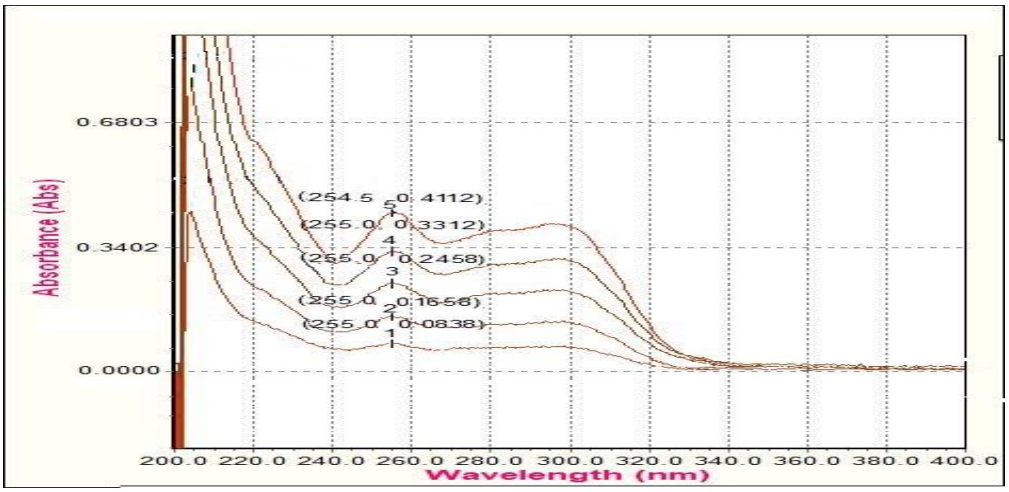

Figure 2a: Ultraviolet Overlain Spectra of Ticagrelor

Linearity

The calibration curve was obtained with five concentrations of the standard solution (2–10 µg/ml for UV method). The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Precision

Precision studies were carried out to check the reproducibility of the method. Repeatability was determined by six replicates of the same concentration of the sample and measuring the absorbance. The intra-day precision study was carried out by analyzing the prepared drug solution at three different times (8-hour samples) in a day. The same procedure was followed for three different days to determined inter-day precision. The results were reported as % RSD. System precision is shown in table 2.

Table 2: Results of system precision

S. No	Absorbance
1	0.2459
2	0.2468
3	0.2458
4	0.2453
5	0.2458
Mean	0.24592
Standard deviation	0.000544977
% Relative Standard deviation	0.221607459

Method precision

Method precision was estimated by conducting the assay of a sample under the test of repeatability (intraday precision) and intermediate precision performed during three successive days for three times. Eventually, the mean, SD, and % Relative standard deviation were determined. Intraday and interday precisions are shown in table 2a and table 2b, respectively.

Table 2a: Results of Method precision (Intraday precision)

Concentration (µg/ml)	Sample absorbance	Mean absorbance ± S. D	% RSD (n=3)
4	0.1655 0.165 0.1652	0.165 ± 0.152	0.152307
6	0.245 0.246 0.2454	0.245 ± 0.205	0.205047
8	0.3303 0.3319 0.3322	0.331 ± 0.030	0.030169

Table 2b: Results of method precision (Interday precision)

Concentration (µg/ml)	Sample absorbance	Mean absorbance ± S. D	% RSD (n=3)
4	0.1659 0.1649 0.1656	0.165 ± 0.0005	0.310129
6	0.2453 0.2457 0.2456	0.245 ± 0.0002	0.084781
8	0.3313 0.3312 0.3311	0.331 ± 0.0001	0.030193

Accuracy (recovery studies)

Recovery studies of Ticagrelor were carried out by using a standard addition method in which estimation of % mean recovery of the sample by % method at three different levels (80 %, 100 %, and 120 %, i.e., 4 µg/ml, 6 µg/ml, 8 µg/ml). These 80 to 120 levels of the sample solutions were prepared as per the procedure given in the methods from the dilutions used for linearity (6 µg/ml). At each level, three analyses were performed. % mean recovery was calculated, as shown in table 3. The accepted limits of

recovery are 98 % - 101 %. In fact, The % recovery and mean % recovery were calculated. Accuracy results of ticagrelor is shown in table 3.

Ruggedness

Ruggedness is done by performing the proposed method on different instruments. In addition to that, this method is carried out by two various analysts, and performing the technique on different days to check the reproducibility Results of ruggedness is shown in table 4.

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD is the lowest amount of analyte in the sample that can be detected. LOQ is the lowest amount of the analyte in the sample that can be quantitatively determined by suitable precision and accuracy. LOD and LOQ were determined by the following equation. LOD = 3.3σ/S, LOQ = 10 σ/S. where σ is the standard deviation of the Y-intercept of the calibration curve and S is the slope of the regression equation. The LOD and LOQ values were found to be 0.189 µg/ml, 0.5746 µg/ml respectively.

Solution Stability

The Solutions of Ticagrelor (Concentration 10 µg/ml) were tested for their stability at ambient temperatures. The absorbance values for 8 hrs, 16 hrs, 24 hrs, 32 hrs, 48 hrs, was reproducible, and absorbance variation was found to be less than 2 % in both conditions. Solution Stability studies of Ticagrelor is shown in table 4a.

Table 3: Accuracy results of Ticagrelor

Level (%)	Absorbance	% Recovery	Mean % Recovery	% RSD
80	0.1964	99.19	99.26	0.078
80	0.1967	99.35		
80	0.1965	99.24		
100	0.2452	99.14	99.29	0.143
100	0.2459	99.43		
100	0.2456	99.31		
120	0.2975	100.29	100.25	0.051
120	0.2972	100.19		
120	0.2974	100.26		

Table 4: Results of ruggedness

Analyst and Instrument	Concentration (µg/ml)	Sample absorbance	Mean absorbance± SD	% RSD
Analyst 1	10	0.4125 0.4115 0.4112	0.4117±0.0006	0.1653
Analyst 2	10	0.411 0.4114 0.4113	0.4112±0.0002	0.0506
Instrument-1	10	0.411 0.4117 0.413	0.4119±0.001	0.2463
Instrument-2	10	0.4113 0.412 0.4114	0.4115±0.0003	0.0919

Table 4a: Solution Stability studies of Ticagrelor

Time (hrs)	Absorbance 10 µg/ml standard in ambient conditions
0	0.4112
8	0.4118
16	0.4122
24	0.4125
32	0.4110
48	0.4108

Analysis of marketed formulation

The developed method was applied to analyze commercially available Ticagrelor tablets (Brilinta). The tablet was having the content of Ticagrelor equivalent to 90 mg. Ten tablets were weighed, and weight equivalent to 100 mg was dissolved in methanol. By frequent shaking, volume was made up to mark with diluent. The solution was then filtered through Whattman filter paper #41. This filtrate was diluted suitability with solvent to get the solution of 5 µg/ml concentration. The absorbance was measured against the solution blank. The amount of Ticagrelor was calculated from the calibration curve. The readings were taken in triplicate. The assay results are shown in Table 5.

Table 5: Result of assay of the pharmaceutical formulation of Ticagrelor

Table formulation	Label amount(mg/tab)	Amount obtained by proposed method*	% Recovery (Amount found)
Brilinta	90	89.85±0.11	99.445

*Average of five determinations.

RESULTS AND DISCUSSION

The ultraviolet spectra of Ticagrelor were scanned in the region between 200-400 nm. The overlay spectra of Ticagrelor at different concentrations were absorbed maximum at 255 nm, which was selected as the detection wavelength. The response of the Ticagrelor was found to be linear in the concentration range of 2-10 µg/ml, with a good correlation coefficient of r^2 = 0.999. The system precision, intermediate precision results, i.e., inter-day and intra-day precision of Ticagrelor, are tabulated in tables 2a and 2b, respectively. The % RSD less than 2 in all precision results, which indicates that the method was precise. In this recovery, study accuracy was carried out by using a standard addition method at three different concentration levels (80 %, 100 %, and 120 %). The mean percentage recovery at each level should be 99.26-100.25%. All the results are well within the acceptance criteria, and results indicate that the method is accurate. Ruggedness was performed to check the reproducibility, which showed the % RSD less than 2, which indicates that the method was rugged. The developed method was eventually applied for the quantification of Ticagrelor in tablets. The mean % assay values were found to be 99.445 %. The amount of the drug in the tablet sample was in good agreement with the label claim of the formulation.

CONCLUSION

A simple, precise, accurate UV spectroscopic method was developed and validated for the estimation of Ticagrelor. This method was found to be economical in terms of usage of solvents and yet to sensitive compared to the existing methods. In this study, the precision and accuracy % RSD was < 2 % in all cases. This method provides reproducible results with high precision, accuracy, and was capable of analyzing Ticagrelor in low concentrations. However, this UV method is simple, quick, sensitive. Hence the developed method can be used in the regular quality control of Ticagrelor in the tablet dosage form.

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