

Development and validation of UV-visible spectrophotometric method for estimation of Ticagrelor in bulk and formulation

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Abstract

A rapid, precise, and cost-effective UV spectrophotometric technique has been established utilizing a solvent acetonitrile for the quantification of Ticagrelor in both bulk and pharmaceutical dosage forms. At a predetermined wavelength of 298 nm, the method demonstrated linearity within the concentration range of 10 to 50 µg/ml, achieving a high correlation coefficient ($R^2 = 0.9956$). This method was effectively utilized to assess the Ticagrelor content in commercially available brands, with the findings aligning well with the stated label claims. The method underwent statistical validation and recovery studies to evaluate its linearity, precision, repeatability, and reproducibility. The results obtained demonstrated that this method is suitable for routine analysis of Ticagrelor in both bulk and commercial formulations.

Keywords: UV- Visible spectrophotometry, Ticagrelor, Analytical method validation

Introduction

Ticagrelor[(1S,2S,3R,5S)-3-(7-{{[(1R,2S)-2-(3,4-difluorophenyl)cyclopropyl]amino}}-5-(propylsulfanyl)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-5-(2-hydroxyethoxy)cyclopentane-1,2-diol]^[1] is a non-competitive and direct-acting P2Y₁₂ receptor antagonist that reversibly binds to the receptor. In the Platelet Inhibition and Patient Outcomes study, Ticagrelor significantly reduces the incidence of cardiovascular death, myocardial infarction, and stroke in patients with the acute coronary syndrome.^[2] Ticagrelor is an antithrombotic medication. It reversibly binds to adenosine diphosphate (ADP) receptors found on platelet cells. Patients with myocardial revascularization require this activity to protect the heart tissue from an elevated plasma adenosine concentration, increasing blood flow to the heart and lowering the risk of embolism.^[3]

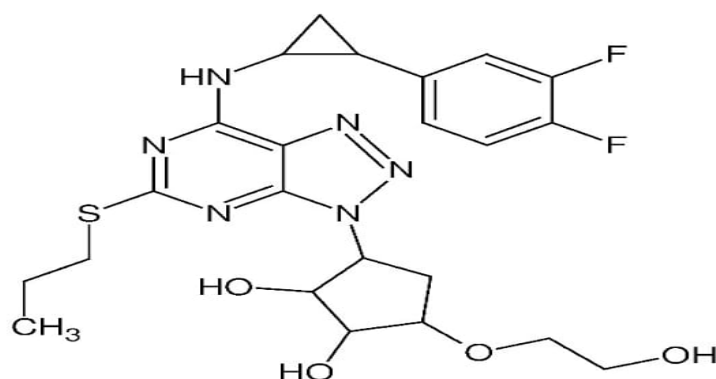


Fig. 1: Ticagrelor

Numerous HPLC assay techniques have been documented for the quantification of Ticagrelor. A review of the literature indicates that a range of analytical approaches, including high-performance thin-layer chromatography (HPTLC)^[4], High performance liquid chromatography(HPLC)^[5]and Voltammetrymethods^[6], have been utilized for the estimation of Ticagrelor. Additionally, recent studies have introduced several UV spectrophotometric methods for the determination of Ticagrelor, employing solvent acetonitrile.

In this research, a straightforward, efficient, and cost-effective UV spectrophotometric technique was established utilizing acetonitrilefor the quantification of Ticagrelorin both raw materials and commercially available dosage forms. The method underwent optimization and validation in accordance with the International Conference on Harmonization (ICH) guidelines,exhibiting remarkable specificity, linearity, precision, and accuracy in the measurement of Ticagrelor.

Materials and methods

Apparatus

Double beam UV–visible spectrophotometer (Systronics 2201)was used for all absorbance measurements with matched quartz cells, volumetric flask, Pipette, Sonicator etc.

Materials

All chemicals and reagents were of analytical or HPLC grade. Ticagrelorwas provided by Smurti Organics Pvt. Ltd., India, Ticagrelor marketed formulation which was used as the reference standard.

Preparation of working standard drug solution

The standard Ticagrelor (10 mg) was precisely weighed and placed into a 10 mL volumetric flask, where it was thoroughly dissolved and diluted to the calibration mark with acetonitrile,

resulting in a final concentration of 1000 µg/ml (Stock-1). Subsequently, Stock-1 was adequately diluted with acetonitrile to produce a 100 µg/ml (Stock-2) solution.

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

The Stock-2 was analyzed using full output mode at a medium scanning speed across the entire range of the UV-VIS Spectrophotometer, spanning from 200 to 800 nm, with a co-solvent system serving as the blank. After acquiring the spectrum, λ_{max} was identified. The above method was repeated thrice.

Preparation of calibration curve

The calibration curve was developed by utilizing Stock-2 to create five separate calibration standards with concentrations of 10, 20, 30, 40, and 50 µg/mL. The absorbance for each standard was recorded at λ_{max} 298 nm, using a fixed wavelength measurement mode. The calibration curves, illustrating the correlation between concentration and absorbance, were generated using Microsoft Excel. This procedure was repeated several times to ensure the reliability and reproducibility of the results.

Method Validation

Developed UV method for the estimation of Ticagrelor was validated in terms of parameters like linearity, range, precision, robustness, ruggedness, accuracy, limit of quantification (LOQ) and limit of detection (LOD) using predefined calibration standards as illustrated below

Linearity and range

The linearity of the proposed UV method was assessed by employing five different calibration standards. Through the analysis of these standards, calibration curves were generated to illustrate the correlation between absorbance and concentration, which were then evaluated using linear least squares regression. The R^2 value was regarded as a key parameter in validating the linearity of the proposed method. The acceptable range of linearity was established by the interval between the upper and lower concentration limits of the proposed UV method.

Accuracy

Accuracy may be represented as the percentage of recovery achieved from the analysis of a known amount of analyte introduced into the sample. Alternatively, it can be defined as the difference between the average value and the accepted true value, along with the relevant confidence intervals.

Intra-day precision and Inter-day precision

Precision of the assay method was assessed in terms of repeatability by carrying out five independent assays of Ticagrelor test arrangement and the % RSD of measurement (intra-

day). Intermediate precision of the method was checked by performing same methodology on three consecutive days.

Stability study

Samples prepared for repeatability study were preserved for 24 hrs.at room temperature and analyzed on the following day to test for short-term stability.

Robustness

The assessment of robustness must be taken into account during the development stage and is contingent upon the specific procedure, which involves intentional alterations in method parameters. If the measurements are vulnerable to fluctuations in analytical conditions, it is essential to either maintain appropriate control over these conditions or to incorporate a cautionary note within the procedure. In the current study, the absorption maxima was decreased and increased by 1 nm and the process was carried for 30µg/ml standard solution. The % RSD was calculated.

Limit of Detection (LOD)

In UV method development LOD was determined by utilizing the following equation

$$\text{LOD} = 3.3 \times \text{SD}/S$$

Where, SD= Standard deviation of Y-intercepts

S= Slope

Limit of Quantification (LOQ)

In UV method development LOQ was determined by utilizing the following equation.

$$\text{LOQ} = 10 \times \text{SD}/S$$

Where, S= slope

SD= Standard deviation of Y-intercepts

Estimation of Ticagrelor content in marketed formulation

Developed and pre-validated UV-Vis method was effectively employed to determine the Ticagrelor content in a commercially available formulation. For this study, AXCER Tablets were acquired from the local market in Solapur, and the tablet contents were collected. Appropriate dilutions were prepared utilizing a pre-optimized solvent system. The prepared samples were then analyzed using the pre-validated UV method, and the results were reported in terms of average percent assay.

Results and Discussion

Method development and optimization

The identification of the wavelength corresponding to maximum absorbance is crucial for performing quantitative UV analysis. Solutions with absorbance values below 1 are generally considered suitable for identifying the wavelength of maximum absorbance. In accordance with this criterion, the maximum wavelength for a Ticagrelor solution at a concentration of 100 µg/mL was ascertained using the full scan mode of a UV-Visible spectrophotometer (see Figure 2). The full scan was conducted with UV software, which aided in pinpointing λ_{max} . The peak absorbance wavelength for Ticagrelor was found to be 298 nm.

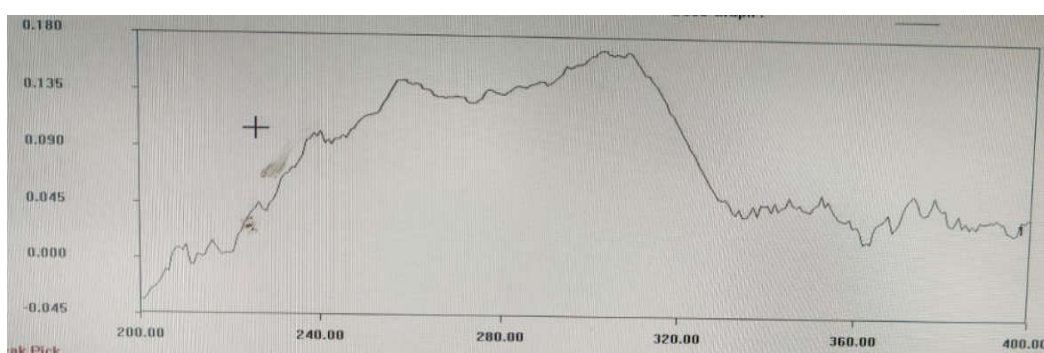


Fig. 2: UV-Visible spectra of Ticagrelor

Preparation of calibration curve

The process of quantifying unknown samples through a UV-Visible spectrophotometer or a similar analytical device requires the development of a consistent calibration curve, along with an associated equation that defines the correlation between concentration and the observed response. In comparison to graphical techniques, this method is widely acknowledged for its reproducibility and reliability. In light of the importance of quantitative analysis for Ticagrelor, a calibration curve was established using five calibration standards. The absorbance readings for these standards were recorded at a wavelength of 298 nm, utilizing the fixed wavelength mode of the UV-Visible spectrophotometer. This calibration curve was created on three different occasions, with the findings presented in Table 1.

Table 1: Results of calibration curve at 298 nm

Standard	Conc. (µg/ml)	Absorbance
STD-1	10	0.492
STD-2	20	0.635
STD-3	30	0.774
STD-4	40	0.895
STD-5	50	0.998

Method Validation

Linearity and Range

The proposed method is intended to achieve optimal performance. A five-point calibration curve for Ticagrelor, covering a concentration range of 10-50 μ g/ml, was established, highlighting the significance of linearity and range. Table 1 presents the specific concentrations along with their corresponding mean absorbance values. Upon conducting least squares regression analysis on the calibration curve, the resulting equation was found to be $y = 0.0127x + 0.3772$, with a correlation coefficient of 0.9956, as illustrated in Figure 3. The linearity study indicated that the developed UV method demonstrated linearity within the specified concentration range of the calibration standards.

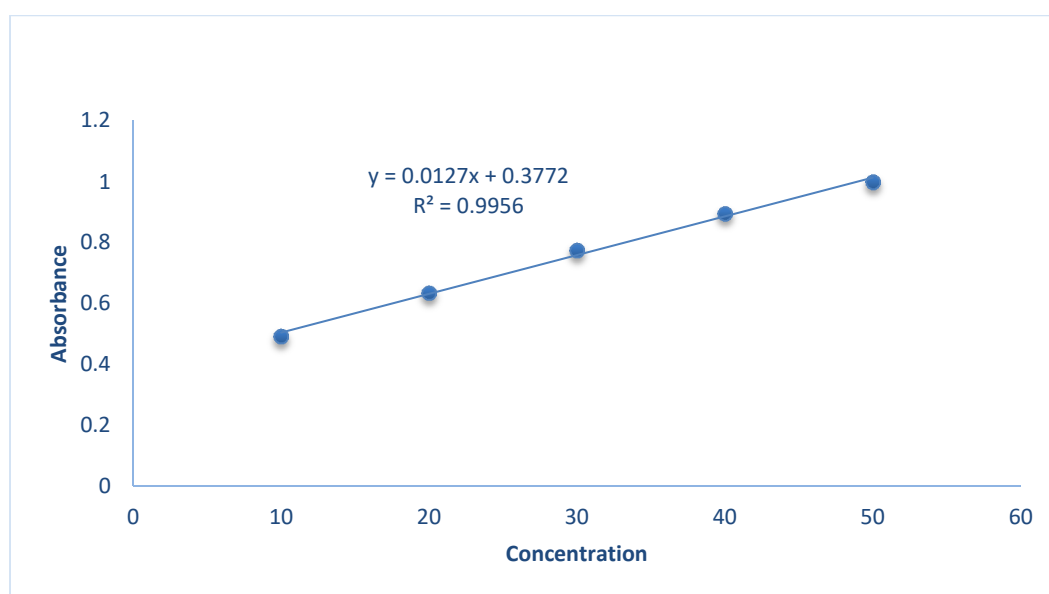


Fig. 3: Calibration curve for Ticagrelor

Accuracy

Accuracy is a crucial metric that reflects the degree to which the experimental value corresponds to the actual concentration of a substance within a specific matrix. It is vital to verify accuracy throughout the entire calibration range of the analytical method to guarantee that results obtained at any measurement point are reliable. In the context of the UV method utilized for Ticagrelor analysis, accuracy was evaluated through recovery studies. For Accuracy, percentage recovery of Ticagrelor was determined. This involved adding the analyte at concentration levels of 80%, 100%, and 120%.

Table 2: Accuracy data of UV method for Ticagrelor

Recovery level (%)	Concentration of sample ($\mu\text{g/ml}$)	Amount added ($\mu\text{g/mL}$)	% Recovery
80	10	8	98.3
100	30	30	98.7
120	50	60	99.5

Precision

Precision is a crucial metric that indicates the degree of variability in measurements, showcasing the reliability of results obtained through a specific analytical technique. It is expected that a robust analytical method will produce results that are consistently reproducible. Achieving precision in an analytical method is vital for ensuring accurate outcomes. Given the importance of obtaining both reproducible and accurate results, the intra- and inter-day precision of the developed UV method was evaluated at concentrations of $5\mu\text{g/ml}$. Results obtained fell within the acceptable limit, demonstrating a relative standard deviation (RSD) of less than 1%.

Table 3: Intra-day precision data of UV method for Ticagrelor

Sr. No	Concentration	Absorbance
1	5 $\mu\text{g/ml}$	0.261
2		0.259
3		0.260
4		0.263
5		0.264
	Mean	0.2614
	SD	0.002073
	%RSD	0.79

Table 4: Inter-day precision data of UV method for Ticagrelor

Sr.No.	Concentration	Absorbance (1st Day)	Absorbance (2nd Day)
1	5 μ g/ml	0.261	0.262
2		0.263	0.259
3		0.261	0.261
4		0.262	0.262
5		0.259	0.260
	Mean	0.2612	0.2608
	SD	0.001483	0.001303
	%RSD	0.56	0.49

Robustness

The robustness of an analytical method is defined by its ability to deliver consistent results even when minor, intentional modifications are made to its parameters. This attribute is essential, as unintentional fluctuations in factors such as solvent composition or pH can arise during standard operations, potentially undermining the method's efficacy. It is expected that these variations should not have a significant effect on the performance of the analytical method. Therefore, a robust analytical method is highly sought after. Robustness shows negligible impact of the absorption level of Ticagrelor solution in acetonitrile at different wavelength (± 2 nm).

Table 5: Robustness data of UV method for Ticagrelor

Sr. No	Absorbance at 295nm	Absorbance at 301nm
1	0.261	0.258
2	0.259	0.257
3	0.262	0.260
SD	0.001078	0.001842
%RSD	0.41	0.71

Limit of Quantitation (LOQ) and Limit of Detection (LOD)

The Limit of Quantification (LOQ) refers to the lowest concentration that can be accurately and precisely measured. Generally, the LOQ acts as the primary calibration standard. In the suggested UV method, the Limit of Detection (LOD) and LOQ were established at 0.495 and 1.502 μ g/ml, respectively, as shown in Table 7. The relatively low LOQ indicates that the proposed method is suitable for the analysis of samples containing even trace amounts of Ticagrelor.

Table 7: LOD & LOQ data for UV method for Ticagrelor

LOD	0.523 μ g/mL
LOQ	1.587 μ g/mL

Estimation of Ticagrelor

The developed UV method was successfully applied for the estimation of Ticagrelor content in AX CER Tablet 90 mg. Average percent assay of Ticagrelor tablet was found to be 97.8 %.

Conclusion

A simple, accurate, and precise UV-Visible spectrophotometric technique for quantifying Ticagrelor has been established and validated. This method exhibited robustness and reliability, proving effective for the estimation of Ticagrelor.

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