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Multivariate Calibration Techniques Using UV Spectrophotometry for Quantifying Ticagrelor in Pharmaceutical Formulations

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Abstract

The aim of this project is to create and verify a straight forward, highly responsive, and precise UV spectrophotometric technique for measuring the amount of ticagrelor in both its raw form and its pharmaceutical form. This will be achieved by utilizing multivariate linear regression analysis. Multivariate linear regression analysis was performed to assess the correlation between concentration and absorbance. Absorbance values were collected at five different wavelengths, and the resulting data were utilized to develop a predictive model for quantifying ticagrelor in pharmaceutical formulations. This analysis facilitated the identification of key wavelengths that contribute to accurate concentration measurements were analyzed using statistical methods. The implemented method exhibited linearity across a concentration range of 5-15 µg/mL, with a correlation coefficient valuation of 0.998. The peak absorption wavelength (λmax) of Ticagrelor was detected at 257 nm. The percentage RSD values for intraday and interday precision were found to be within the ICH recommendations' acceptable range of 2%, namely in the ranges of 0.540 - 0.558 and 0.540 - 0.565, respectively. The created approach was determined to be straightforward, expeditious, precise, and reliable in accordance with the International Council for Harmonisation (ICH) criteria Q2 (R1). Utilizing statistical methods ensures accurate and consistent results, unaffected by instrument errors or experimental variables.Since the drug's absorbance is measured at five different selected wavelengths, the multivariate calibration methodology has been said to be more reliable than the other published procedures. This led to the development of a quick and easy method based on mathematical building blocks.

Keywords: Ticagrelor, Multivariate Linear Regression Analysis, Validation, UV Spectrophotometry and ICH

Introduction

Ticagrelor is a notable compound characterized by its chemical name, (1S,2S,3R,5S)-3-[7-[[(1R,2S)-2-(3,4-difluorophenyl)cyclopropyl]amino]-5-propylsulfanyltriazolo[4,5-d]pyrimidin-3-yl]-5-(2-hydroxyethoxy)cyclopentane-1,2-diol[1]. It has the molecular formula C23H28F2N6O4S and a molecular weight of 522.568 (Figure 1). This drug belongs to the antiplatelet category, specifically acting as an oral antagonist of the adenosine diphosphate (ADP) receptor P2Y12. It inhibits this receptor in a reversible and direct manner, providing a faster. Ticagrelor works by preventing the aggregation of platelets, a process crucial for the formation of blood clots. By doing so, it reduces the risk of heart attacks and strokes[2]. It achieves this by inhibiting the P2Y12 receptor on platelets, which plays a key role in their activation and aggregation.Ticagrelor is widely used in the treatment of patients with coronary artery disease who are at risk of heart attacks or strokes[3]. It is particularly effective in individuals who have experienced a myocardial infarction or have acute coronary syndrome. Studies have shown that

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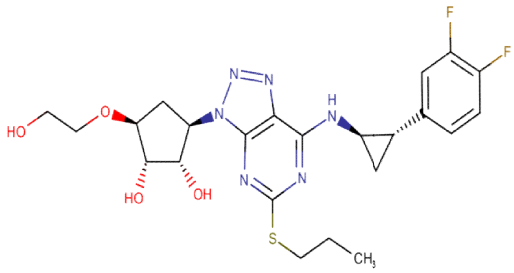

Figure 1: Chemical Structure of Ticagrelor

ticagrelor is effective in preventing first-time heart attacks or strokes in high-risk patients. Several analytical methods have been established for the quantification of ticagrelor in pharmaceutical formulations and biological samples[4]. These include:

1. Liquid Chromatography-Mass Spectrometry (LC-MS)
2. High-Performance Liquid Chromatography (HPLC)
3. Thin Layer Chromatography (TLC)
4. Fourier Transform Infrared Spectroscopy (FTIR)
5. Ultra-Performance Liquid Chromatography (UPLC)
6. High-Performance Thin-Layer Chromatography (HPTLC)
7. Reverse Phase High-Performance Liquid Chromatography (RP-HPLC)
8. Ultraviolet Spectroscopy (UV)

These methods are essential for ensuring the quality, efficacy, and safety of ticagrelor in clinical use. They allow for precise measurement and monitoring of the drug in various formulations and biological fluids.

Most labs employ spectrophotometric methods as their preferred method because to their low cost, accuracy, precision, and reproducibility. The preferred approach is predicated on a high level of precision and accuracy on the direct estimate of Ticagrelor[5].The method is easy to use and reasonably priced, and it may be applied to examine Ticagrelor. The proposed approach outlines the evaluation of Ticagrelor in pharmaceutical formulations through a UV spectral multilinear regression methodology, utilizing fundamental mathematical principles. Multilinear regression, which extends the concept of a single dependent variable to incorporate multiple dependent variables into the calibration model, enhances the model's flexibility and applicability. This statistical technique is particularly valuable under optimal experimental conditions, as it provides substantial sensitivity and resolving power at a relatively low cost, making it suitable for routine quality control analysis[6].

To ensure the reliability and accuracy of the developed method, it is recommended to validate the approach according to the guidelines set forth by the International Conference on Harmonization (ICH). Specifically, the ICH Q2 (R1) guidelines for analytical method validation should be followed to confirm the method's validity and robustness[7].

The current work aimed to establish a speedy, easy-to-use, accurate, precise, sensitive, and fast analytical method for measuring some of the previously described sophisticated analytical techniques of Ticagrelor[8]. A based on the aforementioned claim, a straightforward analytical method based on UV spectrophotometry enabled multivariate calibration process was recommended to be developed[9].

Materials and Methods

Chemicals and solvents employed

- 0.1M NaOH.
- BRILINTA TABLETS – (Label claim – 90 mg, 10 mg and 20 mg of Ticagrelor), manufactured by Samarth Life Sciences Pvt. Ltd., The marketed tablet formulations were procured from the local market.

Solubility

- Insoluble in ethyl acetate.
- Sparingly soluble in methanol .
- Freely soluble in water, 0.1M NaoH, 0.1M HCl.

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Instrumentation

- UV-Vis double beam Spectrophotometer (Lab India UV- 3092).
- Electronic balance (SHIMADZU AY-220H).

Method development

Selection of solvent

Ticagrelor was reported to be freely soluble in 0.1M NaoH, which was employed as the solvent to solubilize the drug for the entire analysis.

Preparation of standard stock solution

After carefully weighing 10 mg of ticagrelor, it was added to a 100 mL volumetric flask. 50mL of the solvent was added in order to dissolve the contents of the volumetric flask. The solvent was thoroughly combined (1 mg/mL) with the final volume, which was 90 mL. Pipetting 3 mL of the aforementioned solution into a 30 mL volumetric flask, the solvent was used to build up the remaining volume to the appropriate level and the mixture was thoroughly stirred. The resultant solution was further diluted with the solvent to achieve concentrations between 5 and 15 µg/mL.

Determination of λ_{max}

The solvent was employed to dilute the standard stock solution of Ticagrelor to a concentration of 10µg/mL. The UV range of 400-200 nm was used to scan this solution. The UV spectra of Ticagrelor is shown in Figure 2 and the absorbance maxima of Ticagrelor was found to be 257 nm. Five wavelengths were selected in and around the absorbance maxima of Ticagrelor, such as 253, 255, 257, 259 and 261 nm for the study.

Preparation of standard solution for linearity

For linearity testing, the solvent was used to further dilute the Ticagrelor standard stock solution to create concentrations of 5, 7.5, 10, 12.5, and 15 µg/mL.

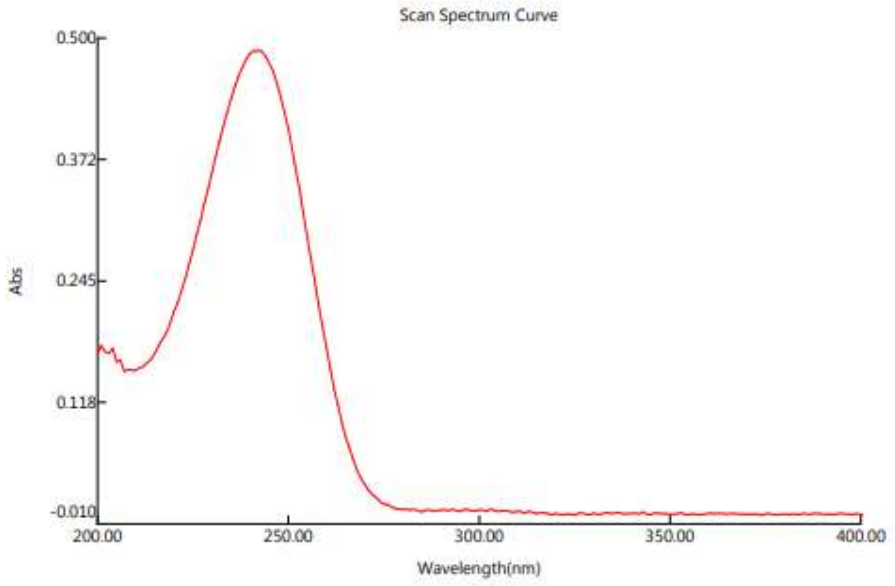

Figure 2: UV spectra of Ticagrelor

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Preparation of sample solution

20 tablets of Ticagrelor (BRILINTA TABLETS – Label claim – 90 mg of Ticagrelor) were measured for weight, and the average weights were established. The tablets were ground into a fine powder and thoroughly combined with the contents. After weighing out 10 mg of Ticagrelor from the combined substance and dissolving it in 90 mL of solvent for 25 minutes using sonication, the solvent was used to bring the volume up to 90 mL. The aforementioned mixture was thoroughly blended and filtered. For additional analysis, the filtrate was appropriately diluted.

Method validation

The developed approach was validated in accordance with the ICH Q2 (R1) protocol, which examined validation parameters such as linearity, precision, and accuracy.

Linearity

To establish a linear correlation and minimize instrumental fluctuations, absorbance measurements were taken for the prepared linearity concentrations at five different wavelengths surrounding the drug's maximum absorbance at 257 nm. These wavelengths were 253, 255, 257, 259, and 261 nm, as detailed in Table 1. The overlay UV spectra, illustrating the linearity at these wavelengths, are presented in Figure 3. Separate correlation coefficient values for the linear regression equations at each of these five wavelengths were calculated and are provided in Table 2. Additionally, Figure 4(a & b) displays the multivariate calibration linearity obtained at these five wavelengths, as well as the cumulative absorbance.

Precision

To evaluate intraday and interday precision, the absorbance of a linearity solution at a 100% concentration (10 µg/mL) was measured across all five wavelengths. For intraday precision, the designated concentration was scanned six times within a single day, while for interday precision, it was scanned across three different days. Figures 5 and 6 illustrate the overlay UV spectra for the intraday and interday precision studies, respectively. The absorbance values obtained at the specified wavelengths for these precision experiments are detailed in

Table 1: Absorbance values at five selected wavelengths

Concentration (µg/mL)	Absorbance				
	253 nm	255 nm	257 nm	259 nm	261 nm
5	0.278	0.284	0.282	0.281	0.277
7.5	0.415	0.423	0.427	0.427	0.415
10	0.543	0.553	0.563	0.557	0.549
12.5	0.692	0.69	0.712	0.711	0.696
15	0.825	0.836	0.848	0.849	0.825

Table 2: Linearity data showing statistical parameters at all five wavelengths

Wavelength	Slope	Intercept	Regression equation	r^2
253	0.0548	0.0026331	y= 0.0548x + 0.0022	0.9996
255	0.0567	0.0032249	Y=0.0567x - 0.0004	0.9998
257	0.0551	0.0023875	y= 0.0551x + 0.0016	0.9997
259	0.0548	0.0031411	y= 0.0548x + 0.0088	0.9996
261	0.0568	0.0026833	y= 0.0568x - 0.003	0.9998

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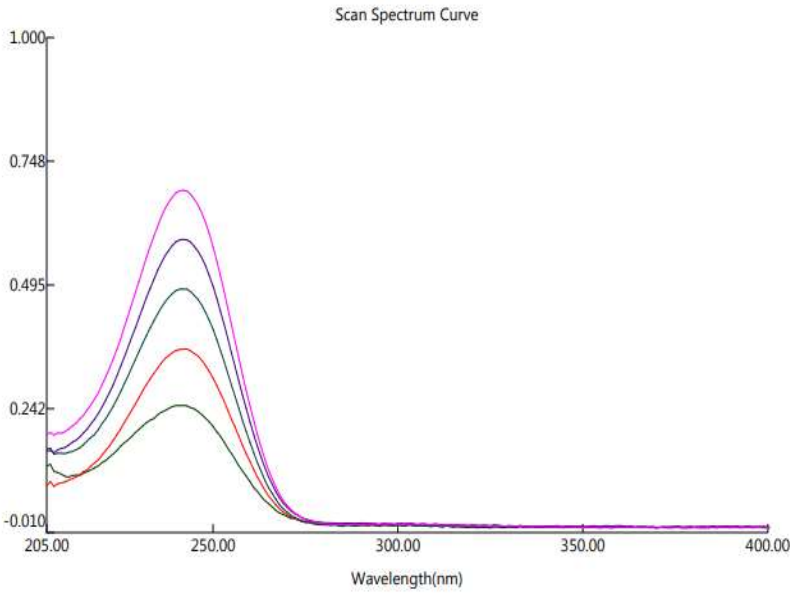

Figure 3: Separate correlation coefficient values

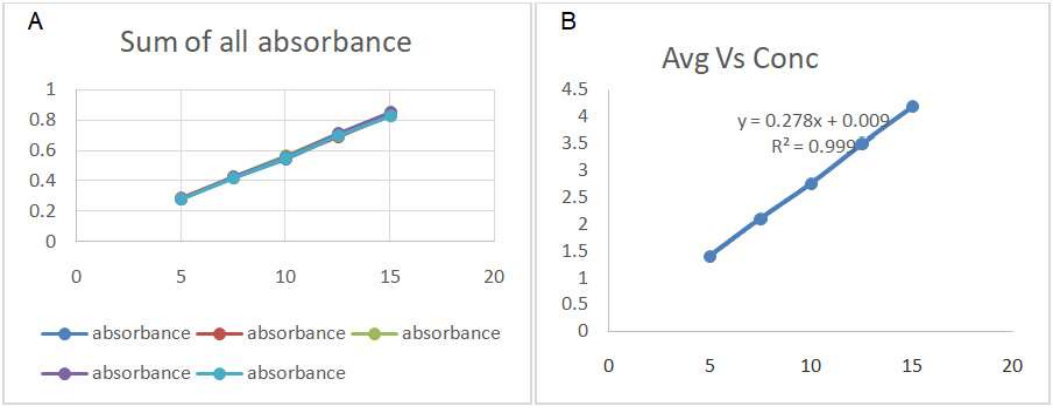

Figure 4: Multivariate Calibration linearity graph (a) and Sum of all Absorbance; and (b) Average *vs* Concentration of Ticagrelor

Tables 3 and 5. Additionally, the calculated standard deviation (SD) and percentage relative standard deviation (% RSD) values for both intraday and interday precision are provided in Tables 4 and 6, respectively.

Accuracy

The accuracy of the developed methodology was evaluated at concentration levels of 50%, 100%, and 15% by recovery studies conducted using the standard addition method. Three distinct 10 mL volumetric flasks were filled with 0.1 mL of the sample solution, and 0.1, 0.5, and 0.7 mL of the standard stock solution were pipetted into the aforementioned volumetric flasks, respectively, from the prepared stock solutions of standard and sample. The final

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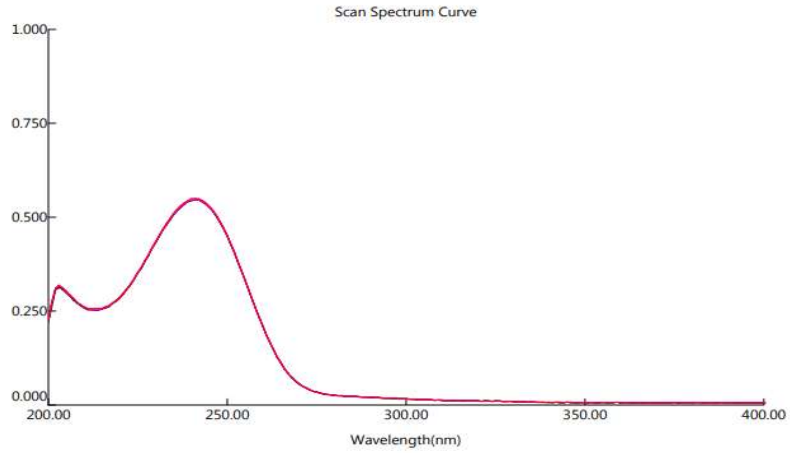

Figure 5: Overlay UV Spectra of Ticagrelor showing intraday precision studies

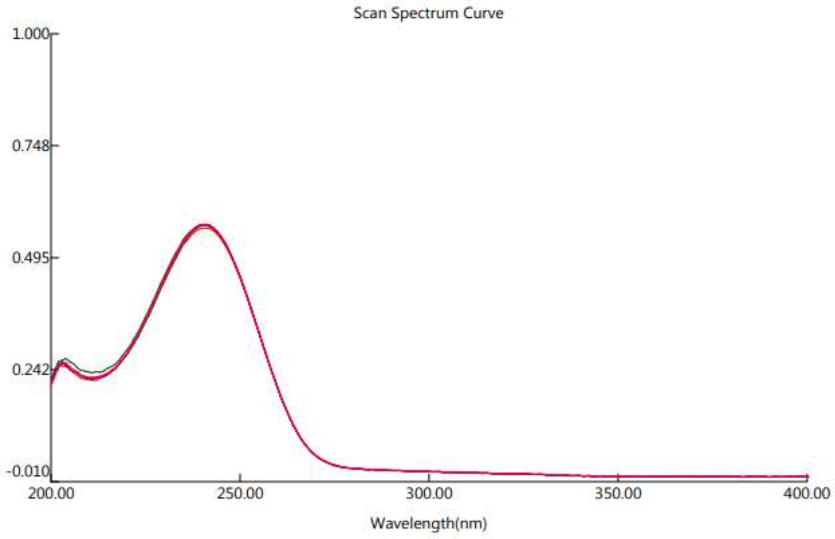

Figure 6: Overlay UV Spectra of Ticagrelor showinginterday precision studies

volume was raised to the necessary level using methanol. It was decided what the recovery percentages were. Table 7 presents a summary of the recovery investigations' results, while Figure 7 shows the overlay UV spectra that demonstrate correctness.

Assay

The absorbance of the extracted sample solutions were recorded at 257 nm. The amount of drug present in the formulations was calculated and the assay results are tabulated in Table 8.

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Table 3: Intraday precision at five selected wavelengths

Concentration (µg/mL)	No. of Repetitions	Absorbance (nm)				
		253nm	255nm	257nm	259nm	261nm
10	1	0.544	0.564	0.548	0.548	0.556
	2	0.543	0.563	0.549	0.553	0.557
	3	0.546	0.562	0.547	0.548	0.556
	4	0.543	0.563	0.547	0.548	0.553
	5	0.546	0.564	0.546	0.556	0.555
	6	0.546	0.565	0.548	0.548	0.553

Table 4: Intraday Precision of TICAGRELOR showing Mean, SD, and % RSD

Conc(µg/mL)	Description	253 nm	255 nm	257 nm	259 nm	261 nm
10	Mean	0.544	0.563	0.547	0.548	0.554
	SD	0.001378	0.001049	0.000816	0.00216	0.001722
	% RSD	0.253151	0.186124	0.149086	0.393727	0.310436

Table 5: Interday precision at five selected wavelengths

Conc (µg/mL)	No. of Repetitions	Absorbance (nm)				
		253 nm	255 nm	257 nm	259 nm	261 nm
10	1	0.543	0.563	0.549	0.553	0.557
	2	0.544	0.564	0.548	0.548	0.556
	3	0.543	0.563	0.547	0.548	0.553
	4	0.546	0.562	0.547	0.548	0.556
	5	0.545	0.564	0.547	0.547	0.554
	6	0.546	0.565	0.548	0.548	0.553

Table 6: Interday Precision of Ticagrelor showing Mean, SD, and % RSD

Wavelength (nm)	Amount present (µg/mL)	Amount added (µg/mL)	Amount recovered (µg/mL)	% Recovery
253	6	4	9.8	98.00
		9	10.2	102.00
		14	19.6	98.00
255	6	4	9.9	99.00
		9	14.8	98.67
		14	19.8	99.00
257	6	4	9.8	98.00
		9	15.2	95.00
		14	19.6	98.00

(*Contd.*)

Table 6: Interday Precision of Ticagrelor showing Mean, SD, and % RSD

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Wavelength (nm)	Amount present (µg/mL)	Amount added (µg/mL)	Amount recovered (µg/mL)	% Recovery
259	6	4	9.9	99.00
		9	15.5	96.88
		14	19.6	98.00
261	6	4	9.8	98.00
		9	14.89	98.90
		14	21	100.8

Table 7: Recovery studies of Ticagrelor at five selected wavelength

Concentration (µg/mL)	Description	253 nm	255 nm	257 nm	259 nm	261 nm
10	Mean	0.544667	0.5635	0.5475	0.550167	0.555
	SD	0.001506	0.001049	0.001049	0.003488	0.001673
	% RSD	0.276416	0.186124	0.191563	0.634003	0.301499

Table 8: Assay of Ticagrelor in marketed pharmaceutical formulations

Label claim (mg)	Amount estimated (mg)	%Assay	Average (n = 3)	SD	% RSD
TICAGRELOR (90MG)	89.98	99.98	99.99	0.0231	0.231
	90.01	100.01			
	89.97	99.97			

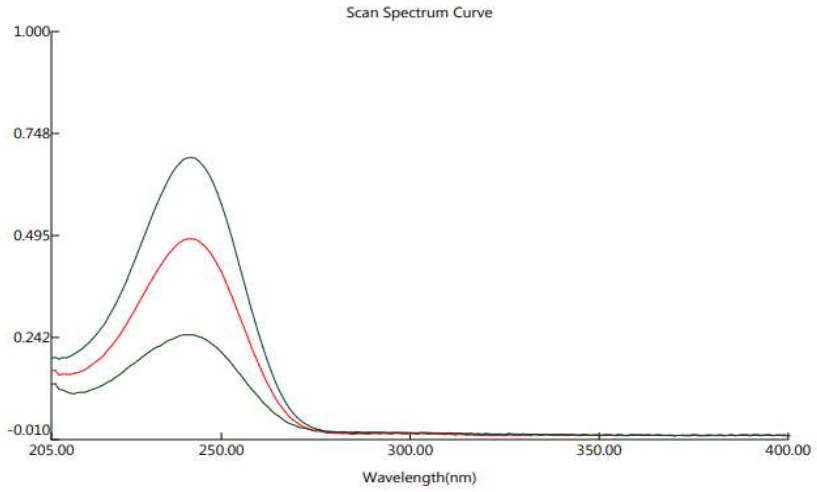

Figure 7: Overlay UV Spectra of Ticagrelor showing Accuracy

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Results and Discussion

The absorption maxima of Ticagrelor were observed at 257 nm using 0.1M NaOH as solvent.

Linearity

Within the defined concentration range of 5– 15 µg/mL, the devised technique was found to be linear. All five wavelengths of 253, 255, 257, 259 & 261 were used to construct a linear regression equation. The resulting correlation coefficient values were determined to be more than 0.998.

Precision

Both intraday and interday precision investigations were conducted. The percentage RSD values for intraday and interday precision were found to be within the ICH recommendations' acceptable range of 2%, namely in the ranges of 0.540 - 0.558 and 0.540 - 0.565, respectively. The low estimated percentage RSD number demonstrates the established approach's accuracy. The mean, standard deviation, and percentage RSD (relative standard deviation) are shown for five different wavelengths in Tables 5 and 6.

Conclusion

For evaluating ticagrelor in pharmaceutical formulation, it was discovered that the established quick and easy UV spectrophotometric-assisted Multivariate calibration approach was linear, sensitive, accurate, and precise. Since the drug's absorbance is measured at five different selected wavelengths, the multivariate calibration methodology has been said to be more reliable than the other published procedures. This led to the development of a quick and easy method based on mathematical building blocks. It is highly recommended for routine quality control testing of Ticagrelor in pharmaceutical formulations as it is more predictable than previous spectrophotometric procedures.

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