Precision Unveiled: Robust RP-HPLC Method for Accurate Estimation of Prasugrel, a Platelet Aggregation Inhibitor, in Final Dosage Form

Asif Khan Sherwani[1], Uzma Asif [2*], Dua Fatima Sherwani[3]

[1]Jamjoom Pharmaceuticals Co. Ltd, Research and development unit, PO Box No. 6267, Jeddah 21442, Saudi Arabia
[2]Department of Biochemistry, Medicine Program, Batterjee Medical College, Jeddah, 21442, Saudi Arabia
[3]Sindh Medical College, Jinnah Sindh Medical University, Karachi 75510, Pakistan

*****Corresponding author's e-mail: uzma.aasif1@gmail.com

ABSTRACT

Background: Administered orally, Prasugrel is a third generation thienopyridine that irreversibly inhibits platelet aggregation by binding with P2Y12 receptor. It is available in coated tablets of 5 and 10 mg commercially, but there are very few analytical methods available for estimation of Prasugrel in final dosage form.

Objective: The current study introduces a simple and accurate high-performance liquid chromatographic technique to determine the amount of Prasugrel in the final dosage form

Method: The mobile phase consists of Methanol – Buffer (700 + 300, v/v). A column containing octadecylsilane chemically bonded to porous silica particles (Purospher star ® 100 ODS 250 x 4.6 mm, 5 µm) was used as stationary phase. Detection was performed using a variable wavelength ultraviolet-visible detector set at 220 nm for Prasugrel. Solutions were injected into the chromatograph under isocratic condition at a constant flow rate of 1.0 mL/min. The stability indicating capability of the method was confirmed through a force degradation study.

Results: The proposed method exhibited acceptable accuracy and precision, with a wide linearity range. Linearity was observed between 100-300 µg/mL for Prasugrel (r2 = 0.999), and the estimated drug concentration using this method was in good agreement with the label claim. Recovery studies at three different levels were conducted to assess the accuracy of the methods, and no interference from commonly encountered pharmaceutical additives was observed during specificity experiments. The method also demonstrated peak purity and stability during force degradation study, indicating its capability. Additionally, the method was found to be precise, with a repeatability analysis showing a %RSD of less than 2.

Conclusion: The HPLC method for estimation of Prasugrel in final dosage form was developed and validated as per guideline and it is found to be precise, specific, accurate and robust.

Highlights: All statistical data confirms the effectiveness of the methods, making them suitable for routine analysis of quality control and stability studies in pharmaceutical dosage form.

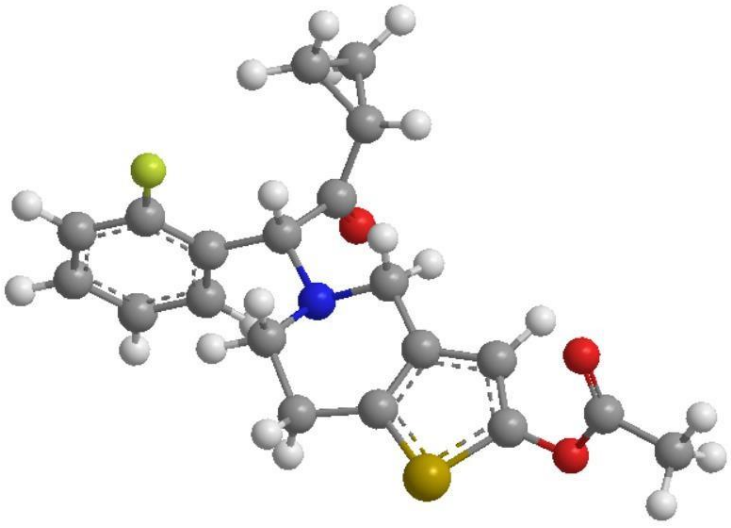
INTRODUCTION

Since the activation and suppression of inflammation are correlated with a number of metabolic processes, inflammation may be viewed as a two-edged sword (1).

Inflammation can lead to various metabolic disorders which include but not limited to cardiovascular disorder, neoplastic, degenerative, infective and autoimmune complications (1, 2). It is a known fact that reduction of inflammation helps in development and managing of cardiovascular event as well as endothelial injury specially in atherosclerotic cardiovascular disorder (3). On the other hand, inflammation can be useful tool in reducing various infection as a useful host response(1, 4). According to recent guidelines proposed by European society of Cardiology in 2017, one of the recommended treatments of acute myocardial infarction is use of Prasugrel as a P_2Y_{12} inhibitor (5). Prasugrel is a highly effective third generation thienopyridine that is administered orally and works by irreversibly inhibiting platelet aggregation through binding with P_2Y_{12} receptor. Its molecular structure is depicted in Figure-1. In comparison to other drugs in its class, such as Ticlopidine and Clopidogrel, Prasugrel has been shown to inhibit adenosine diphosphate-induced platelet aggregation more rapidly, consistently, and to a greater extent in both healthy volunteers and patients with coronary artery diseases (6, 7).

According to a pharmacodynamic study, patients with acute coronary syndrome (ACS) can safely switch from Clopidogrel to Prasugrel, resulting in a further reduction in platelet function after one week. When patients receive a loading dose of Prasugrel prior to switching from Clopidogrel, the reduction in platelet function occurs within two hours. Prasugrel is classified as a BCS class II drug and exhibits pH-dependent solubility, being highly soluble at low pH conditions (8-10).

Figure – 1: **Molecular structure of Prasugrel**



In the pharmaceutical industry, the development of a drug product involves a critical stage known as the optimization of an analytical method. This method plays a vital role in determining the quality of the product. The validation of this analytical method is of utmost importance as it ensures that different HPLC techniques yield consistent and accurate results. Moreover, this validation step is crucial in the creation of new dosage forms as it provides valuable information regarding the method's accuracy, linearity, precision, ruggedness, and specificity (11, 12).

The ICH and USP guidelines dictate that the main goal of validating an analytical procedure is to prove that it is appropriate for its intended use. In the drug development process, it is now required to provide validation data to the relevant authorities. The guidelines for validating analysis methods involve distinguishing and separating degradation products from the compounds of interest under various stress conditions. The testing should encompass the impact of temperature, oxidation, photolysis, and susceptibility (13-15).

The RP-HPLC method proposed in this study is highly specific and represents an enhanced analytical technique when compared to previously reported methods. It offers peak shape of analyte. Various method performance parameters were assessed to understand their impact on the chromatography. The validation of this method confirmed its suitability for analyzing Prasugrel in the final dosage form. Furthermore, this method can be effectively employed in stability studies and quality control applications pertaining to this drug (16, 17)

In this paper, a HPLC method for determining Prasugrel in stability studies and quality control applications is described. The method is simple, selective, precise, and accurate. Various parameters were evaluated to understand their impact on the chromatography. Validating this analytical method is essential for developing new dosage forms and obtaining reliable and precise results. The method was successfully validated and deemed suitable for analyzing Prasugrel in the final dosage form.

Experimental

Apparatus

a) HPLC – HPLC analysis was performed on LC-2030C series instrument (Shimadzu Corporation, Kyoto, Japan) with 4-liquid gradient system, high-speed autosampler, column oven, and UV-visible (UV-Vis) detector. Chromatograms were recorded and integrated with LC solution (Shimadzu) chromatographic PC software.

b) Milli-Q water purification system – A Milli-Q integral 3 system mode was used (Millipore, Billerica, MA).

Standards, Reagents and Chemicals

a) Prasugrel hydrochloride – Standard (Hetero Laboratories, India).
b) 1-Octanesulfonic acid sodium salt - Analytical grade (Panreac Applichem, USA).
c) Methanol – HPLC grade (Fisher scientific, USA).
d) Orthophosphoric acid – Analytical grade (Panreac Applichem, USA).
e) Sodium hydroxide – Analytical grade (Panreac Applichem, USA).
f) Water – Purified in-house using the Milli-Q integral 3 system.
g) Sample – Obtained from University of Karachi, Karachi, Pakistan

a) Mobile phase preparation

Buffer (Dissolved 1.17 g of 1-octanesulfonic acid sodium in 1000 mL of water. Add 1 mL of triethylamine and adjust the pH to 2.5 ± 0.05 with phosphoric acid) and Methanol in the ratio of (30: 70, v/v). Mobile phase was used as diluent.

b) Standard preparation

50.0 mg of Prasugrel working standard was weighed and transferred into a 50 mL volumetric flask. 30 mL of diluent was added and sonicated to dissolve followed by

dilution to volume with diluent. Further diluted 5 mL of this solution into 25 mL volumetric flask and diluted to volume with diluent and mixed well to get final concentrations of 0.1 mg/mL Prasugrel.

c) Sample preparation

1 tablet was transferred into 25 mL volumetric flask. 20 mL of diluent was added and sonicated for 15 minutes with intermittent shaking and diluted to volume with diluent to get desired concentration.

Calculation

(Au/As) x (Wt. std/50) x (5/25) x (25/Spl) x (Av. Wt. /L.C) x (P)

Where,

Au = Area of prasugrel peak obtained with sample solution

As = Area of prasugrel peak obtained with standard solution

Wt. Std = Weight, in mg, of Prasugrel working standard

Spl = Sample (1 tablet)

P = Potency of prasugrel working standard, on as is basis

L.C = Label claim of tablet (5 mg)

Forced degradation study

Acid degradation

The Prasugrel tablet sample and placebo underwent a 24-hour stress test using 0.5 N HCl, followed by neutralization using 0.5 N NaOH.

Base degradation

A base hydrolysis sample and placebo were prepared by treating the sample with 0.5N NaOH for 24 hours and then neutralizing with 0.5 N HCl.

Oxidative degradation

Oxidative stress degradation was performed by sample and placebo treated with 1 mL of 33% hydrogen peroxide at room temperature for 24 hours.

Heat degradation

Sample and placebo were also exposed to 100ºC for 48 hours to obtain thermal degradation products.

Light degradation

The sample and placebo were irradiated with 700 W/ m2 light intensity for 10 hours in the SUNTEST light stress chamber.

Chromatographic conditions

The stationary phase used in the analysis was a Purospher star ® column, measuring 250 x 4.6 mm, made of stainless steel and packed with C18 chemically bonded to porous 5 mm silica particles from Waters Corp. The analysis was conducted at a constant flow rate of 1.0 mL/min, with the UV-Vis detector set at 220 nm. All analyses were performed at ambient sampler temperature, and a volume of 10 µL solution was injected onto the column.

RESULTS AND DISCUSSION

Method development and optimization

The pharmaceutical industry operates under strict regulations, and the creation of analytical method plays a vital role in guaranteeing the safety, effectiveness, and quality of pharmaceutical products. This article focuses on the significance of developing and validating analytical methods in the pharmaceutical sector, using a case study involving the estimation of Prasugrel in final dosage from. It explores the chemical properties of this compound, the selection of an appropriate extraction solvent, the optimization of chromatographic conditions, and the essential validation parameters that must be assessed (18-20)

Before developing an analytical method, it is crucial to have a clear understanding of the chemical nature of the compounds under analysis. In the case of Prasugrel it is soluble in polar solvents such as methanol. The addition of a phosphate buffer increased their solubility. To optimize the chromatographic conditions, a mobile phase composition consisting of Methanol and buffer (70 + 30, v/v) was used on a Purospher star ® C18 column with dimensions of 250 x 4.6 mm. The column was maintained at an ambient temperature in the column oven. This specific mobile phase ratio resulted in symmetrical peak shapes and supported low back pressure and shorter retention time. For detection purposes, a wavelength of 220 nm was selected for Prasugrel. This wavelength is provided maximum absorption when measured by a PDA detector.

Analytical Method validation

The validation of the method was conducted to meet the requirements of the International Conference on Harmonization (ICH) guidelines and the United States Pharmacopeia requirements. Various parameters such as specificity, linearity, accuracy, precision, and robustness were evaluated and deemed satisfactory. It is crucial to validate the method to ensure its specificity, sensitivity, reliability, and suitability for routine analysis, quality control, and stability studies of pharmaceutical preparations (16, 20)

a) System suitability

System suitability tests were performed on chromatograms obtained from standard solution to check parameters such as %RSD between 5 replicate injections, tailing factor and theoretical plates of HPLC column and found satisfactory results. Values obtained from standard solution are provided in (Table-1).

Injection No.	Prasugrel		
	Area	Tailing factor	Theoretical plate
1	3671193	1.35	4362
2	3669646	1.35	4429
3	3673101	1.34	4387
4	3667575	1.36	4393
5	3662526	1.35	4501
Average	3668808	1.35	4362
% RSD	0.11		

Tables -1: Results of system suitability

b) Specificity

The ability to accurately identify the analyte in the presence of other components such as impurities and degradation products is known as specificity. No interference was observed from diluent, placebo and forced degradation samples at the retention time of Prasugrel. Additionally, the chromatogram of Prasugrel gave minimum peak purity index in positive value while peak purity index of all stress conditions found 1.0. Therefore, it can be concluded that the method is specific for determining of Prasugrel in the final dosage form. The peak purity and minimum peak purity index are shown in (Table-2).

Table-2: Peak purity index and minimum peak purity index of Prasugrel by Force degradation study

S. No.	Stress Conditions	Peak purity index	Minimum peak purity index
1	Acid (0.5 N HCl)	1.0	59
2	Base (0.5 N NaOH)	1.0	59
3	Oxidation (33% H_2O_2)	1.0	55
4	Heat (100°C)	1.0	68
5	Light degradation	1.0	59

c) Precision

The degree of agreement between the results determines the precision of a method. The precision of the method was evaluated by examining system precision, method precision, and intermediate precision.

Precision of system

Precision of the system is to determine reproducibility of results obtained by injecting of six replicates of single preparation of standard solution. The relative standard deviation (RSD) for six determination was found to be 0.11%.

Precision of method

Precision of a method is to determine by analyzing six preparation of the same concentration and evaluate the % RSD between these preparations. Six separate test sample solutions were prepared at the specification level for measuring the method precision. The calculated RSD of results was found to be 0.37% as shown in Table-3.

Table -3: Results of method precision

Preparation No.	%Assay
1	99.6
2	99.9
3	100.4
4	100.0
5	99.3
6	99.9
Average (n=6)	99.8

%RSD	0.37

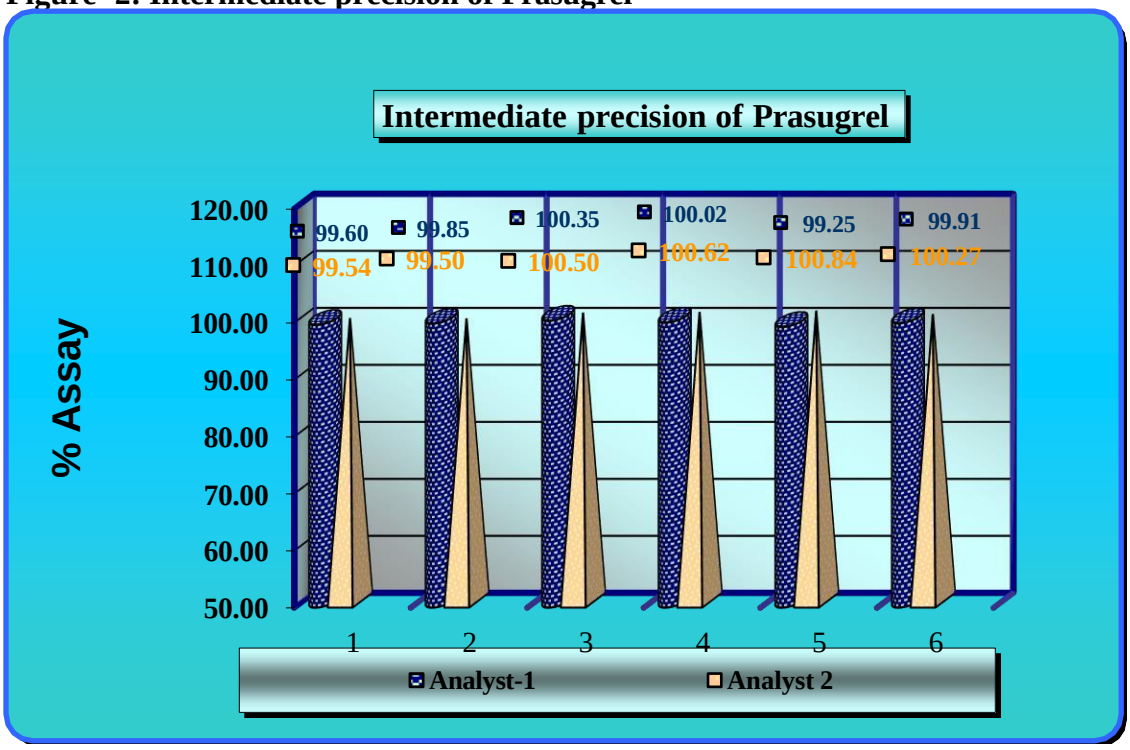
Intermediate precision

The similar procedure of method precision was carried out by a different analyst, using different mobile phase and diluent preparations and instrument on a different day with different lot of same brand column for intermediate precision study. The %RSD of results for intermediate precision study was calculated and compared with the method precision results. RSDs obtained from 12 assay results by 2 analysts were 0.49% as shown in Table-4 and graphical representation in Figure - 2.

Table -4: Results of intermediate precision

Analyst	Analyst-1	Analyst-2
Day	Day-1	Day-2
Preparation No.	%Assay	%Assay
1	99.6	99.5
2	99.9	99.5
3	100.4	100.5
4	100.0	100.6
5	99.3	100.8
6	99.9	100.3
Average (n=12)	100.2	
%RSD	0.49	

Figure -2: Intermediate precision of Prasugrel



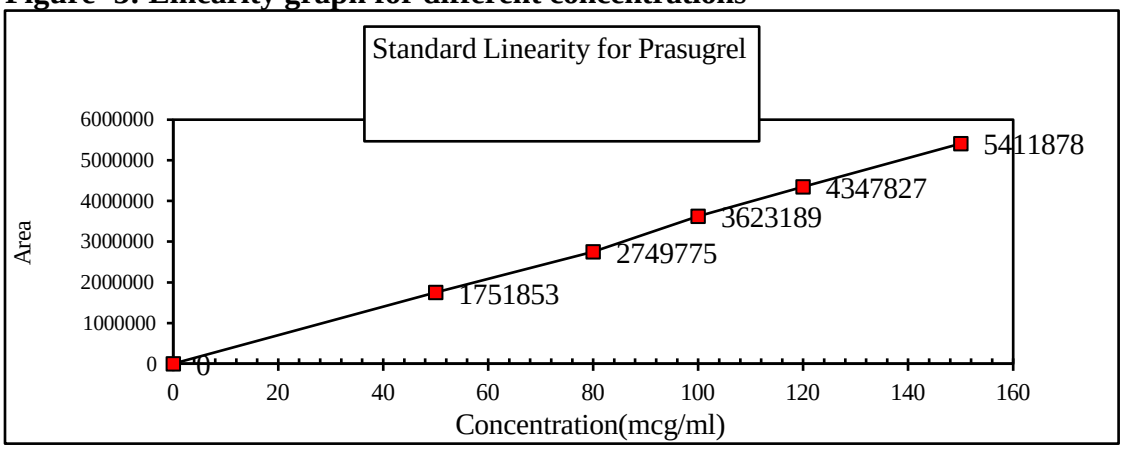
d) Linearity

The linearity of an analytical method refers to its ability to produce test results that are directly proportional to the concentration of the drug substance being tested. In this study, peak areas were plotted against concentrations for Prasugrel within a range of 50-150% of the target level. Calibration graphs, linear regression equations, and the linearity correlation coefficient (r-value) are provided in Tables 5. The r-value, which was found to be greater than 0.999, indicates that the method has a wide linear dynamic range.
Linearity graph for assay concentration range is provided in Figure-3.

Tables -5: Results of Linearity

Linearity level (%)	Concentration (ppm)	Average Area (uAU)
50	100	1751853
80	150	2749775
100	200	3623189
120	250	4347827
150	300	5411878
Slope		**37062.464**
Sigma		**61955.89**
Correlation Coefficient		**0.999**

Figure -3: Linearity graph for different concentrations



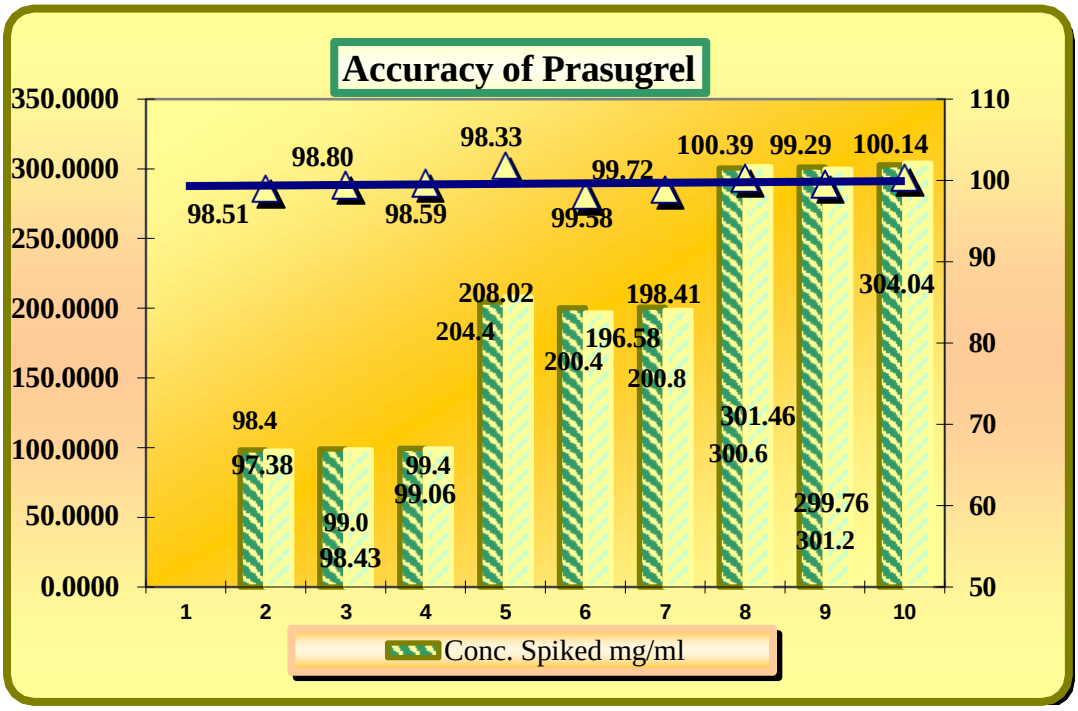
e) Accuracy

The closeness of the analytical method, known as accuracy, is commonly measured by determining the percent recovery through the assay of known amounts of standards or the test of interest. To assess accuracy, equal amounts of placebo matrix were weighed and transferred into separate 50 mL volumetric flasks. The flasks were then spiked with a stock solution of Prasugrel at 50, 100, and 150% of the target level. Each flask was treated according to the test solution preparation and injected twice. All levels of samples were prepared three times, resulting in a mean recovery ranging from 98.09 to 101.77% based on the analysis of 9 samples. Accuracy data at different recovery levels are provided in Table-6 and graphical representation in Figure - 4.

Tables -6: Results of Accuracy at different recovery levels

Accuracy Level (%)	Amount Added	Amount found	% Recovery
50-1	98.4	97.38	98.51
50-2	99.0	98.43	98.80
50-3	99.4	99.06	98.59
100- 1	204.4	208.02	98.33
100- 2	200.4	196.58	99.58
100- 3	200.8	198.41	99.72
150- 1	300.6	301.46	100.39
150- 2	301.2	299.76	99.29
150- 3	303.0	304.04	100.14
Confidence Interval (n=9)			**99.65 ± 1.06**

Figure -4: Accuracy graph for determination of Prasugrel



f) Robustness

The ability to withstand small intentional changes in method parameters and maintain reliability during regular use is known as robustness. To assess the robustness of the method, deliberate modifications were made to the flow rate (from 1.0 mL/minute to 0.9 mL/minute) and column oven temperature (from ambient to 30°C). The test results remained unaffected by these alterations as provided in Table-7.

Tables -7: Results of Robustness

Preparations	Precision data	Robustness - 1	Robustness - 2
1	99.6	99.9	100.2
2	99.9	99.2	99.2

3	100.4	99.7	100.0
4	100.0	100.0	99.6
5	99.3	100.0	99.1
6	99.9	100.2	99.3
Ave. (n=12)	--	99.8	99.6
%RSD	--	0.34	0.42

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

LOD and LOQ for the determination of Prasugrel by the proposed HPLC method were established by signal-to-noise ratio (S/N) obtained from serial dilution of standard solution and injection of blank solution. LOD, the detection limit is determined by the analysis of known concentrations of analyte and by establishing the minimum level at which the analyte can reliably detected. LOQ, the quantitation limit is determined by the analysis of known concentrations of analyte and by established the minimum level at which the analyte can be reliably quantified with acceptable accuracy and precision. The LOD of this method is found to be 5.52 ppm and LOQ is found approximately 16.72 ppm of assay concentration for Prasugrel.

Recovery at Limit of detection (LOQ) level

Prepared 16.72 ppm concentration solution in triplicate. In 3 separate volumetric flasks containing tablet powder matrix, known amounts Prasugrel stock solution was spiked to provide final concentrations of about 16.72 ppm Each solution was analyzed in triplicate, and the recovery was calculated. The data, as presented in Table 9, suggested that the method is accurate for Prasugrel at LOQ concentration levels. The recovery results at LOQ level concentrations are shown in Table - 8.

Table – 8: Accuracy at LOQ concentration level

Analyte name	Amount added (ppm)	Amount found (ppm)	% Recovery
Prasugrel	16.72	16.71	99.9
	16.70	16.68	99.9
	16.72	16.67	99.7

h) Solution stability

The stability of the sample and standard solutions during the analysis period is determined by measuring the percentage difference in peak areas of duplicate injections made 24 hours after the initial injection. The results, shown in Table-9, indicate that there were no significant differences in peak area responses after 24 hours, indicating that the solutions remained stable for this duration.

Tables -9: Stability of standard and sample solutions

Standard solution		Sample solution	
Initial	**After 24 hours**	**Initial**	**After 24 hours**
3671193	3672593	3698588	3699325
Difference in peak area = + 0.038 %		Difference in peak area = + 0.019 %	

Advantages and applications

The evaluation of the commercial formulation sample and bulk drug sample demonstrated that the technique employed is highly specific, selective, and proficient in quantitatively analyzing of Prasugrel in both the bulk drug and final dosage form. This method is characterized by its rapidity, as it can be completed in less than 10 minutes, making it suitable for routine quality control and stability analysis of Prasugrel.

CONCLUSION

The objective of the research was to create and verify a High-Performance Liquid Chromatography (HPLC) technique for measuring Prasugrel in its final dosage form. The validation process adhered to the guidelines set forth by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) and the United States Pharmacopeia (USP). The study examined various parameters, including specificity, linearity, accuracy, precision, Limit of detection, quantitation, and robustness. The optimized method was then put to the test through a forced degraded study of Prasugrel tablets under different stress conditions. The results demonstrated that the method was capable of accurately determining Prasugrel even under these challenging circumstances. The proposed method is considered adequate, feasible, and reliable, making it a valuable tool for routine analysis, quality control, and stability studies of pharmaceutical preparations containing Prasugrel in their final dosage form. Overall, this study provides a robust and validated HPLC method for the estimation of Prasugrel, which can be used in the pharmaceutical industry to ensure the quality and safety of Prasugrel-containing products.

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Conflict of interest

As scientists, we recognize the significance of transparency and objectivity in our scientific pursuits. Hence, we wish to state that we have no vested interests in this project. Our sole aim is to aid in the progress of pharmaceutical research and development, and we trust that our discoveries will prove advantageous to the scientific community.

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