# Analytical method development and validation of prasugrel in bulk and its pharmaceutical formulation using the RP-HPLC method

*Abstract*

**Purpose:** This study was designed to develop and validate a simple, sensitive, precise, and specific reverse phase high-performance liquid chromatographic (HPLC) method for the determination of prasugrel in bulk and its tablet dosage forms. **Materials and Methods:** The HPLC separation was carried out by reverse phase chromatography on an inertsil ODS-3V column (5 µm; 250 × 4.6mm$^2$) with a mobile phase composed of 0.02 M potassium dihydrogen orthophosphate, 0.02 M dipotassium hydrogen orthophosphate in water:acetonitrile (30:70 v/v) in isocratic mode at a flow rate of 1 ml/min. The detection was monitored at 210 nm. **Results:** The calibration curve for prasugrel was linear from 100 to 600 µg/ml. The inter-day and intra-day precision was found to be within limits. The proposed method has adequate sensitivity, reproducibility, and specificity for the determination of prasugrel in bulk and its tablet dosage forms. The limit of detection and limit of quantification for prasugrel were found to be 0.25 µg/ml and 0.75 µg /ml, respectively. Accuracy (recoveries: 99.8–101.2%) and reproducibility were found to be satisfactory. **Conclusion:** The proposed method is simple, fast, accurate, and precise for the simultaneous quantification of prasugrel in the dosage form, bulk drugs as well as for routine analysis in quality control.

**Key words:** Acetonitrile, prasugrel, RP-HPLC method, reverse phase chromatography, validation

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## INTRODUCTION

A number of acute coronary syndrome (ACS) trials have demonstrated a significant regional variation in clinical outcomes and treatment effects.[1-3] Dual antiplatelet therapy with aspirin and a thienopyridine is a cornerstone of treatment to prevent thrombotic complications of ACS and percutaneous coronary intervention (PCI).[4,5] In the trial to assess improvement in therapeutic outcomes by optimizing platelet inhibition with prasugrel-thrombolysis in myocardial infarction 38 (TRITON-TIMI 38), more intensive and consistent antiplatelet therapy with the third-generation thienopyridine prasugrel resulted in a reduction in ischemic events, increase in bleeding and, on balance, an improved net clinical outcome.[6] Prasugrel chemically is 5-[2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-4,5,6,7-tetra hydrothieno[3,2-c]pyridin-2-yl acetate. It is a member of the thienopyridine class of ADP receptor inhibitors, such as ticlopidine and clopidogrel. These agents reduce the aggregation ("clumping") of platelets by irreversibly binding to P2Y12 receptors. Prasugrel inhibits adenosine diphosphate-induced platelet aggregation more rapidly, more consistently, and to a greater extent than do standard and higher doses of clopidogrel in healthy volunteers and in patients with coronary artery disease. Literature survey revealed that only a few analytical methods such as liquid chromatography–mass spectrometric (LC-MS),[7,8] high-performance thin-layer chromatographic (HPTLC),[9] and one high-performance liquid chromatographic (HPLC)[10] method have been reported. Hence, a new sensitive and efficient HPLC method was developed and validated for the assay of the drug in tablets. The structure of prasugrel is shown in Figure 1.

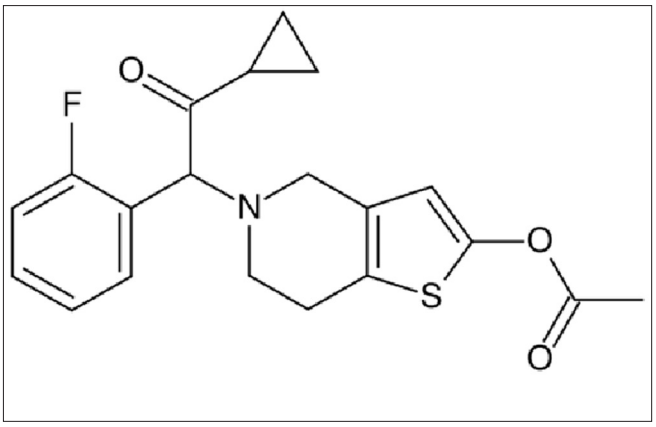


**Figure 1: Chemical structure of prasugrel**

## MATERIALS AND METHODS

### Materials

Prasugrel was provided as a gift sample by MSN Laboratories, Hyderabad, AP, India. Drug was used without any further purification. All other reagents required for experimentation were of analytical reagent (AR) grade. Chemicals used for this experiment: potassium dihydrogen orthophosphate and acetonitrile were purchased from Merck, Mumbai.

### Chromatographic conditions

The HPLC system (Shimadzu Co., Tokyo, Japan) consisted of a Shimadzu model LC-10 ATVp, a Shimadzu model SPD-6AV variable wavelength detector (possessing deuterium lamp with a sensitivity of 0.005 AUFs and adjusted to an absorbency of 210 nm), a Shimadzu model C-R5A chromatograph integrator module (chart speed at 10 mm/min), a Shimadzu model SIL-6A auto injector, and a Shimadzu module SCL-6A system controller.

Isocratic elution of the mobile phase 0.02 M potassium dihydrogen orthophosphate, 0.02 M dipotassium hydrogen orthophosphate in water:acetonitrile (30:70 v/v) with the flow rate of 1 ml/min. Separation was performed on an inertsil ODS-3V analytical column (Thermo Hypersil, 5 μm; 250 × 4.6mm$^2$ i.d. with C18 insert (100 Ao, waters limited) as pre-column to protect the analytical column from strongly bonded material. Integration of the detector output was performed using the Shimadzu Empower software to determine the peak area. The contents of the mobile phase were filtered through a 0.45-μm membrane filter and degassed by sonication before use. The flow rate of the mobile phase was optimized to 1 ml/min which yields a column back pressure of 110–112 kg/cm$^2$. The run time was set at 20 min and a column temperature was maintained at ambient. The volume of injection was 20 μl, prior to injection of the analyte, the column was equilibrated for 30–40 min with the mobile phase. The eluent was detected at 210 nm. The developed method was validated in terms of specificity, linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ), intra-day and inter-day precision and robustness for the assay of prasugrel as per ICH guidelines.[11]

### Diluent

Acetonitrile was used as a diluent.

### Standard preparation

Stock solution of prasugrel was prepared by dissolving 500 mg of prasugrel in a 100 ml volumetric flask, and the volume is made up with the diluents. Subsequent dilutions of this solution ranging from 0.05 to 500 μg/ml were made with the diluent.

### Sample preparation

Twenty tablets were taken, and their average weight was calculated. The tablets were crushed to a fine powder, dose equivalent to 10 mg was transferred to a 100 ml volumetric flask, dissolved in a diluent, and then the solution was made up to the mark with the same and filtered through 0.45 μm membrane filter. Five milliliter of this solution was pipetted into a 50 ml volumetric flask and diluted with the diluent to get a concentration of 500 μg/ml.

### Assay

A mass of not less than 10 tablets was prepared by grinding them to a fine, uniform particle size powder using a mortar and pestle. After calculating the average tablet weight, a composite equivalent to the 10 mg was accurately weighed and quantitatively transferred into a 100-ml volumetric flask. Approximately, 10-ml milli-Q water was added, the solution was sonicated for 10 min, 70 ml diluents was added to it, and mechanically shaken for 10 more minutes. The flask was equilibrated to room temperature, carefully filled to volume with the diluent, and mixed well. A portion of the solution was filtered through a 0.45 mm membrane filter, discarding the first 2–3 ml of the filtrate. A portion of the filtered sample (5.0 ml) was diluted into a 50 ml volumetric flask with the mobile phase and mixed well.

## RESULTS

Several systematic trials were performed to optimize the

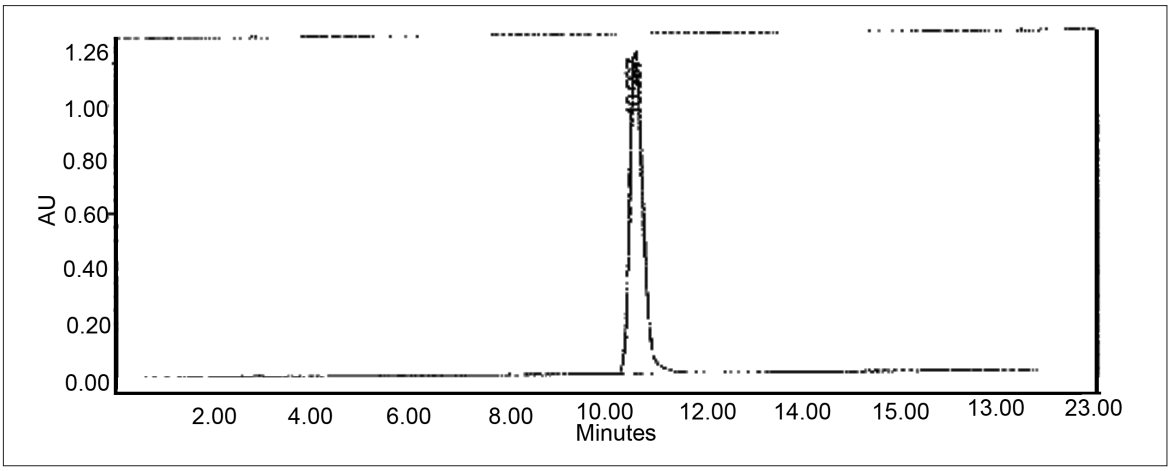


**Figure 2:** Typical chromatogram for prasurel

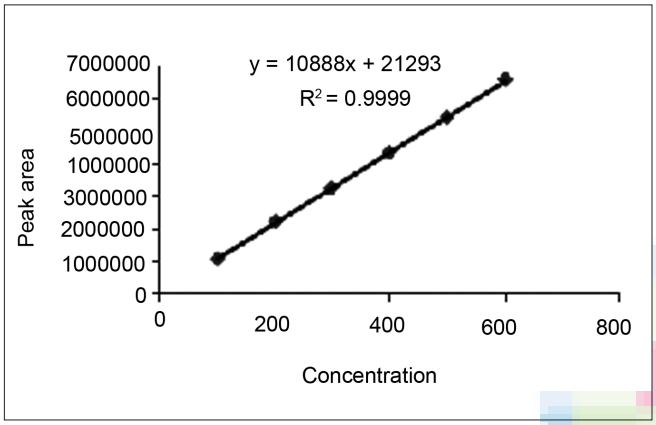


**Figure 3:** Linearity graph of prasugrel

chromatographic conditions for developing a sensitive, precise and accurate RP-HPLC method for the analysis of prasugrel in pharmaceutical dosage forms. This method contains the mobile phase 0.02 M potassium dihydrogen orthophosphate, 0.02 M dipotassium hydrogen orthophosphate in water:acetonitrile (30:70 v/v) which was found to be the most suitable as the chromatographic peaks obtained with this system were better defined and resolved and all almost free from tailing. Under the above conditions, the retention time obtained for prasugrel was 10.597 min. A model chromatogram was shown in Figure 2.

### System suitability

As per the USP 27 System, suitability tests were carried out on a freshly prepared standard solution of prasugrel to check the various parameters such as efficiency, retention time, and peak tailing which was found to comply with USP requirements. The instrumental precisions as determined by six successive injections of the standard solution give RSD below 2% of retention time and area.

**Table 1: Recovery study data**

| Amount of drug added (µg/ml) | Amount of drug found (µg/ml) | % Recovery |
|---|---|---|
| 400 | 380.32 | 95.08 |
| 500 | 428.75 | 85.75 |
| 600 | 622.50 | 103.75 |

### Linearity

The calibration curve for prasugrel was drawn by plotting the mean peak area *versus* concentration yielded a coefficient of regression $r^2$ = 0.9999 over a concentration range (100–600 µg/ml), the representative linear regression equation for prasugrel $Y = 10888X + 21293$ as shown in Figure 3.

### Accuracy

The accuracy of the proposed analytical method was determined by recovery experiments. The recovery studies were carried out at three different concentration levels in triplicate (80, 100, and 120%). The analyzed samples yielded high recovery values from the developed method. The % recovery results of the method are given in Table 1.

### Precision

The precision of the method for the determination of prasugrel was studied using the parameters such as system precision, method precision, and intermediate precision. System precision was determined by six replicate injections of a standard solution injected into the HPLC system. The relative standard deviation was less than 2%. The method precision was determined by the six individual sample preparations injected to the HPLC system. The relative standard deviation was less than 2%. Ruggedness of the method was determined by different analysts, different columns, and different instruments on different days. RSD was found below

2%. The results indicating that the developed HPLC method was found to be precise.

### Robustness

The robustness of the method was studied by small changes in the method such as altering the mobile phase pH, flow rate, and changes in wavelength. It was observed that there were no changes in the chromatograms. System suitability and chromatographic parameters were validated such as asymmetry factor and tailing factor, and a number of theoretical plates were calculated. The results are given in Table 2.

### Limit of detection and limit of quantification

The LOD and LOQ of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (a signal-to-noise ratio of 3). The LOD for prasugrel was found to be 0.25 µg/ml. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (a signal-to-noise ratio of 10). The LOQ of prasugrel was found to be 0.75 µg/ml. It was concluded that the developed method is sensitive.

### Assay

Twenty microliters of standard and sample solutions were injected into an injector of RP-HPLC, peak area of standard amount of drug and the sample were computed. The values are given in Table 3.

### Calculations

% of prasugrel in tablet formulation

$$(\text{Pasugen}) = \frac{\text{At} \times \text{Ws} \times \text{Avg. Wt} \times \text{x}}{\text{As} \times \text{Wt} \times \text{Claimed wt}}$$

where At is the average area due to the Pasugen formulation peak in sample preparation, As is the average area due to the prasugrel peak in STD preparation, Ws is the weight of the working standard (Prasugrel), Wt is the weight of sample (Pasugen formulation), and *P* is the potency of the working standard.

## DISCUSSION

The UV spectrum of prasugrel was recorded, from which 210 nm was selected as wavelength. The flow rate of 1.0 ml/min was selected. 0.02 M potassium dihydrogen orthophosphate and 0.02 M dipotassium hydrogen orthophosphate in water:acetonitrile (30:70 v/v) were selected as the mobile phase. The retention time was found to be 10.597. Prasugrel shown linearity in the range of 100–600 µg/ml, and the co-efficient was found to be 0.999. Recovery studies were performed at 80%, 100%, and 120% levels. The sensitivity of methods LOD and LOQ was found to be 0.25 µg/ml and 0.75 µg /ml, respectively. The stability at room temperature and refrigeration was found to be 3 and 8.5 h, respectively.

From the obtained results, it can be concluded that the proposed method is quite precise and accurate. The low standard deviation and good percentage recovery indicates the reproducibility and accuracy of the method. The absence of additional peaks in the chromatogram indicated that there is no interference of the common excipients used in the tablets. The proposed HPLC method is sensitive and reproducible for the analysis of prasugrel in tablet dosage forms. The method was duly validated by using required statistical parameters. The optimized chromatographic conditions were summarised in Table 4.

**Table 2: System suitability studies**

| Parameters | Results |
|---|---|
| Tailing factor | 1.36 |
| Theoretical plates | 8974.24 |
| Retention time | 10.597 |

**Table 3: Analysis of formulation**

| Amount of drug (mg/tab) | | % Label claimed | % RSD |
|---|---|---|---|
| Labelled | Estimated | | |
| 10 mg | 9.865 | 98.65 | 0.05 |

**Table 4: Optimized chromatographic conditions**

| Parameters | Optimized conditions |
|---|---|
| Chromatograph | HPLC (Shimadzu with SPD-6AV detector) |
| Column | Inertsil ODS-3V (5 µm; 250 × 4.6 mm$^2$) |
| Mobile phase | 0.02 M potassium dihydrogen orthophosphate, 0.02 M dipotassium hydrogen orthophosphate in water:acetonitrile (30:70 v/v) |
| Flow rate | 1 ml/min |
| Detection wave length | 210 nm |
| Injection volume | 20 µl |
| Column temperature | Ambient |
| Rt | 10.597 min |
| Run time | 20 min |

## CONCLUSION

A convenient and rapid RP-HPLC method has been developed for estimation of prasugrel in the tablet dosage form. The assay provides a linear response across a wide range of concentrations. Low intra-day and inter-day % RSD coupled with excellent recoveries. The proposed method is simple, fast, accurate and precise for the simultaneous quantification of prasugrel in dosage form, bulk drugs as well as for routine analysis in quality control.

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