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Research Article

# ESTIMATION OF PRASUGREL IN TABLET DOSAGE FORM BY RP-HPLC

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**ABSTRACT**

A simple, precise, rapid and accurate Reverse Phase HPLC method was developed for the estimation of Prasugrel in tablet dosage form. An Xterra RP C18, 250x4.6 mm, column with 5 µm particle size and the mobile phase consisting of 0.03M $K_2HPO_4$ in water pH: 3.2 adjusted with Acetonitrile (25:75). Acetonitrile in the isocratic mode was used. The flow rate was 1.0 ml/min and the effluents were monitored at 210 nm. The retention time was 4.762 min. The detector response was linear in the concentration of 50-600 mcg/mL for Prasugrel. The respective linear regression equation being Y (158660.8513) = 10312.358x + 237007.9858 for Prasugrel. The Limit of Detection (LOD) and The Limit of Quantification (LOQ) were 2.5 mcg and 7.5 mcg respectively for Prasugrel. The percentage assay of Prasugrel was 99.93 %. The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Prasugrel in bulk drug and in its pharmaceutical dosage forms.

**Keywords:** Prasugrel, RP-HPLC, Estimation, Tablets.

## INTRODUCTION

Prasugrel, chemically is (RS)-5-[2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-4, 5, 6, 7-tetrahydrothieno [3, 2-c] pyridin-2-yl acetate (**Figure 1**).The Empirical formula is $C_{20}H_{20}FNO_3S$ and the Molecular weight is 373.442 g/mol. It is a member of the thienopyridine class of ADP receptor inhibitors. These agents reduce the aggregation ("clumping") of platelets by irreversibly binding to $P2Y_{12}$ receptors. Prasugrel inhibits adenosine diphosphate–induced platelet aggregation more rapidly, more consistently, and to a greater extent [1] than do standard and higher doses of Clopidogrel in healthy volunteers and in patients with coronary artery disease, including those undergoing Precutaneous Coronary Intervention (PCI) [2-3]. Prasugrel is a novel platelet inhibitor prodrug and is rapidly metabolized to a pharmacologically active metabolite and inactive metabolites [4]. Literature survey reveals a few chromatographic methods to determine the Prasugrel in tablet dosage form and in biological fluids. So far, no assay methods by liquid chromatography were reported for the estimation of Prasugrel in pharmaceutical dosage forms. The availability of an HPLC method with high sensitivity and selectivity will be very useful for the determination of Prasugrel in pharmaceutical formulations. The aim of the study was to develop a simple, precise and accurate reverse-phase HPLC method for the estimation of Prasugrel in bulk drug samples and in pharmaceutical dosage form.

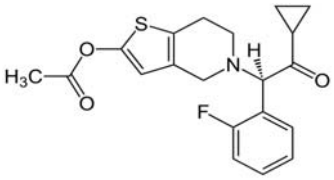


**Fig. 1: Prasugrel**

## MATERIALS AND METHODS

Prasugrel was obtained as a gift sample from Glenmark Pharmaceuticals Limited, Mumbai. Acetonitrile and water used were of HPLC grade (Qualigens). Commercially available tablets Aplet® 10 mg, Glenmark Pharmaceutical Limited, were procured from local market.

### Instrument

Quantitative HPLC was performed on Liquid Chromatograph, Waters separation 2996, PDA detector module equipped with automatic injector with injection volume 5 µl, and 2693 pump. An Xterra RP-C18 column (250x4.6 mm i.d; particle size 5 µm) was used. The HPLC system was equipped with Empower Software.

### HPLC conditions

The contents of the mobile phase were 0.03M $K_2HPO_4$ in water pH: 3.2 adjusted with Acetonitrile (25:75). Acetonitrile in the isocratic mode has been used. They were filtered before use, through a 0.45 µm membrane filter, and pumped from the respective solvent reservoirs to the column at a flow rate of 1.0 ml/min. The run time was set at 30.0 min and the column temperature was ambient. Prior to the injection of the drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The eluents were monitored at 210 nm.

### Preparation of standard stock solution

A standard stock solution of the drug was prepared by dissolving 500 mg of Prasugrel in 100 ml volumetric flask containing 30 ml of diluents (Acetonitrile: HPLC Grade Water 50:50 v/v), sonicated for about 15 min and then made up to 100 ml with Acetonitrile to get standard stock solution of 5 mg/mL of Prasugrel.

### Working standard solution

5 ml of the above stock solution was taken in 50 ml volumetric flask and made up to 50 ml with diluent (Acetonitrile HPLC grade) to get a concentration of 500 µg/ml of Prasugrel.

### Preparation of sample solution

Twenty tablets (Aplet® 10mg, Glenmark, Mumbai) were weighed, and then powdered. A sample of the powdered tablet, equivalent to mixture containing concentration of 500 mg/ml of Prasugrel active ingredient, was mixed with 30 ml of Acetonitrile:HPLC Grade Water-50:50v/v as diluent in 50 ml volumetric flask. The mixture was allowed to stand for 1 hr with intermittent sonication to ensure complete solubility of the drug, and then filtered through a 0.45 µm membrane filter, followed by adding methanol up to100 ml to obtain a stock solution each of 5.0mg/ml of Prasugrel. 5ml of the above sample stock solution was taken in 50 ml volumetric flask and made up to 50 ml with diluent to get a concentration of each 500 µg/ml of Prasugrel.

### Linearity

Aliquot of standard Prasugrel stock solution was taken in a different 10 ml volumetric flask and diluted up to the mark with the mobile

phase such that the final concentration of Prasugrel was in the range of 50-600 µg/ml. Each of the drug solution (5 µL) was injected three times into the column, and the peak area and retention time were recorded. Evaluation was performed with PDA detector at 210 nm and a calibration graph was obtained by plotting peak area versus concentration of Prasugrel **(Figure 2)**.The plot of peak areas of sample against respective concentration of Prasugrel was found to be linear in the range of 50-600 µg/ml with correlation coefficient of 0.999. Linear regression least square fit data obtained from the measurements are given in **Table I**. The respective linear regression equation being Y (158660.8513) = 510312.358x + 237007.9858 for Prasugrel. The regression characteristics, such as slope, intercept, and %RSD was calculated for this method and given in **Table I.**

**Assay**

5 µl of sample solution (Aplet® Tablets 10 mg, Glenmark) was injected into the injector of liquid chromatograph. The retention times was found to be 4.762 min for Prasugrel. The amount of drug present per tablet was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in **Table II**.

**Recovery studies**

Accuracy was determined by recovery studies of Prasugrel. A known amount of standard was added to the preanalysed sample and subjected to the proposed HPLC analysis. Results of recovery study are shown in **Table II**. The study was done at three different concentration levels.

**RESULTS AND DISCUSSION**

The system suitability tests were carried out on freshly prepared standard stock solutions of Prasugrel. Parameters that were studied to evaluate the suitability of the system are given in **Table III.**

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

The Limit of Detection (LOD) and The Limit of Quantification (LOQ) were 2.5 mcg and 7.5 mcg respectively for Prasugrel. From the typical chromatogram of Prasugrel as shown in **Figure 2**, it was found that the retention time was found to be 4.762 min. A mixture of 0.03M $K_2HPO_4$ in water at pH: 3.2 adjusted with Acetonitrile in the isocratic mode was found to be the most suitable as mobile phase to obtain the peaks well defined and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extractions were involved. A good linear relationship (r=0.999) was observed between the concentration range of 50-600 µg/ml for Prasugrel. Low values of standard deviation are indicative of the high precision of the method. The assay of Prasugrel tablets was found to be 99.93 %. From the recovery studies it was found that about 98.00% of Prasugrel was recovered which indicates high accuracy of the method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of bulk and tablet dosage forms of Prasugrel within a short analysis time.

**ACKNOWLEDGEMENT**

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**Table I: Linear regression data of calibration curve**

| Parameter | Prasugrel |
|---|---|
| Concentration range (µg/ml) | 50-600 |
| Slope (m) | 10312.358 |
| Intercept (b) | 158660.8513 |
| Correlation coefficient | 0.999 |
| % RSD | 0.48 |

**Table II: Assay & recovery of prasugrel in tablet dosage form**

| Tablet Formulation | Amount Claim (mg/tablet) | Amount Obtained (mg)* by proposed method | ** % Recovery by the Proposed method |
|---|---|---|---|
| 1 | 10 | 9.3 | 97.21 |
| 2 | 10 | 9.6 | 98.78 |
| 3 | 10 | 9.4 | 98.03 |

* Average of three determinations ; ** After spiking the sample

**Table III: Validation summary: system suitability**

| Parameter | Prasugrel |
|---|---|
| Theoretical Plates (N) | 8070.87 |
| Tailing factor | 1.07 |
| Retention time (Minutes) | 4.762 |
| Resolution | 4.59 |
| % Peak Area | 99.94 |
| LOD (µg/ml) | 2.5 |
| LOQ (µg/ml) | 7.5 |

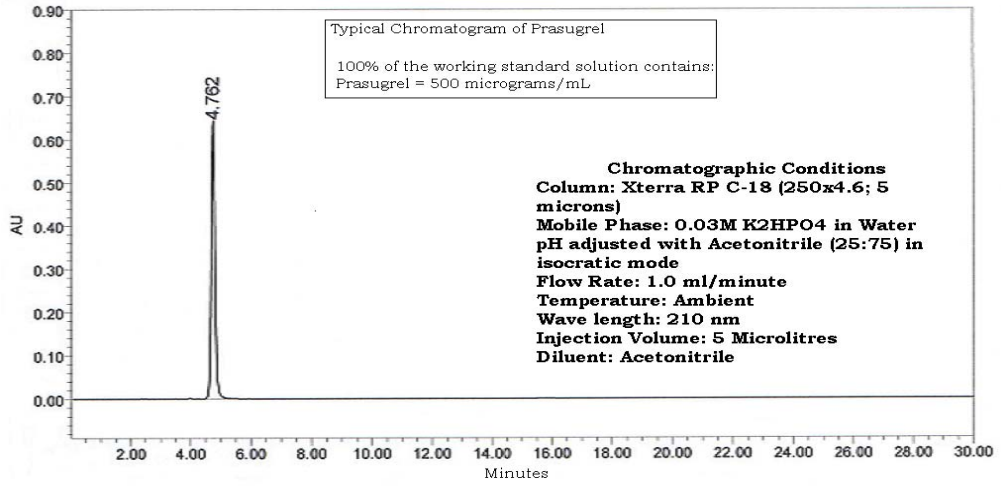


**Fig. 2: Typical chromatogram of Prasugrel by RP-HPLC**

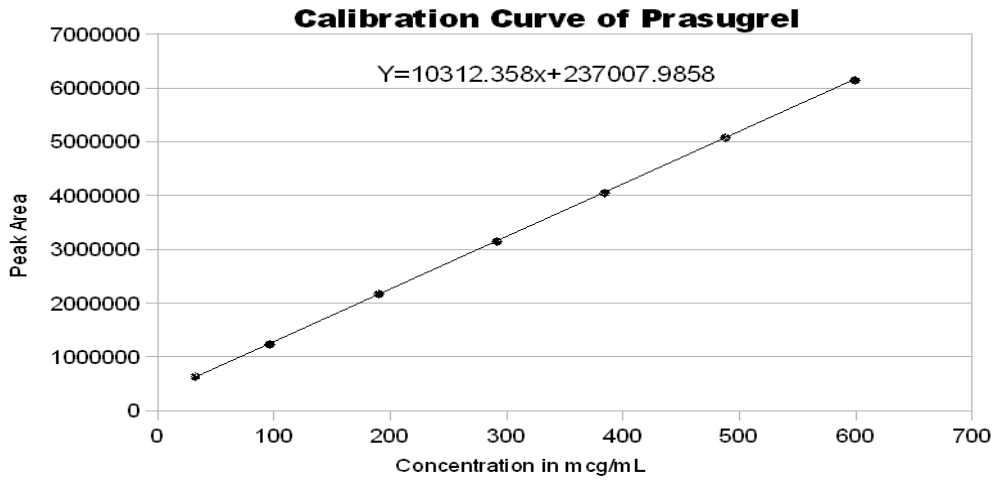


**Fig. 3: Calibration Curve of Prasugrel by RP-HPLC**

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