

## UV-SPECTROPHOTOMETRIC AND RP-HPLC METHODS FOR THE ESTIMATION OF PRASUGREL HYDROCHLORIDE IN BULK AND TABLET FORMULATION

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### ABSTRACT

**Objective:** Prasugrel hydrochloride is used as an anti-platelet drug for the treatment of myocardial infarction prevention, Thrombosis prevention after percutaneous coronary intervention, Platelet-aggregation inhibitors etc. The aim of the present study is to develop a new analytical method for the estimation of Prasugrel hydrochloride in bulk and in tablet dosage form.

**Methods:** Spectroscopic method-1 and RP-HPLC method-2 have been developed for the quantification of Prasugrel hydrochloride in bulk and in the formulation. These methods are simple, cost effective, accurate and precise.

**Results:** In method-1, Prasugrel hydrochloride showed maximum absorbance at 252 nm in methanol which is selected as solvent for analysis based on its stability. Beer's law obeyed in the concentration range of 10-60 mcg/ml. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 1.0195 and 3.0896 mcg/ml respectively. In RP-HPLC (method-2), the mobile phase selected is Acetonitrile: Trifluoroacetic acid in the ratio 45:55% v/v. The flow rate was 0.1 ml/min. The linearity range was found to be 10-60 mcg/ml. The formulation EPIPLT was selected for analysis and the amount present was found to be 100.53% and 106.95% for method 1 and 2 respectively.

**Conclusion:** Both the methods are validated as per ICH guidelines. The methods were found to be simple, accurate, precise and rapid.

**Keywords:** Prasugrel hydrochloride, Myocardial infarction prevention, UV-Spectroscopy, RP-HPLC.

### INTRODUCTION

Prasugrel Hydrochloride is chemically 5-[2-cyclopropyl-1-(2-Fluoro Phenyl)-2-Oxoethyl]-4,5,6,7-tetrahydrothieno [3,2-c] Pyridine -2-yl acetate[1]. Its molecular formula  $C_{20}H_{21}FNO_3S$  and possessing a molecular weight 409.9026 g/mol. It is soluble in methanol, ethanol, acetonitrile, chloroform and insoluble water. Prasugrel hydrochloride is used as an anti-platelet drug for the treatment of Myocardial infarction prevention, Thrombosis prevention after percutaneous coronary intervention, Platelet-aggregation inhibitors etc [2]. The structural formula of Prasugrel Hydrochloride is given below Figure 1.

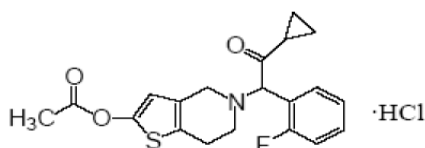


Fig. 1: Chemical Structure Prasugrel Hydrochloride

Analytical method is required to characterize drug substances and drug products composition during all phases of pharmaceutical development in the recent era. Standard analytical procedure for newer drugs or formulation may not be available in pharmacopeia hence, it is essential to develop newer analytical methods which are accurate, precise and specific, linear, simple, and rapid [3].

From the extensive literature survey, it was revealed that there were a very few methods reported for the estimation of Prasugrel hydrochloride from plasma and for pharmaceuticals dosage forms. Therefore, here an attempt was made to develop simple, cost, effective, accurate, precise, sensitive and specific methods for the analysis.

### MATERIALS AND METHODS

#### Reagents and chemicals

Water (HPLC grade), Hydrochloric acid (HPLC grade), Methanol (HPLC grade) Acetonitrile (HPLC grade), Trifluoroacetic acid (AR grade) were purchased from Qualigens India Pvt. Limited and Loba

Chemical (India) Limited. Prasugrel was obtained as a sourenit sample from Alkem india (p) Ltd., Mumbai.

Formulation brand Efilat (Sun pharma India pvt. ltd) containing Prasugrel hydrochloride equivalent to 10 mg was purchased from a local pharmacy.

#### Instrumentation [4]

Shimadzu AUX-220 digital balance, Shimadzu -1700 double beam UV - Visible spectrophotometer consist of halogen lamp and deuterium lamp as light source with silicon photo diode detector, Shimadzu HPLC system consists of deuterium arc lamp as light source equipped with UV detector and C18 column (150 mm×4.6 mm id, 5  $\mu$ ) was used for the analysis. Sonicator-Sonica Ultrasonic cleaner-model 2200MH, Micropipette.

#### HPLC condition

0.1 M acetic acid was selected as a solvent for proposed UV spectroscopic method, the absorbance was measured at 252 nm. It was operated at a temperature of 15 to 35°C. Reverse phase C18 column was employed as stationary phase with a mobile phase consisting of a mixture of Acetonitrile: Trifluoro acetic acid (0.1 ml in 100 ml of water) in the ratio of 45: 55 % v/v and the flow rate of the mobile phase was maintained at 1 ml/min. the column temperature was maintained at ambient. The volume of injection was 20  $\mu$ l, and the eluent was detected at 252 nm.

#### Method development

##### UV spectroscopic method [5]

##### Preparation of standard stock solution

25 mg of Prasugrel Hydrochloride working standard was accurately weighed and transferred into a 50 ml of volumetric flask and dissolved in methanol and made up to 50 ml with methanol. The solution was observed to contain 500  $\mu$ g/ ml.

##### Preparation of working standard solution

1 ml of standard stock solution was pipetted into a 50 ml standard flask and the volume was made up to the mark with distilled water and obtained a concentration of 10  $\mu$ g/ ml.

### Quantification in formulation [6]

Weighed twenty tablets of formulation (EFLIPAT) containing 10 mg of Prasugrel and the average weight was found and powdered. Powdered tablet equivalent to 50 mg of Prasugrel Hydrochloride was transferred into 50 ml volumetric flask, added 10 ml of methanol and sonicated for 15 mins, then shaken vigorously for few min and produced to 50 ml with methanol and filtered through whatmann's filter Paper No:41. From the filtrate, pipetted out 4 ml (40 µg/ ml) and transferred in to 50 ml flask in same direction for six times.

The absorbance was measured at 252 nm and the amount of Prasugrel Hydrochloride present in each formulation was calculated with the respective calibration curve. The peak areas obtained are presented in Figure 2.

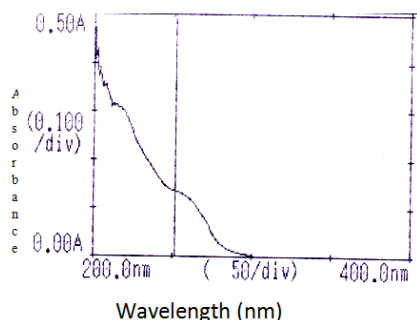


Fig. 2: UV spectrum of prasugrel hydrochloride in Methanol / water at 252 nm

### RP-HPLC Method

#### Preparation of standard Prasugrel Hydrochloride solution

An accurately weighed quantity of 10 mg of Prasugrel Hydrochloride was dissolved in Acetonitrile then made up to the mark 10 ml (1000 µg/ ml) and further diluted 1ml of solution and brought to 10 ml with more mobile phase (10 µg/ ml).

#### Estimation of prasugrel hydrochloride solution

Weighed accurately 20 tablets of formulation (EFLIPAT) contain 10 mg and the average weight of tablet was found and powdered. The powdered tablet equivalent to 25 gm of Prasugrel Hydrochloride was weighed and extracted with acetonitrile and sonicated the solution for 15 mins and shaken well for few minutes and made up to the volume 25 ml. Then filtered through wattman filter paper No: 41 and from the clear solution, further dilution was made up with mobile phase.

### Assay

3 ml of test solution (30 µg/ ml) was taken in to 25 ml volumetric flask and made up to mark with mobile phase with optimized chromatographic conditions. As stated earlier, a steady base line was recorded. After the stabilization of the base line for 30 minutes, test solution of formulation was injected, after filtering through membrane filter and recorded the chromatogram at 252 nm as detection wave length, the concentration of each test solution was determined by using slope and intercept values. Replicate analysis was carried out for setting repeatability value.

### Method validation [7]

The developed method was validated according to ICH and USP guidelines for the validation of analytical procedures.

### System suitability

The system suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatography system. The parameters like tailing factor, asymmetric factor, the number of theoretical plates, capacity factor were calculated.

### Validation of Prasugrel Hydrochloride

#### Method validation of Prasugrel Hydrochloride by UV spectroscopic method [8]

#### Linearity and calibration

To series of six 50 ml volumetric flask, aliquots of (1 to 6 ml) were taken from the above stock solution and made up to the mark using distilled water. The absorbance was measured at 252 nm against distilled water as a blank. The calibration curve was plotted in the concentration range 10-60 µg/ ml using absorbance versus concentration.

#### Recovery studies

To the preanalyzed sample with excipients, a known quantity of standard solution was added. The contents were mixed, finally made up to the volume with methanol and further made up required distilled water. Absorbance was measured at 252 nm and the amount present was calculated by using the slope and intercept. The percentage recovery was determined by using the following formula.

$$\% \text{ RECOVERY} = \frac{(\sum X)(\sum Y) \times 100}{N \sum X^2 - (\sum X)^2}$$

Where,

N = No of observation

X = Amount added in micro gram

Y = Amount recovered in µg/ml.

#### Repeatability

Repeatability is performed by inter day and intra day precision analysis. The assay and recovery procedures were repeated for three times on the same day and one time on three successive days.

#### Limit of detection (lod) and limit of quantification (loq)

Preparation of calibration curve from the serial dilutions of the standard was repeated for six times, the limit of detection and limit of quantification was calculated by using the average values of slope and standard deviation of intercept.

#### Method validation of Prasugrel Hydrochloride by RP-HPLC Method

#### Preparation of calibration graph [9]

In this method, the aliquots of stock solution of Prasugrel Hydrochloride (1-6 ml) are transferred in to 25 ml volumetric flask and made up to the mark with mobile phase. A solution containing 10, 20, 30, 40, 50, and 60 µg/ ml of Prasugrel Hydrochloride in mobile phase was injected and the chromatograms were recorded at 252 nm. It was found that the above concentration range was linear. The procedure was repeated for three times. The peak was plotted against concentration and the calibration curve was constructed and the peak area obtained 30 µg/ ml of Prasugrel Hydrochloride are presented in Figure 3.

|   |                              |           |         |            |
|---|------------------------------|-----------|---------|------------|
| Sample Name:                                  | Data File: ...-11\FR 815.DAT |           |         |            |
| Method File: PR.MET                           |                              |           |         |            |
| Detector: UV-VIS.                             | System: HPLC                 |           |         |            |
| Date: 13 Nov 2010                             | Time: 12:9:50                |           |         |            |
| Run: ch1: 16                                  |                              |           |         |            |
| Type of Analysis : Percent On Area and Height |                              |           |         |            |
| Report printed on : 15/12/2010 at : 11:34:59  |                              |           |         |            |
| Pk.Width                                      | Peak Thrsh.                  | Area Rej. | Ht.Rej. | Time Scale |
| 4   | 30                           | 5         | 4       | 5.0        |

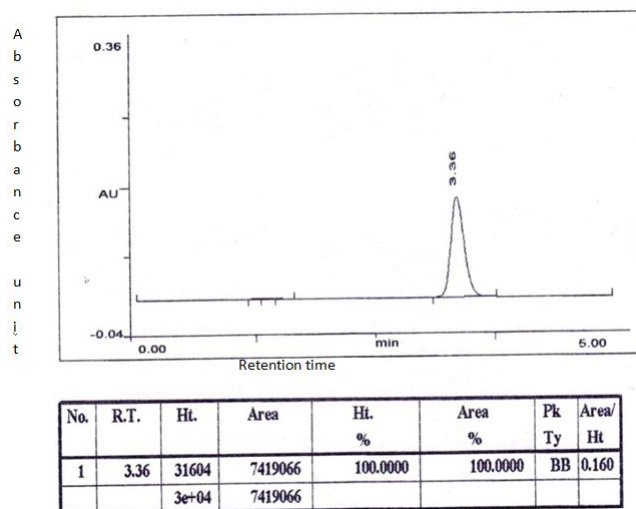


Fig. 3: Linearity Chromatogram of Prasugrel Hydrochloride (30 µg ml<sup>-1</sup>)

### Recovery Studies

To ensure the reliability of the method, recovery studies were carried out by mixing a known quantity of standard drug solution with the pre-analysed sample formulation and the content were mixed and made to the volume with mobile phase and re-analyzed by the proposed method, the percentage recovery was calculated.

### Limit of detection (lod) and limit of quantification (loq)

Calibration of standard was repeated for three times. The limit of detection (lod) and limit of quantification (loq) was calculated by using the average value of the slope and standard deviation of intercept. System stability parameters such as asymmetric factors, capacity factors and tailing factors were validated and the results are given in Table 1.

Table 1: System suitability parameters for the optimized chromatogram by RP-HPLC Method

| Parameters        | Prasugrel hydrochloride |
|-------------------|-------------------------|
| Tailing factor    | 1.30                    |
| Asymmetric factor | 1.53                    |
| Capacity factor   | 1.4                     |

### RESULTS

Two methods were developed for the estimation of Prasugrel Hydrochloride in pure form and in its tablet dosage forms.

#### UV Spectrophotometric method [9]

The solubility of Prasugrel Hydrochloride was determined as per Indian pharmacopeia. The number of polar and non-polar Solvents tried were distilled water, 0.1M hydrochloric acid, 0.1 N sodium hydroxide, 0.05 M hydrochloric acid, acetate buffer, methanol, ethanol, acetonitrile, benzene, acetone, n-hexane, glacial acetic acid, carbon tetra chloride, toluene, ethyl acetate. From the solubility studies, the drug is very soluble in methanol, ethanol, acetonitrile. It is sparingly soluble in dimethyl formamide, dichloromethane and it is practically insoluble in distilled water, acetone, benzene, diethyl ether toluene, pet ether, carbon tetrachloride, isopropyl alcohol. From the solubility data methanol was selected as a solvent for UV method because of its solubility, stability, and easy availability.

Prasugrel Hydrochloride was dissolved in methanol and made further dilution within the Distilled water to get the concentration of 10 µg /ml. The spectrum of Prasugrel Hydrochloride was found and the wavelength maximum was found to be 252 nm. It was observed that Prasugrel Hydrochloride was stable for 1 hr and 45 mins.

Different aliquots of Prasugrel Hydrochloride in methanol were prepared in the concentration range of 10 to 60 µg /ml. The

absorbance's of solutions were measured at 252 nm and the calibration curve was plotted using concentration against absorbance. The procedure was repeated for six times. The optical characteristics like correlation coefficient, slope, intercept, sandell's sensitivity, LOD and LOQ were calculated. These are shown in Table 2.

Table 2: Optical Characteristics of Prasugrel Hydrochloride by UV Spectroscopic Method

| S. No. | Parameters   | Method                     |
|--------|--|----------------------------|
| 1.     | λ max (nm)   | 252                        |
| 2.     | Beers law limit (µg/ ml)                                 | 10-60                      |
| 3.     | Correlation coefficient (r)                              | 0.9996                     |
| 4.     | Regression equation (y=mx+c)                             | y=0.014596786x+0.004710714 |
| 5.     | Slope (m)  | 0.014596786                |
| 6.     | Intercept (c)  | 0.004710714                |
| 7.     | LOD (µg/ ml)   | 1.0195                     |
| 8.     | LOQ (µg/ ml)   | 3.0896                     |
| 9.     | Sandell's sensitivity (µg/cm <sup>2</sup> / 0.001 A. U.) | 0.068508233                |
| 10.    | Standard Error of Mean                                   | 0.008900108                |

The correlation coefficient value for the calibration graph was found to be 0.9996; it indicates that the concentration of prasugrel Hydrochloride has good linearity. The calibration graph is shown in Figure 4.

The amount of test solution was calculated by using slope and intercept. This procedure was repeated for six times to ensure the precision of the method. The percentage label claim of Prasugrel Hydrochloride in the tablet formulation, (EPIPLAT) was found to be 99.82 % and the Percentage Relative Standard Deviation (% R.S.D.) value was found to be 0.7053. The low % R.S.D. value indicates that the method has good precision. The results of analysis are shown in Table 3.

Result of recovery studies of Prasugrel Hydrochloride are tabulated in Table 4.

The precision of the method was further confirmed by Intraday and Interday analysis Table 5.

#### Reverse Phase High Performance Liquid Chromatographic Method

Various mobile phase were prepared and trials were made to determine appropriate chromatographic condition. The mobile phase finalized for HPLC analysis was Acetonitrile : Trifluoroacetic acid (0.1 ml in 100 ml water) in the ratio of 45:55 % v/ v and the chromatogram was developed. The scanning of the development showed a good peak shape.

The linearity of RP-HPLC method development of Prasugrel Hydrochloride was evaluated by injecting the standard drug solutions of different concentration ranging from 10 to 60 µg/ml and the peak areas obtained are presented in Figure 5.

The assay determined the contents of Prasugrel Hydrochloride in formulation, EFIPLAT was found to be 98.76%. The assay results present in the Table 6.

The accuracy and precision was determined for Prasugrel Hydrochloride by fortifying sample with standard drug substance at a concentration ranging 2 to 12 µg/ml and over all recovery

determined. The contents of EFIPLAT 106.95%. The result are presented in the Table 7.

The proposed RP-HPLC method for estimation of Prasugrel Hydrochloride in dosage form is simple, accurate and rapid. The statistical parameters in the method validation studies are precise, accurate with no interference, which was proved by recovery studies.

The various optical parameters, regression equation, slope, intercept, LOD, LOQ, sandell's sensitivity, standard error were calculated and the result are presented in Table 8.

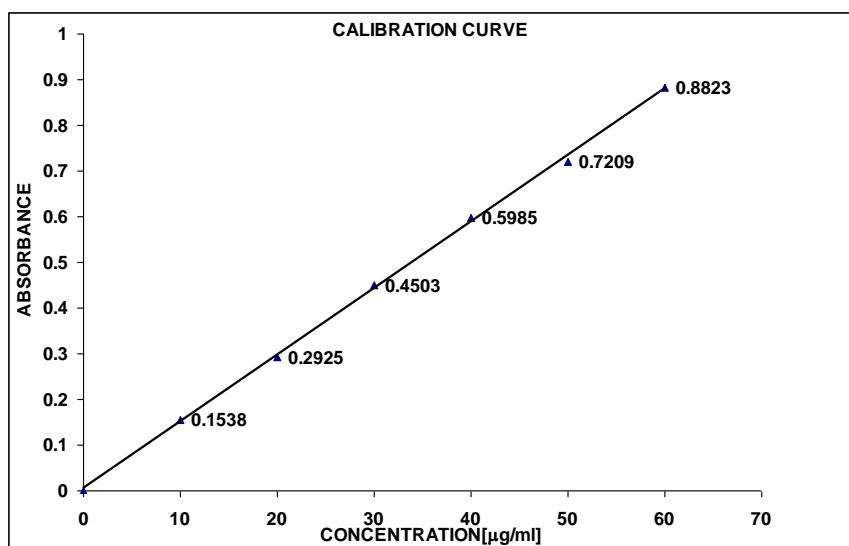


Fig. 4: Calibration curve of prasugrel hydrochloride by uv spectroscopy using methanol / water at 252 nm

Table 3: Quantification of formulation (EFIPLAT) By UV method

| S. No. | Labeled Amount (mg/tab) | Amount Found (mg/tab) | Percentage Obtained (%) | Percentage Found | S.D. (+/-) | % R.S.D. | S.E.    |
|--------|-------------------------|-----------------------|-------------------------|------------------|------------|----------|---------|
| 1.     | 10                      | 11.05                 | 100.66                  | 99.82%           | 0.7093     | 0.7053   | 0.02155 |
| 2.     | 10                      | 10.90                 | 99.29                   |                  |            |          |         |
| 3.     | 10                      | 10.94                 | 99.66                   |                  |            |          |         |
| 4.     | 10                      | 10.99                 | 100.11                  |                  |            |          |         |
| 5.     | 10                      | 11.04                 | 100.57                  |                  |            |          |         |
| 6.     | 10                      | 10.83                 | 98.66                   |                  |            |          |         |

Table 4: Recovery Studies for Formulation (EFIPLAT) by UV Spectroscopic Method

| S. No. | Amount Present (µg/ml) | Amount Added (µg/ml) | Amount Estimated (µg/ml) | Amount Recovered (µg/ml) | Percentage Recovered (%) | Average Percentage Recovery |
|--------|------------------------|----------------------|--------------------------|--------------------------|--------------------------|-----------------------------|
| 1.     | 40.22                  | 2                    | 42.17                    | 1.95                     | 97.50                    | 100.53%                     |
| 2.     | 40.22                  | 4                    | 44.44                    | 4.22                     | 105.50                   |                             |
| 3.     | 40.22                  | 6                    | 46.57                    | 6.35                     | 105.80                   |                             |
| 4.     | 40.22                  | 8                    | 47.45                    | 7.23                     | 96.30                    |                             |
| 5.     | 40.22                  | 10                   | 49.52                    | 9.8                      | 98.00                    |                             |
| 6.     | 40.22                  | 12                   | 52.22                    | 12                       | 100.00                   |                             |

Table 5: Interday and Intraday Analysis of Formulation (EFIPLAT) by UV Spectroscopic Method

| S. No. | Labeled Amount (mg/tab) | Percentage Obtained |           | S.D. (+/-) |           | % R.S.D   |           |
|--------|-------------------------|---------------------|-----------|------------|-----------|-----------|-----------|
|        |                         | Intra day           | Inter day | Intra day  | Inter day | Intra day | Inter day |
| 1.     | 10                      | 100.66              | 100.94    |            |           |           |           |
| 2.     | 10                      | 101.13              | 100.53    | 0.84477    | 0.64478   | 0.65447   | 0.82244   |
| 3.     | 10                      | 100.96              | 100.90    |            |           |           |           |
|        | MEAN                    | 100.91              | 100.79    |            |           |           |           |

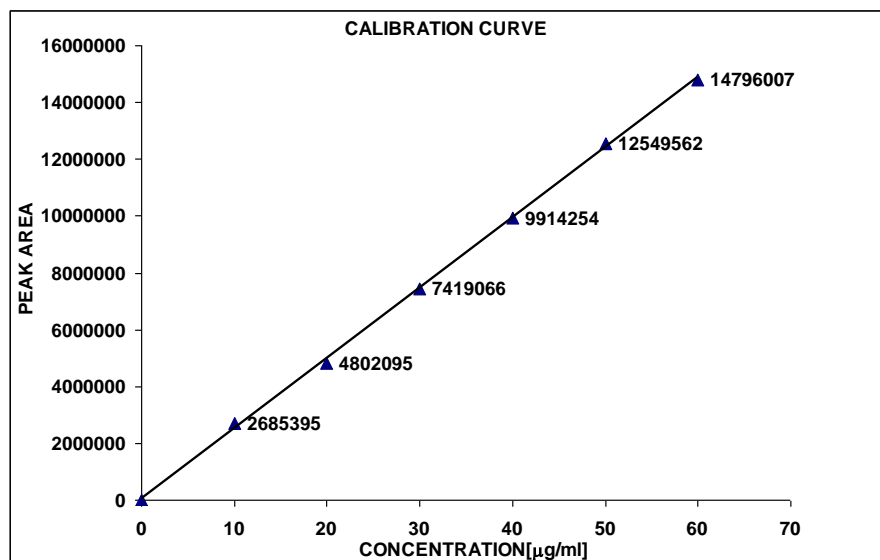


Fig. 5: Calibration curve of prasugrel hydrochloride at 252 nm by RP-HPLC method

Table 6: Quantification of formulation (EFIPLAT) by RP-HPLC method

| S. No. | Labeled Amount (mg/tab) | Amount Found (mg/tab) | Percentage Obtained (%) | Percentage Found | S.D. (+/-) | % R.S.D. | S.E.     |
|--------|-------------------------|-----------------------|-------------------------|------------------|------------|----------|----------|
| 1.     | 10                      | 10.93                 | 99.57                   | 98.76%           | 0.816441   | 0.820558 | 0.022679 |
| 2.     | 10                      | 10.73                 | 97.75                   |                  |            |          |          |
| 3.     | 10                      | 10.92                 | 99.48                   |                  |            |          |          |
| 4.     | 10                      | 10.89                 | 99.20                   |                  |            |          |          |
| 5.     | 10                      | 10.83                 | 98.66                   |                  |            |          |          |
| 6.     | 10                      | 10.75                 | 97.93                   |                  |            |          |          |

Table 7: Recovery Studies for Formulation (EFIPLAT) by RP-HPLC Method

| S. No. | Amount Present (µg/ml) | Amount Added (µg/ml) | Amount Estimated (µg/ml) | Amount Recovered (µg/ml) | Percentage Recovered (%) | Average Percentage Recovery |
|--------|------------------------|----------------------|--------------------------|--------------------------|--------------------------|-----------------------------|
| 1.     | 29.73                  | 2                    | 31.83                    | 2.13                     | 106.50                   | 106.95%                     |
| 2.     | 29.73                  | 4                    | 33.95                    | 4.22                     | 105.50                   |                             |
| 3.     | 29.73                  | 6                    | 36.18                    | 6.45                     | 106.30                   |                             |
| 4.     | 29.73                  | 8                    | 38.14                    | 8.41                     | 105.12                   |                             |
| 5.     | 29.73                  | 10                   | 39.92                    | 10.91                    | 109.10                   |                             |
| 6.     | 29.73                  | 12                   | 41.84                    | 13.11                    | 109.25                   |                             |

Table 8: Optical Characteristics of Prasugrel Hydrochloride by RP-HPLC Method

| S. No. | Parameters   | Method                    |
|--------|--|---------------------------|
| 1.     | λ max (nm)   | 252                       |
| 2.     | Beers law limit (µg/ml)                                  | 10-60                     |
| 3.     | Correlation coefficient (r)                              | 0.9997                    |
| 4.     | Regression equation (y=mx+c)                             | y=247244.6929 x+34999.071 |
| 5.     | Slope (m)  | 247244.6929               |
| 6.     | Intercept (c)  | 34999.07143               |
| 7.     | LOD (µg/ml)  | 3.311158601               |
| 8.     | LOQ (µg/ml)  | 10.03381394               |
| 9.     | Sandell's sensitivity (µg/cm <sup>2</sup> / 0.001 A. U.) | 4.04458                   |
| 10.    | Standard Error of Mean                                   | 137370.0742               |

## CONCLUSION

In recent years, HPLC has emerged as one of the most sophisticated tools for analysis. The estimation of Prasugrel Hydrochloride is done by using UV spectroscopy and reverse phase HPLC. In UV spectroscopy, Prasugrel Hydrochloride showed maximum absorbance at 252 nm in methanol, the percentage label claim of value indicates that the method has good precision. In RP-HPLC, the

employed mobile phase consists of a mixture of Acetonitrile: Trifluoro acetic acid in the ratio of 45: 55%V/V. The detection is carried out using UV detector at 252nm at a flow rate of 1 ml/min, retention time for Prasugrel Hydrochloride was found to be 3.36 min, linearly range for Prasugrel Hydrochloride was found to be 10 – 60 µg/ml.

The quantitative estimation was carried out in the tablet by using both the methods, taking a concentration of 10 µg/ml. Then the quantitative

results obtained were subjected to statistical validation. The value of %RSD are less than 2.0% indicating the accuracy and precision of the developed method. The Percentage recovery of Prasugrel Hydrochloride is 100.53% and the amount present in the formulation (EFIPLAT) was found to be 106.95% in RP-HPLC. The LOD was found to be 3.3111. The LOQ was found to be 10.0338. The stability parameters such as tailing factor, asymmetry factor, capacity factor and resolution were in acceptable limit. The results obtained on the validation parameters met the requirements as per ICH and USP guidelines. The developed methods were found to be simple, precise, specific, linear and proportional i.e it obeys Beer-Lamberts law. Both the methods were found to have a suitable application in routine laboratory analysis with a high degree of accuracy and precision.

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