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STABILITY INDICATING RP-UPLC METHOD FOR THE ESTIMATION OF PRASUGREL HCL IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A simple, rapid and stability indicating RP-UPLC method was developed for the estimation of Prasugrel HCl in pharmaceutical dosage forms. The separation of Prasugrel HCl was achieved by using mobile phase it is a mixture of 0.2% Triethyl amine buffer (pH 2.5 ± 0.05 adjusted with Ortho phosphoric acid), Acetonitrile, Methanol(50:25:25%v/v), with isocratic mode of 0.4 ml/min. The detection of the method at 210nm, Column temperature of 30°C, injection volume of 3.0µl and runtime of 5 minutes are the chromatographic parameters. The method was statistically validated for precision, Linearity, Ruggedness, Robustness & Specificity. Method was showed a linear response in the concentration range of 50-150µg/ml. Quantitative and recovery studies of the dosage form were also carried out and analyzed, the % RSD from recovery studies was found to be less than 1. Due to simplicity, rapidity and accuracy of the method, we believe that the method will be useful for routine quality control analysis.

Keywords: Prasugrel HCl, RP-UPLC

INTRODUCTION

Prasugrel HCl is a novel and potent thienopyridine that targets the P2Y12 ADP receptors and there by inhibiting ADP mediated platelet activation and aggregation **[1]**. A number of acute coronary syndrome (ACS) trials have demonstrated significant regional variation in clinical outcomes and treatment effects. Dual antiplatelet therapy with aspirin and a thieno pyridine is a cornerstone of treatment to prevent throbotic complications of ACS and percutaneous coronary intervention (PCI). 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.

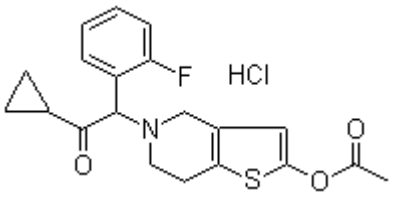

Figure 1: Molecular structure of Prasugrel HCl

Chemically Prasugrel HCl is designated as : 5-[2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-4,5,6,7- tetrahydrothieno[3,2-c]pyridin-2-yl acetate Hydrochloride is shown fig.1. It is a Platelet aggregation inhibitor used in the treatment of acute coronary syndrome[2]. Literature survey reveals HPLC [**3, 4, 5, 6, 7, 8]**, UV [**9,10]** methods, LC-MS [**11, 12],** HPTLC [**13]** and NMR [**14]** methods are available for the estimation of Prasugrel HCl, But there is no Reversed phase Ultra performance Liquid chromatographic method available for estimation of Prasugrel HCl. Taking this point into consideration, an attempt was made to develop a simple ,rapid , accurate and stability indicating RP-UPLC method for the estimation of Prasugrel HCl in pharmaceutical formulations with short run time and by less consumption of solvents.

MATERIALS AND METHODS

Instruments Used

All analytical works performed on Waters Aquity UPLC Quarternary gradient pump with PDA detector, Empower 2 software,Aquity UPLC-BEH C-18 column (100 X 2.1 mm, 1.7 µm particlesize) as stationary phase, a calibrated electronic single pan balance Mettler toledo, a pH meter of Thermo electron corporation and ultra sonicater Bandelin sonorex , also used during the analysis.

Reagents and Chemicals

Analytically pure Prasugrel HCl of known potency was provided by Dr.reddys lab. Tablet of prasugrel 10mg was purchased from the local market. Triethylamine, Acetonitrile, Methanol & Ortho phosphoric acid are of AR grade were used during analysis.

Mobile Phase Preparation

The mobile phase was prepared by mixing 500 ml of 0.2% Triethyl amine (pH adjusted to 2.5. ± 0.01 with Orthophosphoric acid) buffer with Acetonitrile and Methanol in the ratio of (50:25:25 %v/v). The mobile phase was sonicated for 20 min and then it was filtered through a mdi- 0.2µ membrane filter paper.

Standard Preparation

An accurately weighed quantity of 100 mg was transferred to 100 ml volumetric flask, which was then dissolved and made upto volume with diluents [90:10-Acetonitrile:water] to give 1000µg/mL.then pipetted out 10ml to another 100ml volumetric flask and made up to the volume with diluent then filtered with Randisc PVDF 0.22µm filter.

Chromatographic Conditions

RP-UPLC analysis was performed by isocratic elution with flow rate of 0.4 mL/min. The mobile phase containing 0.2% Triethyl amine buffer (pH 2.5 ± 0.05 adjusted with Ortho phosphoric acid), Acetonitrile and Methanol (50:25:25 % v/v) , a flow rate of 0.4 mL/min, Column temperature 30°C and detection of the method of 210nm and runtime of the method is 5 min. By using these chromatographic parameters Prasugrel HCl is eluted of about 2.0 minute. Chromatogram of standard and sample are shown in **Figure 2 & 3**.

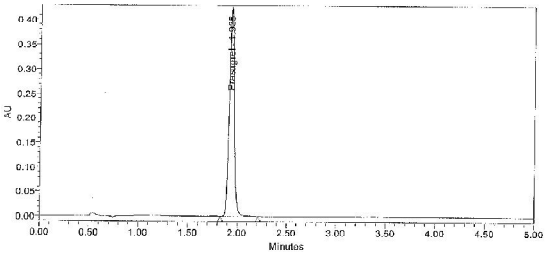

Figure 2: Chromatogram of Standard Prasugrel HCl

Analysis of the Marketed Formulations

Ten tablets were weighed accurately and crushed to form fine powder. Accurately weighed quantity of powder equivalent to about 10 mg of Prasugrel in to a 100 ml of volumetric flask and added 30ml of diluent. The flask was sonicated for 20 minutes and then made up to the mark with diluent then the solution was filtered using PVDF 0.22µm.

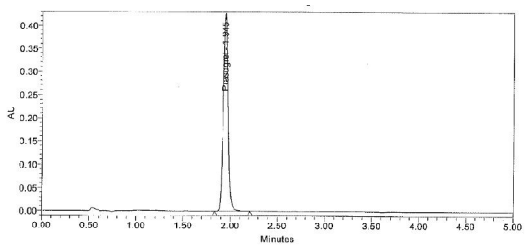

Figure 3: Chromatogram of Sample Prasugrel HCl

Validation of the method

The developed method was validated in terms of method precision, intermediate precision, linearity, accuracy, Solution stability, Mobile phase stability, Robustness and Specificity.

RESULTS AND DISCUSSIONS

System Suitability

Injected five injections of standard solution concentration of 100µg/ml. The RSD for the prasugrel response from five standard injections was found to be 0.19 %.

Table 1: System Suitability Parameters

% of RSD	0.19%
Theoretical plates	7685.2
Tailing factor	1.16

Method Precision

Prepared six sample preparations by taking sample equivalent to 10mg of Prasugrel in to each six 100ml volumetric flasks, Then added 30ml of diluent to each flask and sonicated for 20minutes. Then made up to the mark with diluent. Then samples are filtered with PVDF 0.22µm filter, and injected into UPLC as per the above mentioned chromatographic conditions. The relative standard deviation for the prasugrel peak area was found to be <1%. The results are summarized in **Table 2**.

Intermediate Precision

In the similar way Intermediate precision were assessed by the assay of six sample preparations by another analyst on different day on different column. The result of assay method found to be within 100±2.0%. The results are summarized in **Table 2**.

Linearity

Prepared standard solutions in the concentration range of 50-150µg/ml and injected into UPLC as per test method. Plotted linearity graph of Concentration Vs Area for Prasugrel were plotted, which are linear graphs. The correlation coefficient, Slope and Intercept were found to be 0.99965, 14927.75 and 14055.67 respectively. The linearity plot of Prasugrel is shown in **Figure 4**.

Acuuracy / Recovery

The recovery study of prasugrel was carried out at three different levels, corresponding to 50,100 and 150% of the nominal analytical concentration of 100µg/ml, with triplicate

preparations at each level. Mean recovery of Prasugrel of each level was found > 99%.The results obtained are listed in Table No. 3.

Solution Stability

The stability of standard and sample solutions of prasugrel was assessed by the assay of the corresponding sample solutions immediately after their preparation and then after 2 days with the fresh standard .The difference in percentage of assay of prasugrel in the sample with freshly prepared standard was found to be less than 1% compared to standard 2days before. This indicate that standard and sample are stable for 2 days at room temperature.

Mobile Phase Stability

The stability of the mobile phase was checked for 2 days, the difference in the assay of prasugrel for two sample preparations was found to be less 1% from intial day results by using same mobile phase. So it was found that mobile phase is stable for 2 days. The results obtained are listed in **Table 4**.

Robustness

The robustness of the method was done by altering conditions injecting five injections of standard with such as change in flowrate, temperature, PH and results were found to be satisfactory and are listed in the Table No. 5.

Specificity

This study was carried out in terms of different force degradation studies. Samples were stressed with different conditions and injected into UPLC. With different stress conditions percentage of assay and percentage of degradation are listed in this below **Table 6**.

Table 2: Results of Method Precision and Intermediate Precision Parameters

	Analyst-1	Analyst-2
	% of Assay	% of Assay
Sample Preparation_1	97.7	97.4
Sample Preparation_2	97.3	97.7
Sample Preparation_3	97.3	97.9
Sample Preparation_4	97.0	97.6
Sample Preparation_5	98.1	98.0
Sample Preparation_6	97.8	97.9
Mean % of assay	97.5	97.7
SD	0.37	0.22
% of RSD	0.38	0.22

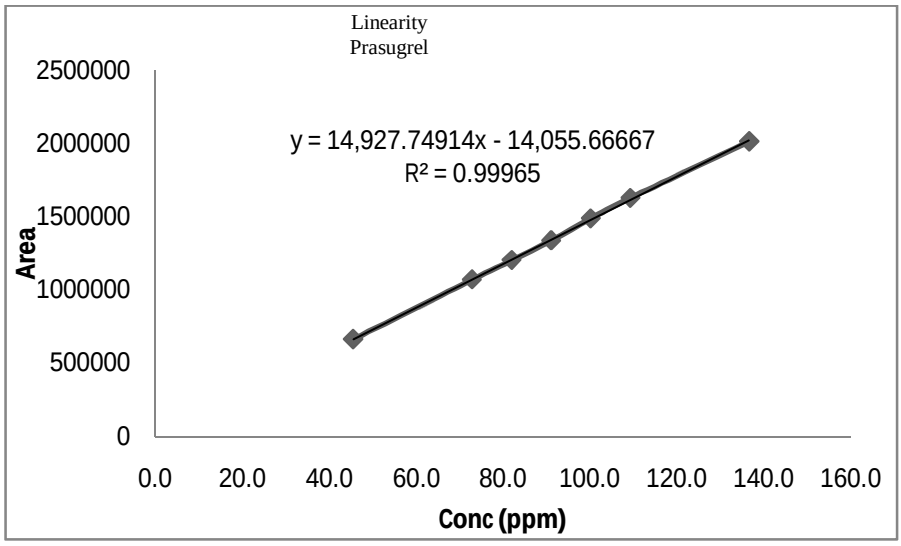

Figure 4: Calibration Curve of Prasugrel HCl

Table 3: Results of Accuracy Study

Accuracy Level	% Assay	Mean % of Recovery	Mean % of RSD
50%-1	49.5	101.34	0.28
50%-2	49.4		
50%-3	49.2		
100%-1	98.6	100.72	0.63
100%-2	98.5		
100%-3	97.5		
150%-1	145.8	99.55	0.07
150%-2	145.6		
150%-3	145.5		

Table 4: Mobile Phase Stability Results

Sample ID	% of Assay	Mean	Mean % Assay Initial	% Difference
Sample-1 after 48hrs	97.3	98.0	97.8	-0.20
Sample-2 after 48hrs	98.6			

Table 5: Results of Robustness

ROBUSTNESS				
Component	**Parameter**	**Tailing factor (NMT 2.0)**	**Theoretical plates NLT(3500)**	**% RSD (NMT 2.0%)**
Prasugrel	**Flow rate[0.45ml/min]**	**1.17**	**7410.1**	**0.15**
	Flow rate[0.35ml/min]	**1.15**	**7997.5**	**0.23**
	column Temperature[35°C]	**1.13**	**8301.2**	**0.11**
	column Temperature[25°C]	**1.18**	**6942.8**	**0.23**
	PH[2.7]	**0.96**	**9877.7**	**0.15**
	PH[2.3]	**1.14**	**3099.1**	**0.22**

Table 6: Degradation Results

Condition	Time	% of Assay	% of Degradants
UV light at-254nm	**7 days**	**99.28**	**0.72**
Humidity-90%RH-25°C	**10days**	**99.58**	**0.42**
Sun light	**55 hrs**	**99.60**	**0.40**
Thermal-105°c	**6hrs**	**98.69**	**1.31**
30%H2O2_60°C	**3hrs**	**95.27**	**4.73**
5N HCl_60°C	**3hrs**	**73.38**	**26.62**
0.02N NaOH at RT	**10 sec**	**84.01**	**15.99**

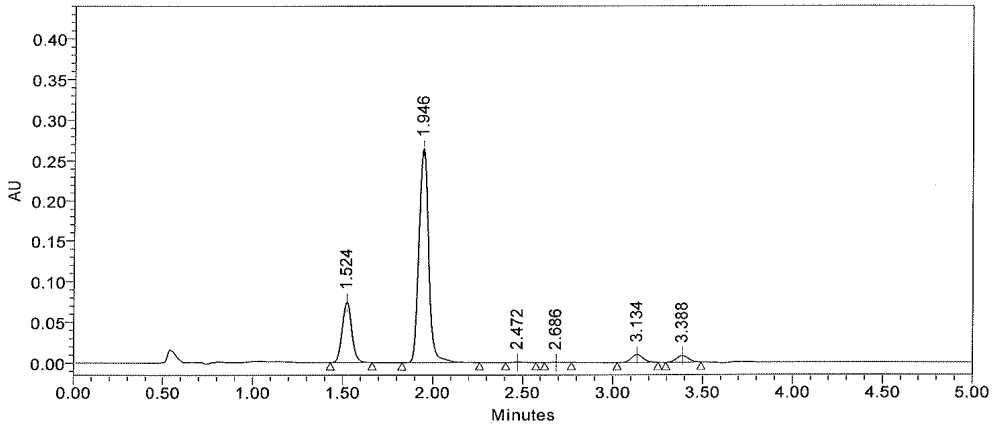

Figure 5: Chromatogram of Acid degradation

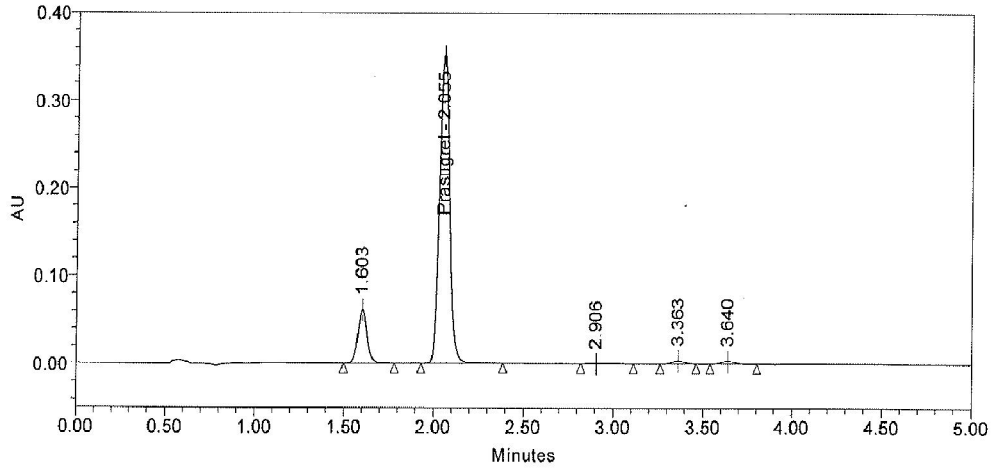

Figure 6: Chromatogram of Base Gradation

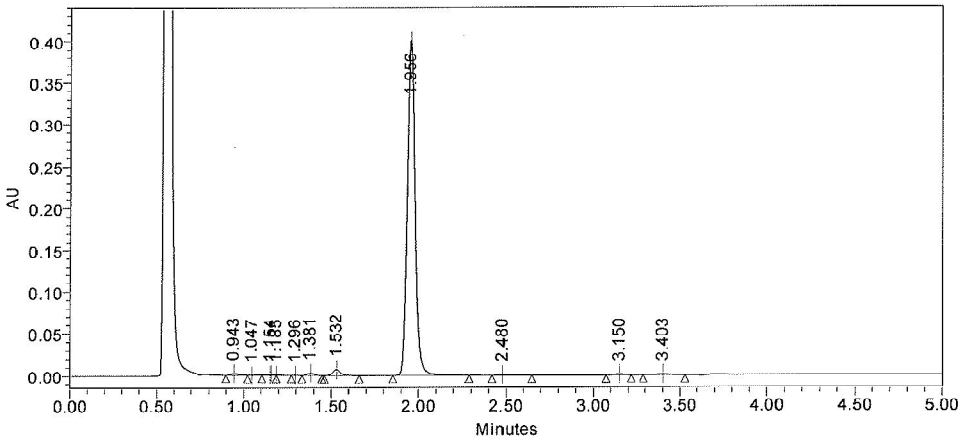

Figure 7: Chromatogram of H_2O_2 Degradation

CONCLUSIONS

Prasugrel HCl is a novel platelet Inhibitor drug used for the treatment of acute coronary syndromes planned for percutaneous coronary invention. An at-tempt was made to develop a simple and accurate RP-HPLC method to deter-mine Prasugrel HCl in the presence of its products.

The mobile phase containing 0.2% Triethyl amine buffer (pH 2.5 ± 0.05 adjusted with Ortho phosphoric acid), acetonitrile and Methanol (50:25:25 % v/v) , UPLC-BEH C-18 column (100 X 2.1 mm, 1.7 µm particlesize) as stationary phase at a flow rate of 0.4 mL/min, Column temperature 30°C and detection of the method with PDA at 210nm and runtime of the method is 5 min. By using these chromatographic parameters

Prasugrel HCl is eluted of about 2.0 min. The run time has set at 5 min. for the UPLC system and was found to be the best for the analysis.

The prepared method is a simple, rapid and stability indicating. Method is showing linear response over the concentration range of 50 to 150µg/ml. The method is showing accuracy of >99.0% .Method is capable of detecting Prasugrel in the presence of degradation peaks, showing that method is stability indicating. Method is validated for Method precision, Intermediate precision, solution stability &mobile phase stability for 2 days. Due to simplicity and rapidity, method can be used for routine quality control analysis in pharmaceutical industry.

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