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Method Development and Validation Of Prasugrel Tablets By RP- HPLC

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Abstract:

Prasugrel hydrochloride is a platelet inhibitor and anti-thrombosis drug[1]. Mortality rate due to thrombosis has been rising and so the need to develop methods for the analysis of the drug. The scope of the study was development and validation of Prasugrel Hydrochloride 5mg tablet by RP-HPLC for precision, accuracy linearity and robustness. A RP-HPLC method was developed and validated for the estimation of Prasugrel Hydrochloride in tablet dosage form. A Zorbax, SB-Phenyl 250×4.6mm, 5µ in isocratic mode, with mobile phase containing buffer (pH 6.5 with Triethylamine and potassium dihydrogen ortho phosphate) Acetonitrile: Water (90:10) [40-60 (v/v)] was used. The flow rate was 1.2 ml/min and the analyte was monitored at 235 nm. The retention time for Prasugrel HCL was 11.5 minutes. The method was validated for system suitability, specificity, precision, accuracy, linearity, ruggedness and robustness. Linearity was obtained in the concentration range of 50ppm to 150ppm, with correlation coefficient of 0.999. The percentage recovery of Prasugrel was found to be in the range of 98%-102%. The method was robust and rugged as observed from insignificant variation in the results of analysis by changes in the flow rate and temperature separately and analysis being performed by different analyst, on different systems and by using different columns respectively. There was no interference due to excipients and mobile phase, the method was found was to be specific. The system suitability parameters were within limit, hence it was concluded that the system was suitable to perform the assay.

Key words: Prasugrel Hydrochloride, Method development validation and RP-HPLC.

INTRODUCTION

Pharmaceutical analysis plays a vital role in the quality assurance and quality assurance of drugs. Qualitative analysis reveals the chemical identity of the sample, whilst quantitative analysis establishes the relative amount of the species. High Performance Liquid Chromatography is one of the most common methods of analysis for pharmaceuticals[2]. HPLC is a type of liquid chromatography that employs a liquid mobile phase and a very finely divided stationary phase. The HPLC method is a method of choice for analytical chemistry because it can yield the required results in a specific, robust, linear, precise and accurate way. Other advantages to this method are that it is a very quick method (speed), greater resolution, improved resolution, and re-usable columns, ideal for substances of low viscosity, easy sample recovery and maintenance. In normal phase chromatography, the stationary phase is a polar adsorbent, whilst the mobile phase is a mixture of the non-aqueous solvents[3].

In new method development a good strategy should be employed as many experimental runs are required in order to achieve the desired results. Important factors to consider in order obtaining reliable analysis results are careful sample preparation and careful sample preparation, appropriate column choice and appropriate operating conditions[4]. Also the performance of the recording and data handling systems should be reliable. Most importantly the developed method should be validated. Validation parameters for High performance liquid chromatography include system suitability, accuracy, specificity, linearity, precision, limit of quantitation, limit of detection and robustness. According to the USP, system suitability tests are done to verify that the resolution and reproducibility of the system that is to be analysed are adequate it is generally hinged on the fact that the analytical operations, the equipment, electronics and samples are part of an integral system which can be evaluated as a whole[5]. ICH defines selectivity as a method which provides responses for many or a number of chemical entities which may not, or may be distinguished from each other. Specificity on the other hand refers to that method which produces a response for only one analyte. Accuracy refers to a measure of how close the values of the experiment are to the true value. Sensitivity is also very important and it defines the ability to detect very small variations in the concentration of the sample analyte. Precision measures the closeness of data values to each other under the same experimental conditions. Reproducibility then defines precision between laboratories. A linear relationship should exist between samples and assays. Limit of detection defines the lowest concentration of an analyte that can be detected in a sample, whilst the limit of quantitation defines that concentration that can be quantified under the given set of conditions. Finally robustness defines that capability to remain unaffected by small deliberate variations in the parameters of the method. These parameters are very important in the development of an analytical procedure[6].

No method has been documented therefore for the analysis of Prasugrel tablets using the Reverse HPLC method. Prasugrel Hydrochloride is an antithrombotic drug, mainly used to treat acute coronary syndrome in those patients that are undergoing percutaneous coronary intervention, angina, atherosclerosis and other few conditions.

MATERIALS AND METHODS

Acetonitrile, Potassium dihydrogen ortho phosphate, Triethyl amine and purified water were used are of HPLC Grade. Prasugrel Hydrochloride Marketed Formulation-

Prax 05 was procured from (MSN LABS). The instrument used is weighing balance (Sartorius), pH metre (Polmon), Sonicator (fast clean), HPLC (Agilent Technologies 1200 Series).

Selection of Suitable mobile phase, diluent and wave length:

The mobile phase solution A is prepared by dissolving 1.36 g of Potassium Dihydrogen orthophosphate in 100ml of water and mix. Adjusted pH to 6.5 with triethylamine. Filtered and degassed the solution. The Solution B: Degassed mixture of acetonitrile and water in the ratio 90:10. The wavelength is 235nm and the mobile phase ratios for Solution A: Solution B is (40:60)

The Chromatographic conditions:

The experiment is carried out using column (Inertsil C18, 5 m, 150 mm x 4.6 mm) to Zorbax SB-Phenyl 250 x 4.6mm, 5µ at Flow rate of 1.0ml/min using detector wave length of 235nm, column temperature used is ambient, injection volume is 10µl, run time of 25 minutes.

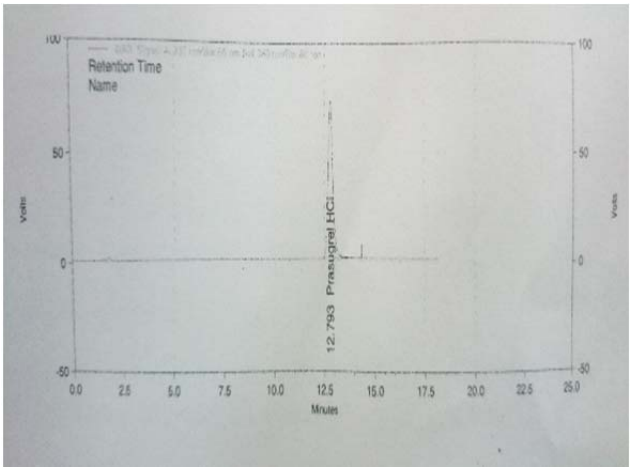


Fig 1: Retention Time has increased.

RESULTS & DISCUSSIONS

The developed method was within limits. The results have also shown that the excipients and the mobile phase did not affect the validation parameters. This therefore showed that there was no interference due to these parameters and therefore it can be concluded that the method was specific. System suitability was also proven as every value was within the recommended limits

VALIDATION PARAMETRES

SYSTEM SUITABILITY: To verify the analytical system is working the system suitability parameters are to be set.

Standard preparation 1: weighed 11.24 mg of Prasugrel HCL WS into 100ml volumetric flask, added 60 ml of diluents, to dissolved for about 10 minutes, further made up to volume with the diluents.

Standard preparation 2: Weighed 11.32 mg of Prasugrel Hydrochloride into 100ml volumetric flask, added 60ml diluent and further diluted with the diluent up to 100ml of the volumetric flask. The results were tabulated in table 1 & 2.

Table 1: Data of System Suitability parameter

Injection	RT	Peak Area	USP Plate Count	USP tailing
1	11.647	2147703	7945	1.217
2	11.640	2145329	7965	1.202
3	11.560	2148786	8011	1.207
4	11.645	2146980	8022	1.200
5	11.640	2149104	7900	1.208
Mean	11.6264	2147580	7968	1.207
SD	0.037246	1518.2		
%RSD	58.132	1357.87		

Table 2: System Suitability

System Suitability	Observed value	Acceptance criteria
Theoretical Plate for Prasugrel Std 1	9562	Should be NLT 2500
Tailing factor for Prasugrel Std 1	1.20	Should be NMT 2.0
RSD for peak area for Prasugrel for five injection of Std 1	0.07	Should be NMT 2.0%
Similarity factor between two Prasugrel Std preparation	1.001	0.985 to 1.015

SPECIFICITY

Standard preparation : weighed 11.76g of Prasugrel HCL into 100ml volumetric flask, add 60ml of diluent, to dissolve for 10 minutes. Further made up the volume up to 100 ml with diluent.**Fig-2**

Impurity stock preparation: 2.53 mg of Desacetyl impurity was weighed into a 25 ml volumetric flask. 15 ml of the diluent was added, dissolved for 10 minutes and made up to volume with the diluent. **Fig-3**

Impurity spiked preparation: transferred 1ml of the standard stock solution and 1ml of impurity stock solution into 20 ml volumetric flask made up to the volume with the diluent. **Fig-4**

Placebo preparation: weighed 174.61 mg of the Prasugrel HCL 10 mg Placebo into 100ml volumetric flask, added 60ml of diluent, to dissolve for about 10 minutes and further make up to volume with the diluent. 0.45μm and the filtrate were used after the discarding of the first 5ml. **Fig-5**

LINEARITY

Standard stock preparation: 10 mg of Prasugrel HCL was weighed into 50ml volumetric flask; 30 ml of the diluent was added, left for 10 minutes to dissolve. The volume was made such that it was 200mg/L. Further dilutions were made of different concentrations as below:

Standard solution 50% preparation (50mg/L): diluted 2.50 ml of the standard stock preparation to 10ml with the diluent.

Standard solution 75% preparation (**75mg/L):** diluted 3.75 ml of the standard stock preparation to 10 ml with the diluent.

Standard solution 100% Preparation (100mg/L): Diluted 5.00 ml of the standard stock Preparation to 10 ml with diluent.

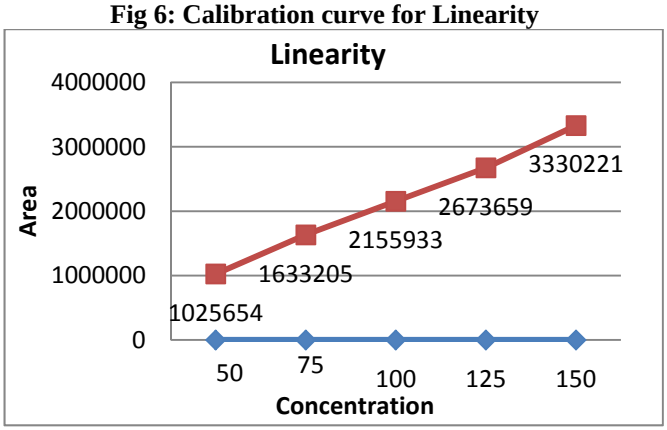
Standard Solution 125% preparation (125mg/L): Diluted 6.25 ml of the standard stock preparation to 10ml with diluent.

Standard Solution 150% preparation (150.0mg/L): Diluted 7.50mL of the standard stock preparation to 10ml with diluents. The results were tabulated in table 4 and Calibration curve for linearity is in fig -6.

Table 3: Linearity

S.No.	Concentration in (mg/L)	Area	Acceptance criteria
1	50.0	1025654	Correlation Coefficient (r2) should not be less than 0.999
2	75.0	1633205	
3	100.0	2155933	
4	125.0	2673659	
5	150.0	3330221	

Fig 6: Calibration curve for Linearity



PRECISION:

Standard preparation:

Weighed 11.76mg of Prasugrel HCL into 100ml volumetric flask, add 60ml of diluent, to dissolve for 10 minutes. Further made up the volume up to 100 ml with diluent. The standard solution was prepared and results were tabulated.

Sample preparation -1:

180.23mg of tablet powder were weighed and transferred into 100mL volumetric flask, 60mL of diluent, sonicated to dissolve for about 15min, further made up the volume with the diluent and mixed. Filtered through 0.45µm.

Sample preparation -2:

180.08mg of tablet powder were weighed and transferred into 100mL volumetric flask, 60mL of diluent, sonicated to dissolve for about 15min, further made up the volume with the diluent and mixed. Filtered through 0.45µm.

Sample preparation -3:

180.18mg of tablet powder were weighed and transferred into 100mL volumetric flask, 60mL of diluent, sonicated to dissolve for about 15min, further made up the volume with the diluent and mix. Filtered through 0.45µm.

Sample preparation -4:

180.47mg of tablet powder were weighed and transferred into 100mL volumetric flask, 60mL of diluent, sonicated to dissolve for about 15min, further made up the volume with the diluent and mixed. Filtered through 0.45µm.

Sample preparation -5:

181.27mg of tablet powder were weighed and transferred into 100mL volumetric flask, 60mL of diluent, sonicated to dissolve for about 15min, further made up the volume with the diluent and mixed. Filtered through 0.45µm.

Sample preparation -6:

180.67mg of tablet powder were weighed and transferred into 100mL volumetric flask, 60mL of diluent, sonicated to dissolve for about 15min, further made up the volume with the diluent and mixed. Filtered through 0.45µm.

The Prasugrel tablets were powdered and spiked and results were tabulated.

ACCURACY:

Standard preparation:

Weighed 11.32mg of Prasugrel HCL into 100ml volumetric flask, add 60ml of diluent, to dissolve for 10 minutes. Further made up the volume up to 100 ml with diluent.

Sample preparation 50% (50.0mg/L):

92.16mg of tablet powder were weighed and transferred into 100mL volumetric flask, 60mL of diluent were added, sonicated to dissolve for about 15min, further made up the volume with the diluent and mixed. Filtered through 0.45µm.

Sample preparation 75% (75.0mg/L):

143.82mg of tablet powder were weighed and transferred into 100mL volumetric flask, 60mL of diluent, sonicated to dissolve for about 15min, further made up the volume with the diluent and mixed. Filtered through 0.45µm.

Sample preparation 100%(100.0mg/L):

183.49mg of tablet powder were weighed and transferred into 100mL volumetric flask, 60mL of diluent, sonicated to dissolve for about 15min, further made up the volume with the diluent and mix. Filtered through 0.45µm.

Sample preparation 125 %(125.0mg/L):

230.40mg of tablet powder were weighed and transferred into 100mL volumetric flask, 60mL of diluent, sonicated to dissolve for about 15min, further made up the volume with the diluent and mixed. Filtered through 0.45µm.

Sample preparation 150 %(150.0mg/L):

291.59mg of tablet powder were weighed and transferred into 100mL volumetric flask, 60mL of diluent, sonicated to dissolve for about 15min, further made up the volume with the diluent and mixed. Filtered through 0.45µm.the results were tabulated in table 5a &5b.

Table 5 a: Accuracy

System suitability	Observed values	Acceptance Criteria
Theoretical plates for Prasugrel standard	4507	NLT 2500
Tailing factor for Prasugrel Standard	1.43	NMT 2.0
RSD for peak area of Pasugrel from 5 injections of the standard	0.08	NMT 2.0%

Table 5 b: Accuracy data

Sample No	Spike level	µg/ml added	µg/ml found	% Recovery
1	50%	48.6	48.2	99.1
2	50%	48.6	48.1	
3	50%	48.6	48.1	
1	75%	76.1	79.1	101.1
2	75%	76.1	79.0	
3	75%	76.1	79.1	
1	100%	98.4	99.5	99.9
2	100%	98.4	99.5	
3	100%	98.4	99.4	
1	125%	126.2	123.9	101.9
2	125%	126.2	126.1	
3	125%	126.2	125.9	
1	150%	158.2	160.1	101.2
2	150%	158.2	160.1	
3	150%	158.2	160.1	
Acceptance criteria	The accuracy (recovery) for the average of triplicate at each concentration level should be within 98.0% to 102.0%			

RUGGEDNESS:

1. Analysis with different analyst:

Standard preparation:

Weighed 11.72mg of Prasugrel HCL into 100ml volumetric flask, add 60ml of diluent, to dissolve for 10 minutes. Further made up the volume up to 100 ml with diluent.

Sample preparation 1:

183.23mg of tablet powder were weighed and transferred into 100mL volumetric flask, 60mL of diluent were added, sonicated to dissolve for about 15min, further made up the volume with the diluent and mixed. Filtered through 0.45µm.

Sample preparation 2:

185.08mg of tablet powder were weighed and transferred into 100mL volumetric flask, 60mL of diluent, sonicated to dissolve for about 15min, further made up the volume with the diluent and mixed. Filtered through 0.45µm.

Sample preparation 3:

186.82mg of tablet powder were weighed and transferred into 100mL volumetric flask, 60mL of diluent, sonicated to dissolve for about 15min, further made up the volume with the diluent and mix. Filtered through 0.45µm.

Sample preparation 4:

185.44mg of tablet powder were weighed and transferred into 100mL volumetric flask, 60mL of diluent, sonicated to dissolve for about 15min, further made up the volume with the diluent and mixed. Filtered through 0.45µm.

Sample preparation 5:

181.27mg of tablet powder were weighed and transferred into 100mL volumetric flask, 60mL of diluent, sonicated to dissolve for about 15min, further made up the volume with the diluent and mixed. Filtered through 0.45µm.

Sample preparation 6:

180.51mg of tablet powder were weighed and transferred into 100mL volumetric flask, 60mL of diluent, sonicated to dissolve for about 15min, further made up the volume with the diluent and mixed. Filtered through 0.45µm.

The results were tabulated in table 6a1 &6a2.

Table 6a1: System Precision

System suitability	Observed value	Acceptance criteria
Theoretical Plates for Prasugrel standard	8823	NLT 2500
Tailing factor for Prasugrel standard	1.31	NMT 2.0
RSD for peak area of Prasugrel from five injections	0.14	NMT 2.0%

Table 6a2: Data for System Precision

Sample Number	% of Assay		Acceptance criteria
	Analyst-1	Analyst-2	
1	99.7	99.9	All-individual assays of Prasugrel tablets should be within 98.0% to 102.0% by the analyst, columns and systems.
2	100.4	99.6	
3	99.2	98.7	
4	100.1	98.7	
5	98.5	99.7	
6	98.9	98.9	
Mean	99.4	99.3	
% RSD	0.74	0.53	

2. Solution stability:

Standard preparation (Initial):

Weighed 11.61mg of Prasugrel HCL into 100ml volumetric flask, add 60ml of diluent, to dissolve for 10 minutes. Further made up the volume up to 100 ml with diluent.

Standard preparation (12th hour):

11.76mg of tablet powder were weighed and transferred into 100mL volumetric flask, 60mL of diluent were added, sonicated to dissolve for about 15min, further made up the volume with the diluent and mixed. Filtered through 0.45µm.

Standard preparation (24th hour):

11.96mg of tablet powder were weighed and transferred into 100mL volumetric flask, 60mL of diluent, sonicated to dissolve for about 15min, further made up the volume with the diluent and mixed. Filtered through 0.45µm.

Standard preparation (36th hour):

11.42mg of tablet powder were weighed and transferred into 100mL volumetric flask, 60mL of diluent, sonicated to dissolve for about 15min, further made up the volume with the diluent and mix. Filtered through 0.45µm.

The results were tabulated in table 6b1.

Table 6b1: Bench top stability

Time in hours	Standard (similarity factor)	%Assay of Prasugrel		% of Difference	
		Test-1	Test-2	Test-3	Test-4
Initial	NA	99.7	100.4	NA	NA
12th hour	1.003	100.4	100.9	0.7	0.5
24th hour	0.997	100.8	101.6	1.1	1.2
36th hour	1.001	101.0	101.8	1.3	1.4
Acceptance criteria	Similarity factor between two Prasugrel standard preparations 0.985-1.015 Acceptable difference between initial and stability samples against fresh standard is NMT 3.0%.				

ROBUSTNESS:

1. Filter variation:

Standard preparation :

Weighed 11.61mg of Prasugrel HCL into 100ml volumetric flask, add 60ml of diluent, to dissolve for 10 minutes. Further made up the volume up to 100 ml with diluent.

Sample preparation 1:
180.23mg of tablet powder were weighed and transferred into 100mL volumetric flask, 60mL of diluent were added, sonicated to dissolve for about 15min, further made up the volume with the diluent and mixed. Filtered through 0.45µm PTFE filter.
Sample preparation 2:
180.08mg of tablet powder were weighed and transferred into 100mL volumetric flask, 60mL of diluent, sonicated to dissolve for about 15min, further made up the volume with the diluent and mixed. Filtered through 0.45µm Nylon filter. The results were tabulated in table 7.

Table 7 a. Filter variation

S.No.	Flow Rate		%RSD
	At 1.2ml	At 1.4ml	Acceptance criteria
	Std Areas	Std Areas	
1	2232059	1935847	Relative standard deviation for peak area of prasugrel from five injections of standard should not be more than 2.0%
2	2233969	1946697	
3	2235380	1966582	
4	2235410	1887082	
5	2195067	1882595	
AVG	2226377	1923761	
SD	17556	37235	
% RSD	0.53	1.94	

2. **Effect of variation in flow rate:**
Standard preparation:
Weighed 11.61mg of Prasugrel HCL into 100ml volumetric flask, add 60ml of diluent, to dissolve for 10 minutes. Further made up the volume up to 100 ml with diluent.
Sample preparation 1:
180.23mg of tablet powder were weighed and transferred into 100mL volumetric flask, 60mL of diluent were added, sonicated to dissolve for about 15min, further made up the volume with the diluent and mixed. Filtered through 0.45µm filter and use the filtrate after discarding the first 5mL and inject into the system at a flow rate of 1.2mL.
Sample preparation 2:
180.23mg of tablet powder were weighed and transferred into 100mL volumetric flask, 60mL of diluent were added, sonicated to dissolve for about 15min, further made up the volume with the diluent and mixed. Filtered through 0.45µm filter and use the filtrate after discarding the first 5mL and inject into the system at a flow rate of 1.4mL.the results were tabulated in table 7b.

Table 7 b. Effect of variation in flow rate

Sample No	% of Assay of Prasugrel as % of labelled amount		
	0.45µm Nylon	0.45µm PTFE	% of Difference
1	99.7	100.1	0.4
2	100.4	98.6	1.8
3	99.2	97.4	1.8
Acceptance criteria	The difference between the filtered sample solutions should not be more than 3.0%		

3. Effect of variation in column temperature:
Standard preparation:
Weighed 11.42mg of Prasugrel HCL into 100ml volumetric flask, add 60ml of diluent, to dissolve for 10 minutes. Further made up the volume up to 100 ml with diluent.
Sample preparation 1:
180.23mg of tablet powder were weighed and transferred into 100mL volumetric flask, 60mL of diluent were added, sonicated to dissolve for about 15min, further made up the volume with the diluent and mixed. Filtered through 0.45µm filter and use the filtrate after discarding the first 5mL and maintain a column temperature of 25ºC.
Sample preparation 2:
180.23mg of tablet powder were weighed and transferred into 100mL volumetric flask, 60mL of diluent were added, sonicated to dissolve for about 15min, further made up the volume with the diluent and mixed. Filtered through 0.45µm filter and use the filtrate after discarding the first 5mL and maintain a column temperature of 30ºC.
Sample preparation 2:
180.23mg of tablet powder were weighed and transferred into 100mL volumetric flask, 60mL of diluent were added, sonicated to dissolve for about 15min, further made up the volume with the diluent and mixed. Filtered through 0.45µm filter and use the filtrate after discarding the first 5mL and maintain a column temperature of 35ºC.
inject into the system at a flow rate of 1.4mL.the results were tabulated in table 7b.

Table 7 c. Effect of variation in column temperature

S.No.	Temperature			%RSD
	At 25^0	At 30^0	At 35^0	Acceptance criteria
	Std Areas	Std Areas	Std Areas	
1	2144313	2098262	2122540	Relative standard deviation for peak area of prasugrel from five injections of standard should not be more than 2.0%
2	2145023	2097886	2122996	
3	2138191	2097184	2121340	
4	2138596	2094386	2118118	
5	2141137	2094325	2113378	
AVG	2141452	2096409	2119674	
SD	3155	1914	4003	
RSD	0.15	0.09	0.19	

CONCLUSION

A simple, rapid and reproducible RP-HPLC method was developed for the estimation of Prasugrel Hydrochloride in the tablet dosage form. The method was validated for system suitability, specificity, precision, accuracy, linearity, ruggedness and robustness. The conclusion is therefore that this method can be adopted as a method of analysis for Prasugrel Hydrochloride.

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