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ANALYTICAL HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ASSAY OF PRASUGREL TABLETS

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Abstract:

Prasugrel hydrochloride functions as an agent that inhibits platelet activity and prevents the formation of blood clots. The mortality rate attributed to thrombosis has been increasing, therefore highlighting the need to develop methodologies for the evaluation of pharmaceutical interventions. The objective of this study was to develop a Prasugrel Hydrochloride 5mg tablet with high reliability, accuracy, and precision using RP-HPLC methodology. In order to quantify the amount of Prasugrel Hydrochloride present in tablets, a reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated. A C18 SunfireTM (5 µm, 250 mm × 4.6 mm) analytical column was used in an isocratic mode. The mobile phase consisted of a mixture of methanol and water in a ratio of 80:20 (v/v) and $KH_2PO_4$with a pH of 6.5. The analyte was monitored at a wavelength of 245 nm with a flow rate of 1.0 ml/min. The technique was validated to assess the system's applicability, specificity, precision, accuracy, linearity, ruggedness, and robustness. Linearity was successfully attained within the range of 25 parts per million (ppm) to 125 ppm, as shown by a high correlation coefficient of 0.9999. The reliability and durability of the technique were shown by the consistent findings obtained despite variations in flow rate and temperature, as well as when the analysis was conducted by a different analyst, on a different system, and with a different column. Given that all the criteria for system suitability were within the permissible range, it was concluded that the system has the capability to conduct the test.

Keywords: Prasugrel, Method development, Methodvalidation and RP-HPLC

Introduction:

Pharmaceutical analysis is of utmost importance in ensuring the quality and reliability of pharmaceuticals. Qualitative analysis is used to determine the chemical composition of the sample, whilst quantitative analysis is utilised to ascertain the proportional quantity of the different species present. High Performance Liquid Chromatography (HPLC) is widely used

as a prevalent analytical technique in the pharmaceutical industry. High-performance liquid chromatography (HPLC) is a liquid chromatographic technique that utilises a liquid mobile phase and a finely split stationary phase. The high-performance liquid chromatography (HPLC) technique is widely used in the field of analytical chemistry due to its ability to consistently provide the desired outcomes with a high degree of reliability, linearity, precision, and accuracy. Additional benefits of this approach are its rapidity, enhanced resolution, and the ability to reuse columns. These attributes make it particularly well-suited for compounds with low viscosity, facilitating effortless sample retrieval and upkeep. In conventional phase chromatography, the stationary phase consists of a polar adsorbent, whereas the mobile phase is composed of a blend of non-aqueous solvents [1-3].

In the creation of a novel technique, it is advisable to apply a sound approach due to the need of doing several experimental runs in order to get the desired outcomes. In order to acquire trustworthy analytical findings, it is crucial to consider important criteria such as meticulous sample preparation, judicious selection of the proper column, and the establishment of suitable operating conditions4. Additionally, it is essential that the performance of the recording and data handling systems be characterised by a high degree of reliability. Validation of the proposed approach is of utmost importance. Validation parameters for High Performance Liquid Chromatography (HPLC) encompass several key aspects, namely system suitability, accuracy, specificity, linearity, precision, limit of quantitation, and limit of detection. It is crucial to note that these parameters rely on the premise that the analytical operations, equipment, electronics, and samples form an interconnected system that can be evaluated holistically.The user's text is too short to be rewritten academically. According to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), selectivity is a technique that yields distinct responses for many chemical entities, allowing for differentiation between them. On the contrary, specificity refers to a methodology that generates a result only for a single analyte. Accuracy is defined as the degree to which the obtained values of an experiment align with the real value. The attribute of sensitivity has significant importance as it delineates the capacity to discern minute fluctuations in the concentration of the analyte within the sample. Precision refers to the degree of proximity between data values obtained under identical experimental circumstances. Reproducibility is a crucial factor that determines the level of accuracy achievable across different labs. It is expected that a linear correlation exists between the samples and tests. The limit of detection refers to the minimum concentration of an analyte

that can be observed in a sample, while the limit of quantitation refers to the concentration at which the analyte can be accurately measured within a certain set of circumstances[4-7].

Robustness, in essence, refers to the capacity of a technique to stay unaffected by minor intentional modifications in its parameters. The aforementioned factors have significant importance in the formulation of an analytical technique. There is currently no defined method available for the examination of Prasugrel tablets using the Reverse High-Performance Liquid Chromatography (HPLC) technique. Prasugrel Hydrochloride is a pharmacological agent with antithrombotic properties, primarily indicated for the management of acute coronary syndrome in individuals having percutaneous coronary intervention, as well as for the treatment of angina, atherosclerosis, and a limited number of other medical disorders.

Materials & Method:

Methanol, KH_2PO_4, $(C_2H_5)_3N$ and purified water were used are of HPLC Grade. The Prasugrel Hydrochloride Marketed Formulation, known as Prax 05, was obtained from the pharmaceutical company MSN LABS. The experimental apparatus used in this study consists of a weighing scale manufactured by Shimadzu, a pH metre produced by Mettler-Toledo, a Sonicator device known for its quick cleaning capabilities, and an HPLC system from the Shimadzu LC Series.

Selection of wavelength, diluent and Suitable mobile phase:

The mobile phase is composed of two solutions. Solution A was made by dissolving 1.36 grammes of KH_2PO_4 in 100 millilitres of water. The resulting mixture was then adjusted to a pH of 6.5 using triethylamine. The solution underwent filtration and degassing processes. Solution B involves a degassed combination of mthanol and water in a ratio of 80:20. The wavelength used in this study is 245nm, whereas the mobile phase ratios for Solution A : Solution B is 50:50.

The optimized chromatographic conditions:

The experiment is carried out using column C18 SunfireTM (5 µm, 250 mm × 4.6 mm)at a Flow rate of 1.0ml/min using a detection wavelength of 245nm, ambientcolumn temperature was used, the injection volume is 20µl, run time of 08 minutes. A typical chromatogram is shown in Fig.No: 01.

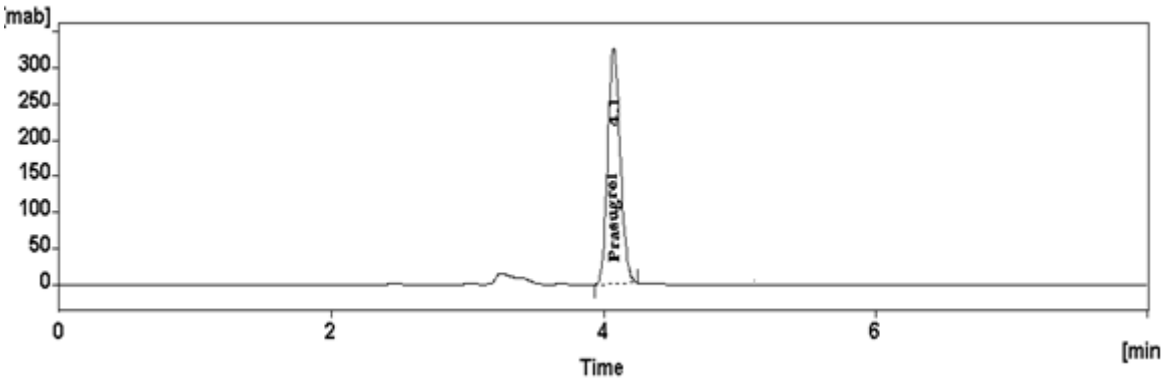

Fig.No. 01: Typical Chromatogram of Standard Prasugrel

Results andDiscussions:

The procedure that was devised fell inside the limits that had been established. The findings have also shown that the technique exhibits precision and accuracy, and the validation parameters are unaffected . This finding indicates that the parameters under investigation did not cause any interference, therefore leading to the conclusion that the procedure used was specific. The adequacy of the system was also shown, since each value fell within the prescribed limits.

Validation Parameters:

System Suitability:

In order to ensure the functionality of the analytical system, it is necessary to establish the system suitability parameters. The standard preparation included the measurement of about 11 mg of Prasugrel HCL, which was then placed into a 100 ml volumetric flask. Subsequently, 50 ml of diluent was added to facilitate dissolution, and the mixture was thoroughly stirred for a duration of approximately 10 minutes. Finally, the volume of the solution was adjusted to the desired level by adding more diluent. The results show that % RSD is less than 2, plate count was more than 8000 and the peak symmetry is less than 1.2. The results are tabulated in Table No.:01.

Table No. 01: System Suitability Data

Injection Number	Retention Time	Peak Area	Plate Count	Peak Symmetry
1	4.1	2149104	9234	1.127
2	4	2150126	9789	1.123

3	4.2	2149255	9567	1.134
4	4.1	2146567	9123	1.012
5	4	2150789	9078	1.056
6	4.1	2148432	9654	1.078
Average	4.1	2149046		
Standard Deviation	0.0753	1467.9189		
% RSD	1.84	0.07		

Linearity and Range

The concentrations of prasugrelshowed a linear relationship with peak area was 25-100 µg/ml. Results are shown in (Fig.No.: 02), (Table 2), and the linearity of the calibration curve is confirmed by the high value of the correlation coefficient of the regression equation.

Table 2: Linearity data

S.No.	Concentration (µg/ml)	Peak Area
1	0	0
2	25	1074675
3	50	2189104
4	75	3223678
5	100	4292198
6	125	5382867
Slope		42973
Intercept		7933.8
Regression		0.9999

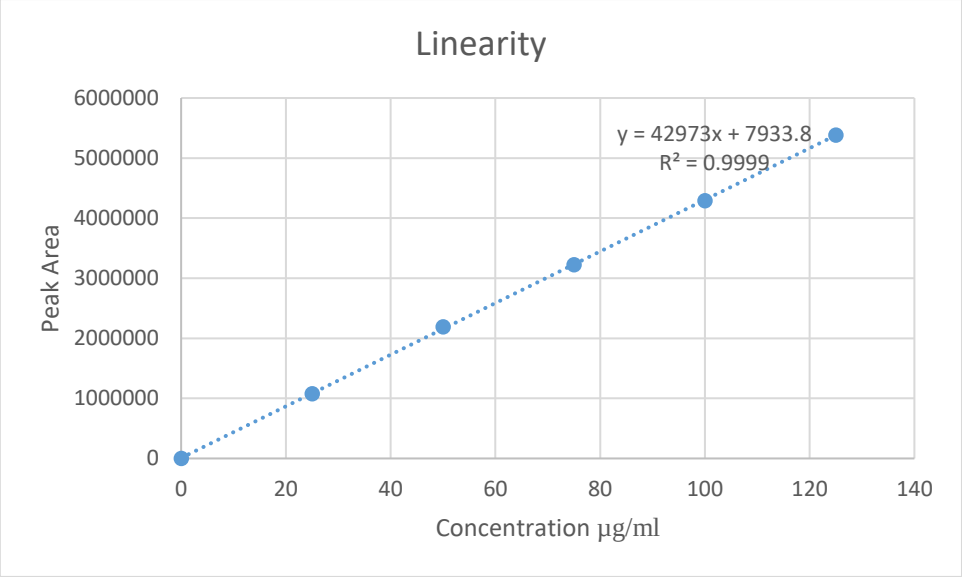

Fig.No. 01: Linearity data

Precision:

Standard preparation: Approximately 11 milligrammes of Prasugrelwas carefully weighed and placed into a volumetric flask with a capacity of 100 millilitres. Subsequently, 50 millilitres of a suitable diluent were added to the flask in order to facilitate dissolution. The mixture was allowed to dissolve for a duration of 10 minutes. The capacity was then increased to 100 ml by adding a diluent. The standard solution was made in six duplicates, and the resulting data was recorded in a tabular format in Table No.03.

Table 3: Precision data

Sample Preparation No.	Assay (%)
1	100.03
2	100.12
3	99.93
4	99.87
5	100.49
6	100.66
Mean	100.18

SD	0.3197
RSD (%)	0.31914

Accuracy

To check the accuracy of the proposed method, recovery studies were carried out at 70, 100 and 130 % of the test concentration as per ICH guidelines. The recovery study was performed three times at each level. The results of the recovery studies are given in Table 4.

Table 4. Recovery Studies

Level	Amount found (mg/ml)	Amount added (mg/ml)	Recovery (%)	Mean (%)
Level-1 (70%)	0.0351	0.0352	99.72	99.53
	0.0352	0.0358	98.32	
	0.0363	0.0361	100.55	
Level-2 (100%)	0.0511	0.0512	99.80	98.37
	0.0511	0.0534	95.69	
	0.052	0.0522	99.62	
Level-3 (130%)	0.0656	0.0665	98.65	98.37
	0.0666	0.0668	99.70	
	0.0656	0.0678	96.76	
Mean			98.76	
SD			1.5997	
% RSD			1.6198	

Robustness of method

The robustness of the developed method was studied by making small deliberate variations in the method parameters such as the small components in the mobile phase, flow rate and wave length. The solution containing 100 µg/mL of standardwas injected into sample injector of HPLC under the different conditions. The results of the robustness study are given in Table 5

Table 5: Method Robustness

Condition	% RSD	Tailing Factor	% Recovery
1) Change in Flow rate			
Normal Condition (1.0 ml per minute)	0.05	1.09	99.48
Flow rate (0.8 ml per minute)	0.06	1.08	99.17
Flow rate (1.2 ml per minute)	0.09	1.08	98.38
2) Change in minor component in the mobile phase			
Normal Condition (Mobile Phase A : Mobile Phase B) (50 : 50))	0.05	1.08	99.59
(Mobile Phase A : Mobile Phase B) (40 : 60))	0.18	1.08	99.43
(Mobile Phase A : Mobile Phase B) (60 : 40))	0.09	1.06	101.57
3) Change in Wave Length			
Normal: Wave Length 245 nm	0.04	1.09	99.89
Wave Length 240 nm	0.09	1.05	98.97
Wave Length 250 nm	0.08	1.07	98.83

Ruggedness

Ruggedness test was determined between two different analysts, instruments and columns. The value of percentage RSD was below 2.0%, exhibits the ruggedness of developed analytical method and results are presented in Table 6.

Table 6. Method Ruggedness

Sample Preparation No.	Assay (%)
1.	99.47
2.	99.06
3.	98.92
4.	99.10
5.	99.47
6.	97.48
Mean	98.92
SD	0.88
RSD (%)	0.89
Difference between method precision and intermediate precision assay	**1.26**

Conclusion

A robust and efficient reversed-phase high-performance liquid chromatography (RP-HPLC) approach was devised to quantitatively determine the concentration of Prasugrel in tablet formulations. The method demonstrated excellent precision and accuracy, making it suitable for routine analysis in pharmaceutical laboratories. The approach underwent validation to assess its applicability for system performance, precision, accuracy, linearity, ruggedness, and robustness. Hence, it can be inferred that this approach may be used as an analytical technique for the analysis of Prasugrel Hydrochloride.

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