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Development And Validation Of Reverse Phase HPLC Method For The Determination Of Impurities In Prasugrel Hydrochloride

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Abstract: The present paper describes the development of reverse phase HPLC method for Prasugrel hydrochloride in the presence of impurities and degradation products generated from the forced degradation studies. The drug substance was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal degradation. The degradation of Prasugrel hydrochloride was observed under neutral, acid, base and oxidation environment. The drug was found more sensitive to basic condition. Successful separation of the drug from the process related impurities and degradation products were achieved on Gemini C18 (250 x 4.6 mm) 5 µm particle size column using reverse phase HPLC method. The isocratic method employed with a mixture of buffer and (10%v/v water in acetonitrile) mixture of ratio 30:70 respectively. Potassium dihydrogen orthophosphate (0.05M) is used as buffer. The HPLC method was developed and validated with respect to linearity, accuracy, precision, specificity and robustness, which is useful for the routine determination of Prasugrel hydrochloride.

Key words: Prasugrel hydrochloride, RP-HPLC, LC Method development and validation, Forced degradation studies.

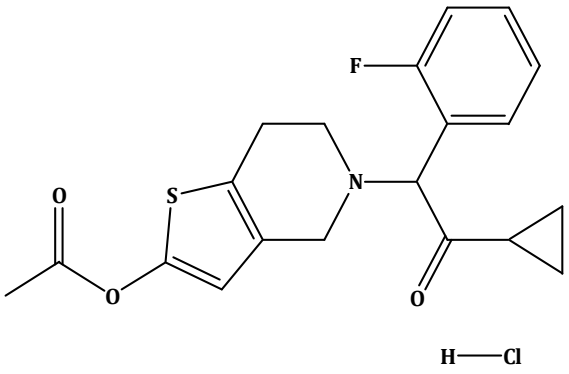
1. INTRODUCTION

Prasugrel Hydrochloride [1-7] is a novel platelet inhibitor used for acute coronary syndromes planned for percutaneous coronary intervention. Prasugrel hydrochloride is used to reduce the risk of thrombotic cardiovascular events (e.g., stent thrombosis, myocardial infarction [MI]) in patients with acute coronary syndromes (ACS) undergoing percutaneous coronary intervention (PCI). Prasugrel (marketing name “Effient” in the US and India, and “Efient” in the EU) is a platelet inhibitor developed by Daiichi Sankyo Co. and produced by Ube and currently marketed in the United States in cooperation with Eli Lilly and Company for acute coronary syndromes planned for percutaneous coronary intervention (PCI).

Prasugrel is a member of the thienopyridine class of ADP receptor inhibitors, like ticlopidine (trade name Ticlid) and clopidogrel (trade name Plavix). These agents reduce the aggregation ("clumping")

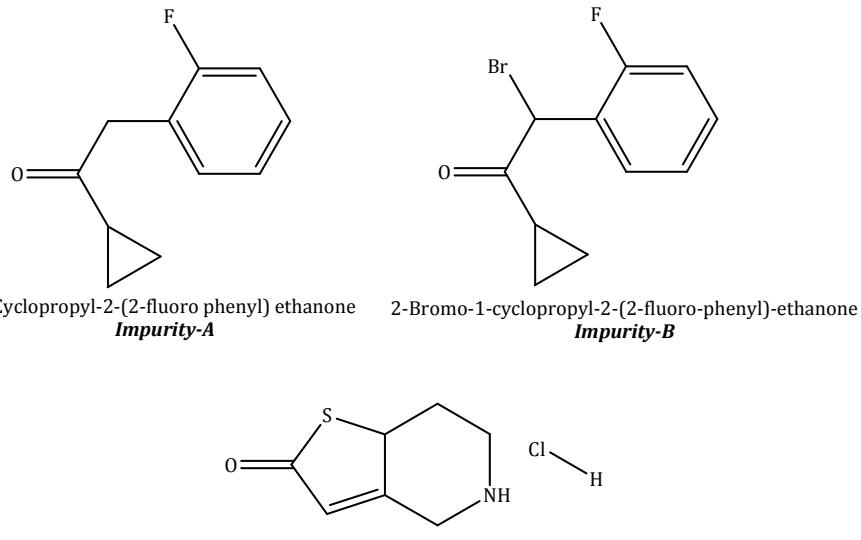
of platelets by irreversibly binding to $P2Y_{12}$ receptors. Compared to clopidogrel, prasugrel inhibits adenosine diphosphate–induced platelet aggregation more rapidly, more consistently, and to a greater extent than do standard and higher doses of clopidogrel in healthy volunteers and in patients with coronary artery disease, including those undergoing PCI". Clopidogrel, unlike prasugrel, was issued a black box warning from the FDA on March 12, 2010, as the estimated 2-14% of the US population that have low levels of the CYP2C19 liver enzyme needed to activate clopidogrel may not get the full effect. Tests are available to predict if a patient would be susceptible to this problem or not. Unlike clopidogrel, Prasugrel is effective in most individuals, although there have been several case reports of decreased responsiveness to Prasugrel. Different analytical techniques reported[8-19] so far for the determination of this drug substance and drug product. The typical structure of Prasugrel hydrochloride is mentioned in fig.1.

HPLC method was developed for the determination of Prasugrel and the impurities arising during its manufacturing. In the present study, we describe a reverse phase liquid chromatography method for the separation of process and degradation impurities of Prasugrel. The accuracy, linearity, precision, limit of detection (LOD), limit of quantification (LOQ) and robustness of the method were determined in accordance with ICH guidelines [20]. This paper reports, a rapid, efficient, simple and validated LC method for the separation of impurities and degradation products.



2-[2-(Acetyloxy)-6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl]-1-cyclopropyl-2-(2-fluorophenyl)ethanone hydrochloride

(Fig. 1: Structure of Prasugrel Hydrochloride)



1-Cyclopropyl-2-(2-fluoro phenyl) ethanone
Impurity-A

2-Bromo-1-cyclopropyl-2-(2-fluoro-phenyl)-ethanone
Impurity-B

5,6,7,7a-Tetrahydrothieno[3,2-c]pyridine-2(4H)-one hydrochloride
Impurity-C

(Fig. 2: Impurities of Prasugrel Hydrochloride)

2. EXPERIMENTAL SECTION

2. Experimental

2.1. Standards and Reagents

Prasugrel hydrochloride sample and impurities namely Impurity-A, Impurity-B and Impurity-C {Fig. 2} were developed and prepared in Research Division of Vindhya. All reagents used were of analytical reagent grade stated otherwise. Milli Q water, HPLC-grade acetonitrile, AR-grade Potassium dihydrogen ortho phosphate were purchased from Merck.

2.2. Instruments

The HPLC system used was equipped with quaternary gradient pumps with auto sampler injector (Shimadzu LC 2010, Japan) and controlled with LC solutions software (Shimadzu). Essae analytical balance was used for all weighing.

2.3. Preparation of system suitability solution

Accurately weighed 25mg of Prasugrel hydrochloride into a 50 mL volumetric flask, added about 10 mL of diluent, sonicated to dissolve and diluted up to the mark with diluent.

2.4. Preparation of sample solution

Accurately weighed 25mg of Prasugrel hydrochloride sample into a 50 mL volumetric flask, added about 10 mL of diluent, sonicated to dissolve and diluted up to the mark with diluent.

2.5. Chromatographic conditions

The chromatographic separation was achieved on Gemini C-18 250 x 4.6 mm, 5 µm particle size column. The Isocratic LC method employs with the composition of solution A and B as mobile phase in the ration of 30:70 respectively. Solution A contains 0.05M Potassium dihydrogen ortho phosphate. Solution B contains HPLC grade acetonitrile and water 90:10. The flow rate of the mobile phase was 1.2 mL per min and the peak shape of the Prasugrel hydrochloride peak was found to be symmetrical. Typical system suitability chromatogram is presented as figure.4. The detection was performed at UV λ_{220} nm. The injection volume was 20 µL. A mixture of water and acetonitrile (50 : 50 v/v) was used as a diluent.

3. VALIDATION OF THE METHOD

3.1. Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. The specificity of the developed LC method for Prasugrel Hydrochloride was carried out in the presence of its impurities namely, Impurity-A, Impurity-B and Impurity-C. Stress studies were performed for Prasugrel Hydrochloride drug substance to provide an indication of the stability indicating property and specificity of the proposed method. Degradation study was performed on 0.50 mg per mL solution of the drug in diluent. Neutral, acid hydrolysis, base hydrolysis and oxidation study was carried out by using diluent, 5 mL of 1N hydrochloric acid, 5 mL of 1N sodium hydroxide and 2 mL of 50 % hydrogen peroxide solution respectively. These solutions were prepared and each was heated in water bath at 60 °C for 0.5 hr, 1 hr, 1.5 hr and 2 hr respectively. Photolytic and thermal degradation was carried out on drug substance by exposing it separately on short wavelength light (254 nm) and heat (60 °C) for 4 days. Samples were withdrawn at appropriate time and subjected to analysis. All the impurities and degradation products were separated with appropriate resolution by the developed method.

3.2. Precision

The precision of the related substance method was checked by injecting six individual preparations of Prasugrel Hydrochloride spiked with 0.5 % of each impurity with respect to the Prasugrel Hydrochloride analyte concentration. The % RSD of the area of each impurity was calculated. The intermediate precision of the method was also evaluated using different analyst and instrument in the same laboratory.

3.3. LOD and LOQ

The LOD and LOQ were determined by measuring the standard deviation of the response and slope. The LOD and LOQ for Prasugrel Hydrochloride, Impurity-A, Impurity-B and Impurity-C were determined by injecting a series of dilute solutions with known concentrations. The precision study was also carried out at the LOQ level by injecting five injections of Prasugrel Hydrochloride, Impurity-A, Impurity-B and Impurity-C, calculating % RSD for the areas of each impurity.

3.4. Accuracy

The accuracy of the method for all the related substances was determined by analyzing Prasugrel Hydrochloride sample solutions spiked with all the related substances at four different concentration levels of LOQ, 50, 100 and 150 % of each in triplicate at the specified limit. The percentage of recoveries for the impurities was calculated by injecting the standard solution for each level.

3.5. Linearity

The Linearity of the method for all the related substances was determined by analyzing dilute solution of Prasugrel Hydrochloride and its related substances at four different concentration levels of LOQ, 50, 100 and 150 % of each in triplicate at the specified limit. The correlation coefficient was calculated for each substance.

3.6. Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered and the resolution between the Prasugrel Hydrochloride, Impurity-A, Impurity-B and Impurity-C was recorded. The parameters selected were mobile phase composition and flow rate (± 0.2 mL/min).

3.7. Solution and mobile phase stability

To determine the stability of sample solution, Prasugrel Hydrochloride samples spiked with related substances at specified level were prepared and analyzed after 48 hr. The results of these studies indicated the stability of sample solution at room temperature for 48 hr. The mobile phase prepared and kept at room temperature for 48 hr. After 48 hr Prasugrel Hydrochloride sample spiked with impurities at specified level were prepared and analyzed. The results are statistically evaluated and meeting system suitability and precision requirement which indicates the mobile phase is stable for 48 hr at room temperature.

4. RESULTS AND DISCUSSION

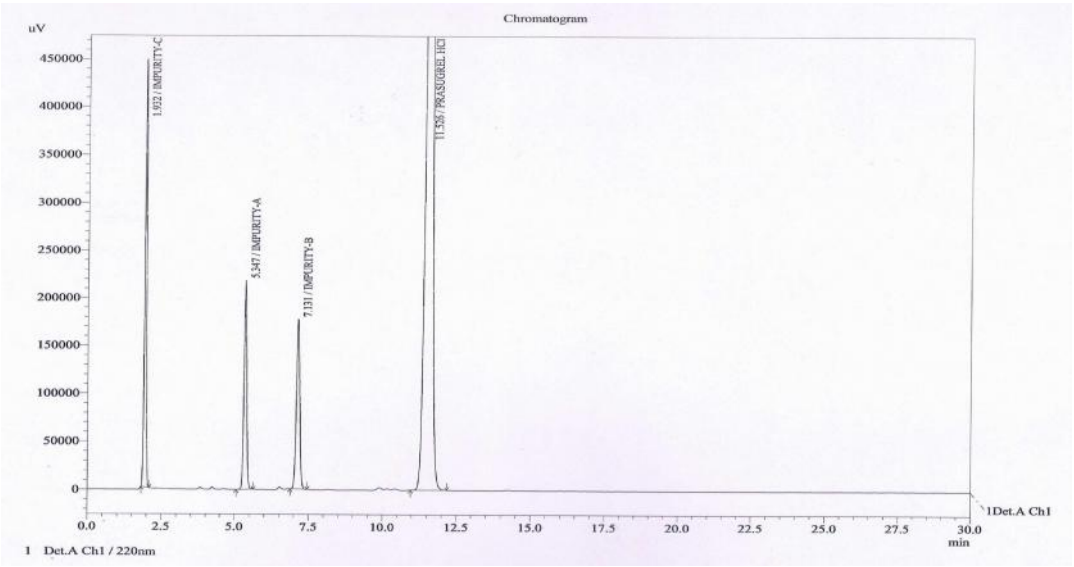
4.1. Optimization of chromatographic conditions

The main objective of the chromatographic method was to separate Prasugrel Hydrochloride from Impurity-A, Impurity-B and Impurity-C. Impurities were co-eluted using different stationary phases such as C-8, Cyano, phenyl column with different mobile phases. During evaluation of different column chemistries, Phenomenex Gemini C-18 column was observed to give better resolution with the 0.05M phosphate buffer. A good resolution and peak shape was optimized as mentioned under section “Chromatographic conditions”. In optimized chromatographic conditions Prasugrel Hydrochloride, Impurity-A, Impurity-B and Impurity-C were separated with a good resolution greater than 2, typical relative retention times for Impurity-A, Impurity-B and Impurity-C were approximately 0.17, 0.46 and 0.62 respectively {Fig. 3}. Specificity details are mentioned in the Table I.

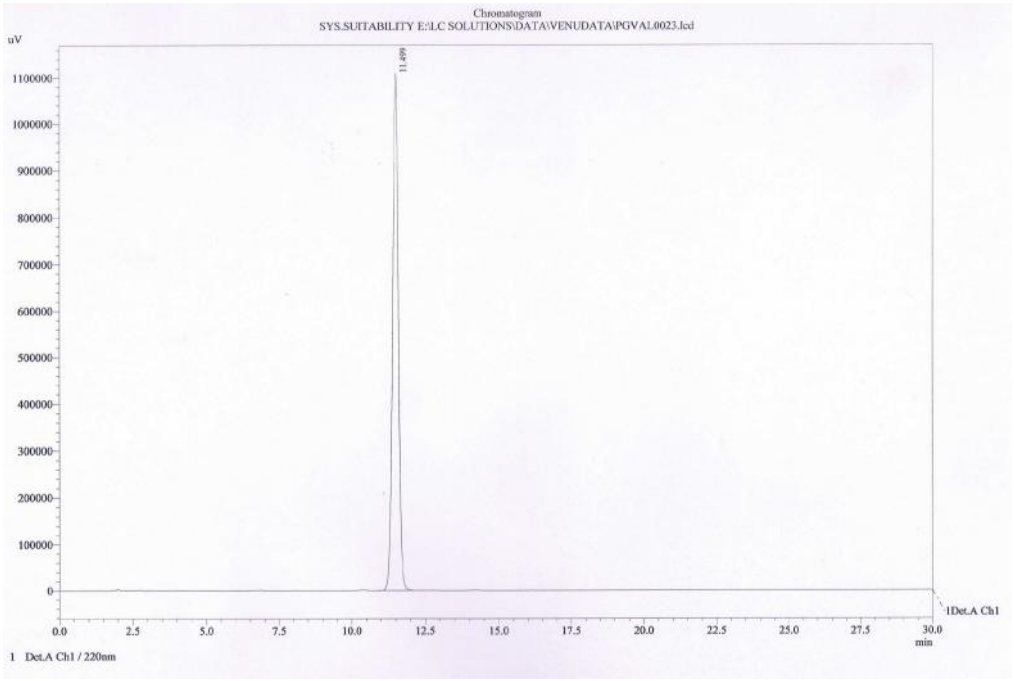
Table I. Specificity details

Parameter	Prasugrel HCl	Impurity-A	Impurity-B	Impurity-C
RT	11.59	5.36	7.16	1.93
RRT	1.000	0.46	0.62	0.17
N	17847	14959	17615	4424
TF	1.008	1.086	1.063	1.114

RT= Retention time, RRT=Relative retention time, N= Theoretical plates & TF= Tailing factor.



{ Fig.3 : Prasugrel Hydrochloride spiked with impurities }

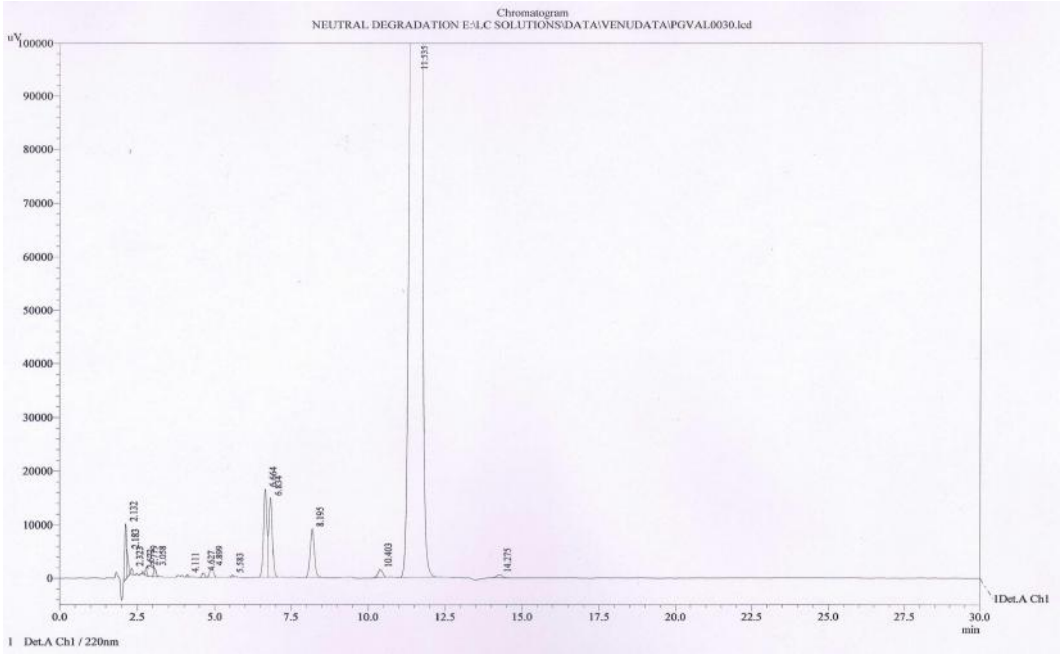


{ Fig.4 : Prasugrel hydrochloride system suitability chromatogram}

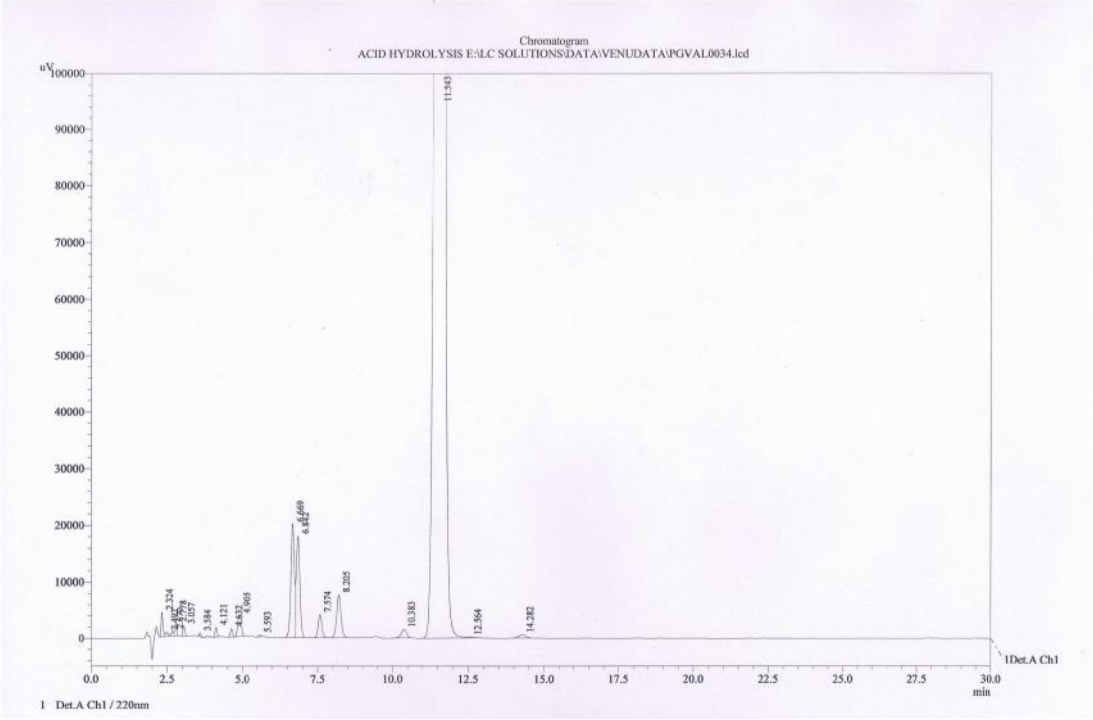
4.2. Validation of the method

4.2.1. Forced degradation

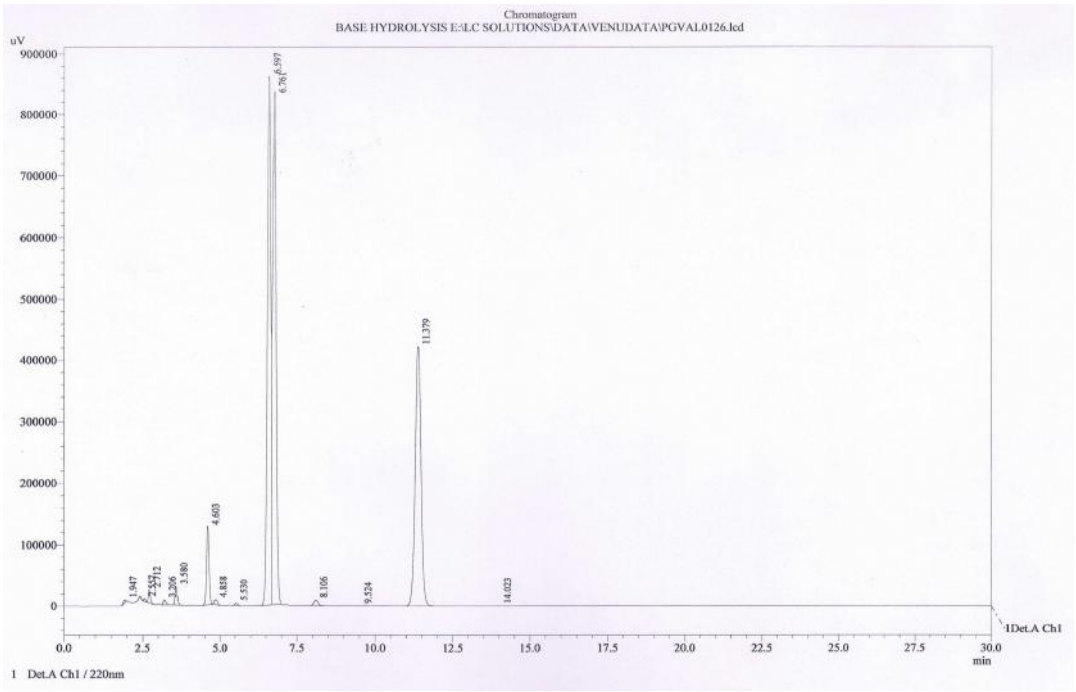
Degradation was not observed in Prasugrel Hydrochloride sample when subjected to neutral, thermal and photolytic stress conditions. Prasugrel Hydrochloride was degraded under neutral, acid, base and oxidation condition {Fig.4}. The summary of the forced degradation study is mentioned in Table II. Prasugrel found more sensitive to base medium and oxidation condition. Significant degradation was observed in base and oxidation condition. Initially the base hydrolysis conducted with 5 ml of 1N Sodium hydroxide solution at 60°C and the product is completely degraded then 0.1N sodium hydroxide was used for the study of one hour with 15 minutes sampling interval. For oxidation initially 2 mL of 50% hydrogen peroxide was used and significant degradation was observed at 60°C, then the study was performed using 1 mL of 50% hydrogen peroxide.



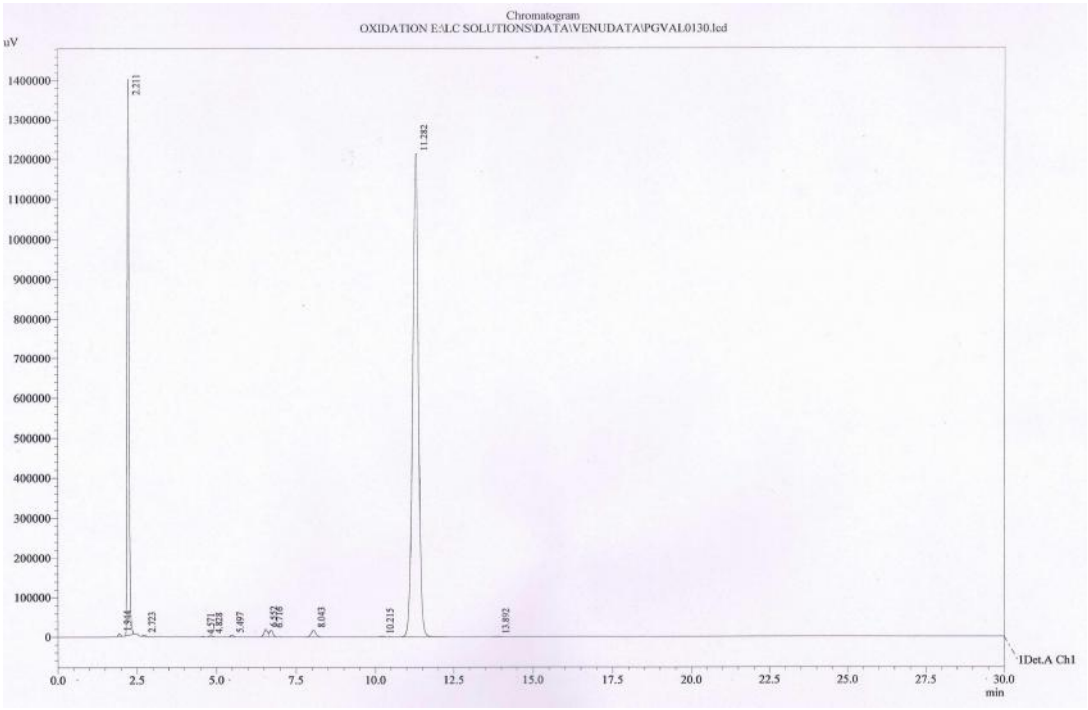
{ Fig.5: Prasugrel Hydrochloride neutral degradation sample with diluent (2hr at 60°C)- Major degradation products were observed at RT 2.13, 6.66 and 8.19 min.}



{ Fig.6: Prasugrel Hydrochloride acid hydrolysis study sample with 5 mL of 1N HCl (2 hr at 60 °C)- Major degradation products were observed at RT 2.13, 4.12, 4.63, 6.66, 6.84, 7.57, 8.20 and 14.28 min.}



{ Fig.7: Prasugrel Hydrochloride base hydrolysis study sample with 5 mL of 0.1N sodium hydroxide (15 min at 60 °C)- Major degradation products were observed at RT 3.58, 4.60, 6.59, 6.76, 8.10 and 14.02 min.}



{ Fig.8: Prasugrel Hydrochloride peroxide degradation study sample with 1 mL of 50% hydrogen peroxide (30 min at 60 °C)- Major degradation products were observed at RT 2.21, 6.55, 6.71, 8.04 and 13.89 min.}

Table II. Forced degradation study result of Prasugrel hydrochloride

Degradation condition	% Purity	RRT of major degradation peaks
Control sample	99.63	-
Neutral condition (60 °C, 2 hr only diluent)	97.36	0.15, 0.48, 0.49 & 0.59
Acid hydrolysis (60 °C, 2 hr, 5 mL, 1 N HCl)	96.77	0.20, 0.42, 0.58, 0.59,0.66 & 0.71
Base hydrolysis (60 °C, 15 min, 5 mL, 0.1 N NaOH)	27.27	0.24, 0.28, 0.31, 0.40, 0.43, 0.58, 0.59 & 0.71
Oxidation (60 °C, 30 min, 1 mL, 50 % Hydrogen peroxide)	70.75	0.20, 0.24, 0.43, 0.49, 0.58, 0.60 & 0.71
Photolytic degradation (UV λ_{254}, 4 days)	99.48	No major degradation peak was observed
Thermal degradation (60 °C, 4 days)	99.54	No major degradation peak was observed

4.2.2. Precision

The Precision was determined at the LOQ to 150% concentration for Prasugrel Hydrochloride, Impurity-A, Impurity-B and Impurity-C and the % RSD was found to be below 6.0 for all impurities.

4.2.3. Limit of detection and limit of quantification

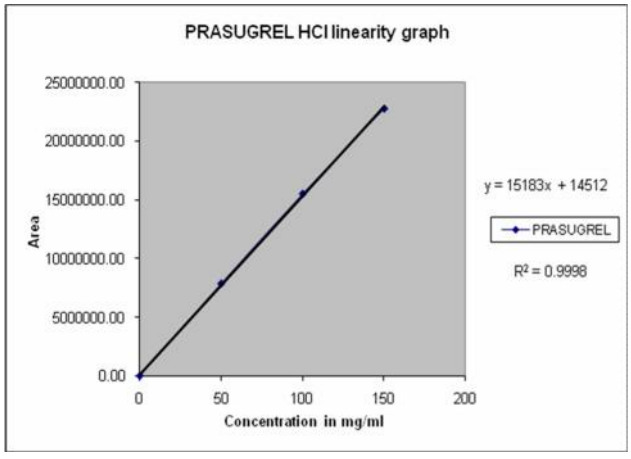
The values of LOD and LOQ for Prasugrel Hydrochloride, Impurity-A, Impurity-B and Impurity-C are mentioned in the Table. III.

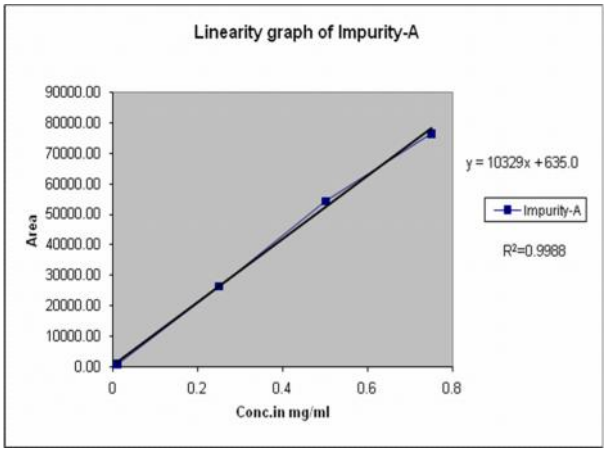
Table III. Validation results summary

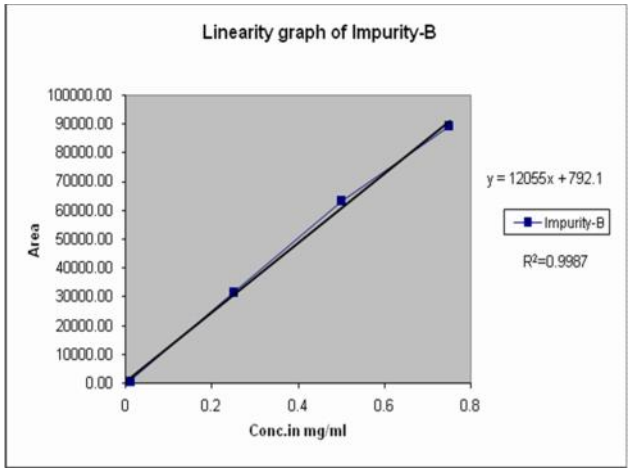
Parameter	Prasugrel HCl	Impurity-A	Impurity-B	Impurity-C
R^2 value	0.9998	0.9988	0.9987	0.9990
LOD in µg/ml	0.0167	0.0081	0.0077	0.0041
LOQ in µg/ml	0.0493	0.0246	0.0234	0.0125
%RSD at LOQ	2.016	4.504	5.758	1.743
Precision	0.198	0.984	1.933	0.870
Accuracy at LOQ	--	104.54	95.73	97.02
Accuracy at 50%	--	97.08	92.52	106.22
Accuracy at 100%	--	97.20	96.38	103.79
Accuracy at 150%	--	97.94	95.49	96.63

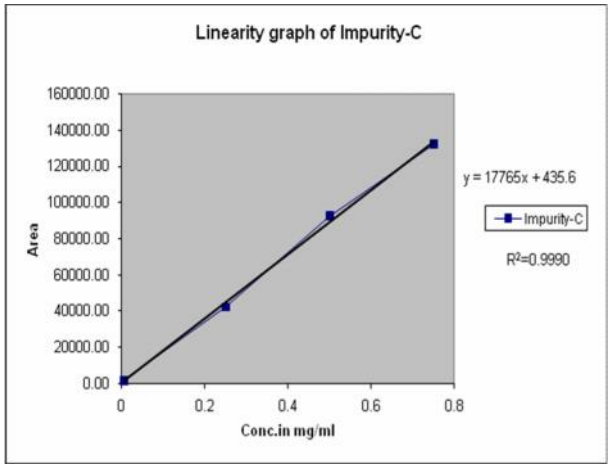
4.2.4. Linearity

Linearity calibration plot for the related substances method was obtained over the calibration ranges tested *i.e*. LOQ, 50 %, 100 % and 150 % of the specification limit. The correlation co-efficient obtained was greater than 0.99. The above result shows that an excellent correlation existed between the peak area and the concentration of three impurities. Linearity graphs for Prasugrel hydrochloride, Impurity-A, Impurity-B and Impurity were presented below. Linearity details were mentioned table III.









4.2.5. Accuracy
The Accuracy of all these related substances was found to be in between the predefined acceptance criteria of 80 to 120 % and the data given in Table III.

4.2.6. Robustness
When the chromatographic conditions like flow rate and mobile phase composition were deliberately varied and resolution between any two peaks is found greater than 2.0, which illustrates the robustness of the method.

4.2.7. Solution stability
There were no significant changes in the amount of the impurities during solution stability experiment performed using the related substances method. The results from the studies indicated, the sample solution was stable at room temperature for 48 hours.

5. CONCLUSION

A new, accurate and selective isocratic HPLC method was developed for the determination of impurities in Prasugrel hydrochloride and validated as per the ICH guidelines. The method was found to be simple, selective, precise, accurate and robust. Therefore, this method can be used for routine testing as well as stability analysis of Prasugrel hydrochloride. All statistical results were within the acceptance criteria.

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