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STABILITY INDICATING METHOD FOR QUANTITATION OF PRASUGREL HYDROCHLORIDE IN PRESENCE OF ITS DEGRADATION PRODUCTS

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Abstract

A stability indicating HPLC assay method has been developed and validated for the estimation of prasugrel hydrochloride in bulk and pharmaceutical dosage form. A RP-HPLC isocratic separation was achieved on C18 column (250×4.6 mm, 5µm) utilizing a mobile phase comprising of methanol and acetonitrile in the ratio of 90: 10 (v/v) and the eluents from the column were detected using a variable wavelength detector at 240 nm. The stress testing of prasugrel hydrochloride was carried out under acidic, alkaline, neutral hydrolysis, oxidation, photolytic and thermal degradation (dry heat) conditions and prasugrel hydrochloride was well resolved from its degradation products. The proposed method has permitted the quantification of prasugrel hydrochloride in the linearity range of 10-160 µg/ml and the flow rate was maintained at 1ml/min. The column was maintained at ambient temperature and the complete separation was achieved for prasugrel hydrochloride with all degradation products in an overall analytical run time of approximately 15 min. The retention time of prasugrel hydrochloride was found to be 3.78 min. The method was validated as per ICH guidelines.

Key Words: Prasugrel hydrochloride, Stability indicating assay, Forced degradation, ICH guidelines.

Introduction

Prasugrel hydrochloride (PRL) chemically is 5-[2-cyclopropyl-1-(2-fluoro-phenyl)-2 oxoethyl] 4,5,6,7-tetra hydrothieno [3,2-c] pyridin-2-yl acetate hydrochloride (Figure 1). It is a novel platelet inhibitor prodrug, member of

third generation thienopyridine class of adenosine diphosphate (ADP) receptor inhibitors which rapidly gets metabolized to an irreversible $P2Y_{12}$ receptor inhibitor.

PRL reduces the aggregation ("clumping") of platelets by irreversibly binding to $P2Y_{12}$ receptors**.** It is used as an antiplatelet therapy in patients with acute coronary syndromes. Compared to the standard therapy (Clopidogrel, a second generation thienopyridine), PRL has a more rapid, predictable and potent antiplatelet effect with a longer duration of platelet inhibition. [1-3]

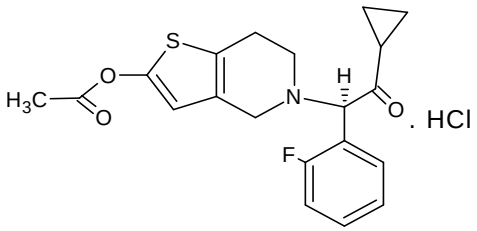

Figure 1: Chemical structure of Prasugrel HCl.

Literature survey reveals that a very few analytical methods like LC-MS [4], HPTLC [5], HPLC [6-7] and some spectroscopic methods [8-9] and one forced degradation study [10] have been reported for the estimation of PRL.

By our knowledge, there was no method has been developed so precise, accurate and specific for estimation of PRL. So, the main objective of this work was to develop a new, simple, economic, rapid, precise, and accurate stability-indicating HPLC method for quantitative analysis of PRL and to validate the method in accordance with ICH guidelines. Forced degradation of PRL was performed under different stress conditions (acid, alkaline, neutral, photolytic, thermal and oxidative). The proposed RP-HPLC method was validated by assessing its specificity, linearity, accuracy and precision, robustness, limit of detection and quantification.

Experimental

Materials and methods

Prasugrel hydrochloride was obtained as a gift sample. The commercially available tablet, “Prasusafe” 5 mg (MSN Laboratories Ltd., Bollaram) containing 5 mg of PRL was procured from the local market and used for analysis. Acetonitrile, methanol and water used were of HPLC grade and purchased from Merck specialties Pvt Ltd., Mumbai, India. Hydrochloric acid, sodium hydroxide and hydrogen peroxide used were of analytical reagent grade. All the

glassware employed in the study was cleaned with hot water, followed by acetone and dried in hot air oven whenever required.

Instrumentation

Analysis of samples were performed by using JASCO PU 2075 plus HPLC system consisted of a 2080 plus intelligent pump, a variable wavelength programmable UV/VIS detector with precision loop injector (Rhenodyne, 20 µl). The data was processed by using BORWIN software. All samples were filtered through 0.45 µm membrane Millipore filtration apparatus with vacuum pump.

Chromatography

The isocratic method was employed with the mobile phase consisting of 90 volumes of methanol and 10 volumes of acetonitrile. The chromatographic column used was a HiQ sil C-18 HS with dimensions of 250×4.6 mm with 5µm particle size. The column was maintained at ambient temperature and detection was performed at a wavelength of 240 nm. Prior to injection of analyte, the column was equilibrated for 30-40 min with mobile phase. The injection volume was 20 µL. Methanol was used as diluent for preparation of solutions.

Preparation of mobile phase

The HPLC grade solvents of methanol and acetonitrile were used for the preparation of mobile phase in the ratio of 90:10 (v/v). The contents of the mobile phase were filtered through a 0.45µm membrane filter, degassed by sonication and pumped from the solvent reservoir to the column at a flow rate of 1 ml/min throughout the analysis.

Preparation of standard stock solution

Standard stock solution was prepared by dissolving the 10 mg of PRL in 10 ml methanol to obtain primary stock solution of 1000 µg/ml. From the primary stock solution; 5 ml of the solution was pipette out and diluted to 10 ml with methanol to get concentration of 500 µg/ml. 4 ml of the resultant solution was again diluted to 10 ml with methanol to get final stock solution of 200 µg/ml. 100 µg/ml solution was prepared from the above final stock solution and injected to chromatographic system to obtain chromatogram (Figure 2).

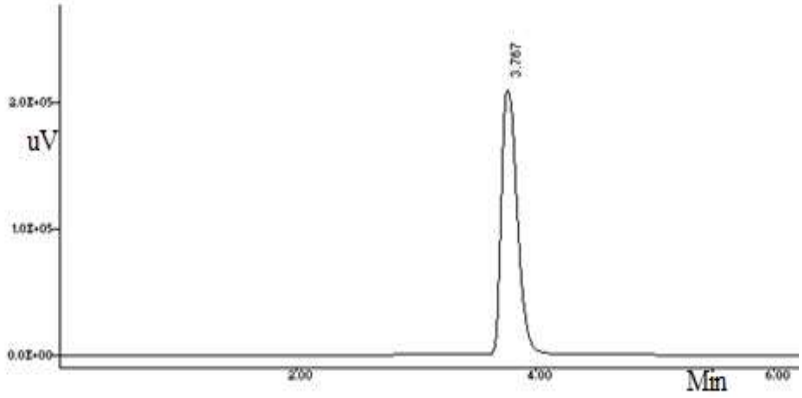

Figure 2: Chromatogram of standard API of Prasugrel HCl.

Analysis of tablet formulation

Twenty tablets were accurately weighed and triturate thoroughly to get fine powder. The powder equivalent to 5 mg of PRL was weighed and transferred into 10 ml volumetric flask. The contents of the flask were dissolved in the 5 ml of the methanol with the aid of ultrasonication for 10 min. The solution was filtered through whatmann filter paper no. 41 and volume was made up to 10 ml with methanol. From the resultant solution, further dilutions were prepared with methanol to get final concentration of 100 µg/ml in triplicate. The solution was injected into the chromatographic system and chromatogram was obtained. The concentration of analyte was determined with the equation obtained from calibration curve. The results of assay of tablets are shown in Table 1.

Table 1: Analysis of tablet formulation.

Tablet	Concentration (µg/ml)	% of drug estimated	Statistical analysis	
			Mean	%RSD
Prasusafe	100	100.15	98.68	1.3
	100	98.13		
	100	97.77		

Forced degradation (stress degradation) study

In order to establish whether the analytical method was stability indicating, the standard active pharmaceutical ingredient of PRL was exposed under various stressed conditions to promote degradation. [11]. The exposed sample solution was filtered through whatmann filter paper No. 41 and injected in the chromatographic system. The degradants were separated in the chromatogram with good resolution of PRL.

For acid hydrolysis, about 1 ml of standard primary stock solution (1000 µg/ml) of PRL was transferred to 10 ml volumetric flask. Then 1 ml of 0.1 N HCl was added and kept at room temperature (RT) for about 15 min. After the degradation, the solution was neutralized with 0.1 N NaOH to pH 7 and the volume made up to 10 ml with methanol. The solution was prepared to achieve a final concentration 100 µg/ml.

For alkaline hydrolysis, about 1 ml of standard primary stock solution (1000 µg/ml) of PRL was transferred to 10 ml volumetric flask. Then 1 ml of 0.1 N NaOH was added and kept at room temperature for about 15 min. After the degradation, the solution was neutralized with 0.1 N HCl to pH 7 and the volume made up to 10 ml with methanol. The solution was prepared to achieve a final concentration of 100 µg/ml.

For neutral hydrolysis, about 1 ml of standard primary stock solution (1000 µg/ml) of PRL was transferred to 10 ml volumetric flask. Then 4 ml of water was added and kept at room temperature for about 1 hour. After the degradation, the volume was made up to 10 ml with methanol. The solution was prepared to achieve a final concentration 100 µg/ml.

To study the effect of oxidative conditions, about 1 ml of standard primary stock solution (1000 µg/ml) of PRL was transferred to 10 ml volumetric flask. Then 1 ml of 15 % H_2O_2 was added and kept at room temperature for about 15 min. After the degradation, the volume was made up to 10 ml with methanol. The solution was prepared to achieve a final concentration 100 µg/ml.

To study the effect of temperature, the bulk drug was subjected to heat treatment for the assessment of the thermal degradation and was exposed to dry heat in an oven at 60^0c for about 3 hours. Then the drug was removed from the oven and allowed to attain room temperature. The powder equivalent to 10 mg of active ingredient was dissolved in 10 ml methanol. 1 ml of the resultant solution was diluted up to 10 ml with methanol to get final concentration 100 µg/ml.

For photolytic degradation, about 15 mg of bulk drug was sufficiently spread on a Petri plate (1 mm thick layer) and exposed to photo degradation test for 3 hours. Then the solution was prepared to achieve a final concentration 100 µg/ml.

Method validation

The developed method was validated as per the ICH guidelines. The method was validated for its specificity, linearity, accuracy, precision, LOD and LOQ [12].

The linearity of the method is its ability to elicit test results that are directly proportional to the concentration of the analyte in the samples. From the standard stock solution (200 µg/ml), series of dilutions were made to get concentrations in the range of 10, 20, 40, 60, 80, 100, 120, 140 and 160 µg/ml for PRL. The standard plot for the PRL was constructed by plotting the peak area of PRL against concentration. It was found to be linear in the range of 10-160 µg/ml with a correlation coefficient (r^2) of 0.999.

LOD and LOQ were based on the standard deviation of the response (σ) and the slope of the corresponding curve (S) using the following equation:

$$LOD = 3.3\sigma/S, \quad LOQ = 10\sigma/S$$

Where, σ is the standard deviation of the response of blank,

S is the slope of calibration curve.

The LOD and LOQ of PRL were found to be 0.56 µg/ml and 1.69 µg/ml respectively. The results are given in the **Table 2.**

Table-2: Statistical data for linearity and calibration range.

Parameters	Prasugrel hydrochloride
Linearity range	10-160 µg/ml
Correlation coefficient (r^2)	0.999
Slope (m)	17621
Intercept (c)	53783
LOD (µg/ml)	0.558
LOQ (µg/ml)	1.69

Precision is the measure of how close the data values to each other for a number of measurements under the same analytical conditions. Precision of the method was determined by performing inter day variation, intraday variation and repeatability studies. Three replicate injections of three different concentrations were injected at various time intervals on the same day for intraday and on three different days for inter day precision. The % RSD was also found to be within the limits for PRL. In repeatability study, six determinations of the fixed concentration of PRL were analyzed separately. The results of precision data are given in Table 3.

Table-3: Precision study of PRL.

Parameters	Fortified amount (µg/ml)	Amount found (µg/ml)	% RSD
Intra day (n = 3)	40	39.30	0.80
	80	80.43	0.87
	120	120.37	0.55
Inter day (n = 3)	40	39.73	1.60
	80	77.01	1.40
	120	119.84	0.80
Repeatability (n = 6)	100	98.12	1.46

To determine the robustness of the developed method, experimental conditions were purposely altered and the resolution was evaluated. The flow rate of the mobile phase was 1.0 ml/min. To study the effect of flow rate on resolution, it was changed by 0.1 units from 0.9 to 1.1 ml/min. The effect of percent organic strength on resolution was studied by varying composition of mobile phase by 2 units from methanol: acetonitrile (92:8 v/v) to methanol: acetonitrile (88:12 v/v). The results of robustness study are shown in Table 4.

Table-4: Results of robustness study of PRL.

Chromatographic conditions	Normal	Variation	Drug estimated	Rt of PRL (min)
Mobile phase (methanol : acetonitrile, v/v)	90:10	88 : 12	99.13	3.60
		92 : 08	100.56	3.72
Flow rate (ml/min)	1.0	0.9	98.27	3.80
		1.1	94.80	3.48

The accuracy of the proposed method was determined by calculating the recoveries of PRL by the standard addition method. It was determined by preparing solutions of different concentrations at 80%, 100% and 120% of label claim. The amount of PRL recovered was estimated by applying obtained values to the regression line equation. The results of % recovery are given in Table 5.

Table-5: Results of recovery study of PRL.

% level	Sample No.	Amount of drug added (µg/ml)		Amount of pure drug recovered (µg/ml)	% Recovery	Statistical analysis	
		Formulation	Pure Drug			%Mean	%RSD
80	1	50	14	13.93	99.56	99.75	1.73
	2	50	14	14.22	101.57		
	3	50	14	13.73	98.13		

100	1	50	30	30.05	100.16	100.61	1.4
	2	50	30	30.67	102.20		
	3	50	30	29.84	99.49		
120	1	50	46	46.60	101.30	100.11	1.09
	2	50	46	45.60	99.14		
	3	50	46	45.95	99.89		

Results and Discussions

Optimization of chromatographic conditions

A simple stability-indicating RP-HPLC method has been developed for determination of PRL in presence of its degradation products. The method was optimized to provide a good separation of the components with acceptable theoretical plates, sufficient sensitivity and suitable peak symmetry in a short run. For this purpose, the analytical column, solvent selection, mobile phase composition, flow rate, and detector wavelength were studied. The chromatographic separation was achieved using an RP C18 column so as to resolved the degradation products from PRL with adequate resolution and gave symmetrical peak shapes. The mobile phase used was methanol: acetonitrile 90:10 (v/v) eluted PRL in a significant shorter time of 3.78 min. with a good peak shape (peak symmetry). The method has many advantages, e.g., simplicity, isocratic conditions and absence of buffers in the mobile phase that could damage the chromatographic column and equipment.

Forced degradation study

Degradation study was carried out by exposing the samples to various stress conditions. It was observed that PRL undergoes significant degradation (17.94 %) with additional peaks at Rt 2.975 min (Figure 3) after exposure to oxidative condition. The % degradation in acidic (Figure 4), basic hydrolysis (Figure 5) and heat exposure sample were 10 %; 9.85 % and 7 % respectively indicates that PRL is quite susceptible under these exposed conditions. Negligible degradation was observed in neutral and photolytic degradation study indicates PRL is light stable. (Table 6)

Table-6: Degradation study of PRL.

Sr. No	Stress Degradation	Exposure condition	% Degradation	R_t (min)	
				PRL	Degradants
1	Acid	0.1 N HCl for 30 min at RT	10.0	3.83	2.77
2	Alkali	0.1N NaOH for 30 min at RT	9.85	3.81	2.50

3	Neutral	Water for 60 min at RT	2.0	3.79	2.59, 2.96
4	Oxidative	15% H_2O_2 for 15 min at RT	17.94	3.83	2.96
5	Thermal	3 hours at 60ºc in oven	7.0	3.81	2.57
6	Photolytic	3 hours in sunlight	-	3.81	-

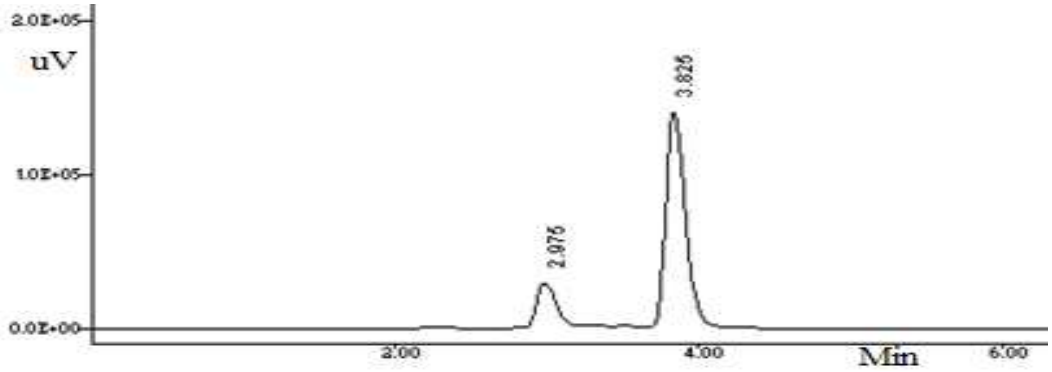

Figure 3: Chromatogram of oxidative degradation (15% H_2O_2).

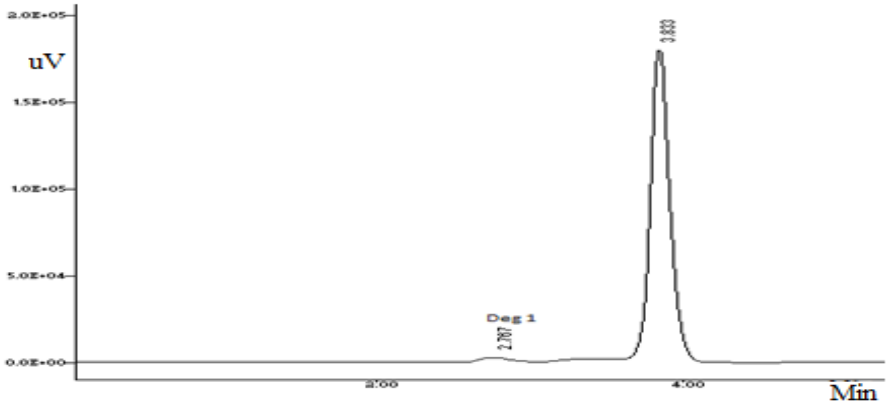

Figure 4: Chromatogram of acid degradation (0.1 N HCl).

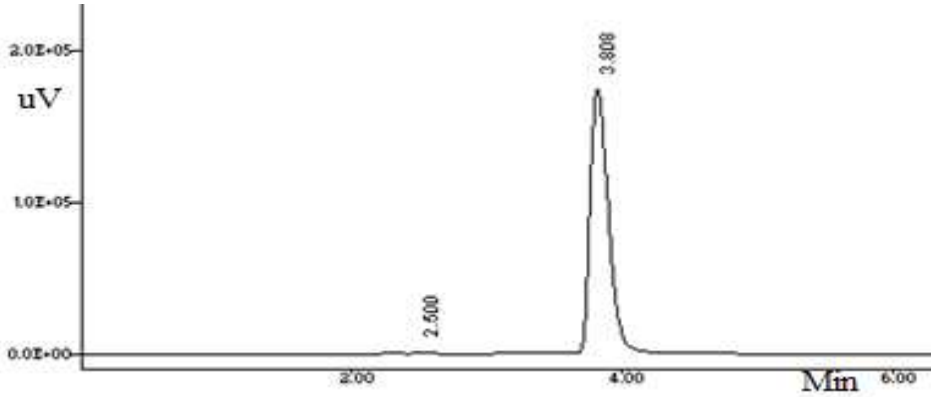

Figure 5: Chromatogram of base degradation (0.1 N NaOH).

Conclusion

A validated stability-indicating HPLC analytical method has been developed for the determination of PRL in bulk and dosage form. The results of stress testing undertaken according to the International Conference on Harmonization (ICH) guidelines reveal that the method was selective and stability-indicating. The proposed method was simple, accurate, precise, specific, and has the ability to separate the drug from degradation products. In the absence of a stability indicating assay in the literature, the proposed method was suitable to use for the routine analysis of PRL in either bulk API powder or in pharmaceutical dosage forms. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments such as LC–MS and or GC–MS. These methods are complicated, costly and time consuming rather than a simple HPLC-UV method. In addition, the HPLC procedure can be applied to the analysis of samples obtained during accelerated stability experiments to predict expiry dates of pharmaceuticals. The method had proved its importance in terms of sensitivity, rapidity, economy in the stability indicating estimation of PRL.

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References

1. J.A. Jakubowski, J.T. Brandt, *Platelets*, 2006, *17*, pp209.
2. S.D. Wiviott, C.H. McCabe, *N. Engl. J. Med.*, 2007, *357*, pp2001
3. M.T. Martin, S.A. Spinler, E.A. Nutescu, *Clin. Ther.*, 2011, *33*, pp425.
4. E.R. Wickremsinhe, K.J. Ruterbories, E.M. Verburg, *Drug. Metab. Dispos.*, 2007, *35*, pp917.
5. T.C. Borole, M.C. Damle, K.G. Bothara, *J. Chem. Pharm. Res.*, 2010, *2*, pp907.
6. G.U. Rani, N. Devanna, *J. Appl. Pharm. Sci.*, 2011, *1*, pp172.
7. R.P. Pulla, B.S. Sastry, Y.R. Prasad, *Int. J. Chem. Res.*, 2011, *2*, pp34.
8. A.A. Kumar, M. Laxmiram, B.B. Kumar, D.G. Sankar, *Int. J. Adv. Pharm. Sci.*, 2011, *2*, pp55.
9. R.K. Viriyala, M.M. Annapurna, S.P. Bisht, F.M. Jena, B.R. Kumar, *Int. J. Comp. Pharm.*, 2011, *2*, pp1.

10. K. Sahu, C. Karthikeyan, N. S. Hari Narayana Moorthy, P. Trivedi, *J. Liq. Chromatogr. Related Technol.*, 2011, *34*, 17, pp1870-1884.
11. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Stability testing of New Drug Substances and Products. QIA (R2), August, 2003.
12. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Validation of Analytical Procedures: Text and Methodology. Q2 (R1).

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