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Development and Validation of UV Spectrophotometric Method for Quantitative Estimation of Prasugrel Hydrochloride in Bulk and Tablet Dosage Forms

B. Famida*, S. Muneer, S. Parveen, S. Praveena, P. Sathya Sowmya, B. Mohammed Ishaq, Hindustan Abdul Ahad

Department of Pharmaceutical Analysis, Balaji College of Pharmacy, Anantapur –515001, AP. India.

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Abstract

Prasugrel Hydrochloride is a novel platelet inhibitor used for acute coronary syndromes planned for percutaneous coronary intervention. The present research work discusses the development and validation of a simple and cost effective UV spectrophotometric method for the quantitative estimation of Prasugrel in bulk and its tablet dosage form. The optimum conditions for the analysis of the drug were established. The maximum wavelength (λ_{max}) was found to be 222 nm in 0.1N HCl as a solvent. The percentage recovery of prasugrel was found to be in range 99-100 %. Beers law was obeyed in the concentration range of 5-50 µg/ml. Calibration curves shows a linear relationship between the absorbance and concentration. The line equation y = 0.018x + 0.0949 and R^2 = 0.9973 was obtained. Validation was performed as ICH guidelines for Linearity, accuracy, precision, LOD and LOQ. The proposed method may be suitable for the analysis of ritonavir in bulk and tablet formulation for routine quality control purposes.

Keywords: Prasugrel, UV spectrophotometer, platelet inhibitor, ICH guidelines

Contents

***Corresponding author**
B. Famida
Dept. of Pharmaceutical Analysis,
Balaji College of Pharmacy,
Anantapur – 515001, AP. India.
E-mail: fahmida456@gmail.com
Manuscript ID: AJMPS2141

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1. Introduction

Prasugrel Hydrochloride chemically is 5-[2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-4, 5, 6, 7-tetrahydrothieno [3, 2- c] pyridin-2-yl acetate hydrochloride [1]. The chemical structure was shown in figure 1. It is a member of the thienopyridine class of ADP receptor inhibitors. These agents reduce the aggregation ("clumping") of platelets by irreversibly binding to P2Y12 receptors. 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 [2, 3]. 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 [4]. Literature review revealed that few analytical methods have been reported like UV & HPTLC, HPLC for its analysis of pure drug [5, 6, 7, 8, 9 and 10]. The purpose of this study was to develop simple, rapid, precise, specific and accurate UV spectrophotometric method for the estimation of the drug in pure and in pharmaceutical dosage forms. The method was validated by evaluation of the linearity, precision, accuracy as per ICH guidelines [11 and 12].

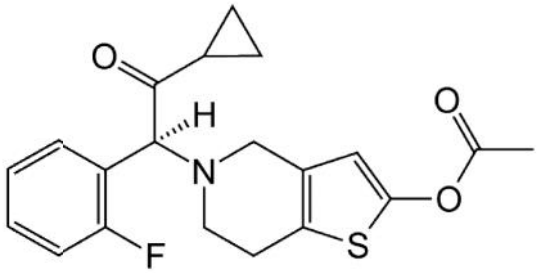


Figure 1. Chemical structure of Prasugrel

2. Materials and Method

Standards and Reagents

Prasugrel hydrochloride sample was obtained as a gift sample from MSN Laboratories, Hyderabad, AP, India. Drug was used without any further purification. HCl was purchased from Merck, Mumbai, India. Distilled water was prepared from Milli-Q water purification system.

Instrumentation

A PG Instruments T-60 model double beam UV/Visible spectrophotometer with spectral width of 1 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Win system software. An Electronic analytical balance (Contech, Mumabi) and an ultrasonic bath were used in the study.

Preparation of standard solution:

An accurately weighed quantity of prasugrel (10 mg) was transferred to a 10 ml volumetric flask and dissolved and diluted to the mark with 0.1 N HCl to obtain standard solution having concentration of prasugrel (1000 µg/ml). Further 0.25 ml was diluted to 10 with 0.1 N HCl to get the final concentration of 25 µg/ml.

Preparation of sample solution (Assay of Tablets):

Take tablet powder equivalent to 10 mg of prasugrel (184.4 mg) was transferred in 10 ml volumetric flask, dissolved and diluted up to mark with 0.1 N HCl. The solution was sonicated for 5 minutes. Filter the solution through Whatman filter paper no.42 and discard first few drops of filtrate. Pipette out 0.25 ml of the above solution in 10ml volumetric flask and diluted to mark with 0.1 N HCl to obtain standard solution having concentration of prasugrel 25µg/ml.

Method validation:

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. The method was validated for different parameters like Linearity, Specificity, Accuracy, Precision, LOD and LOQ.

Linearity:

Various aliquots were prepared form the stock solution (1000 µg/ml) ranging from 2-75 µg/ml. The samples were scanned in UV-VIS Spectrophotometer using 0.1 N HCl as blank. It was found that the selected drug shows linearity between the 5-50 µg/ml.

Accuracy:

The accuracy of the method was determined by preparing solutions of Different concentrations that is 50%, 100% and 150%. 50 and 150 % concentrated solutions were prepared in 6 replicates where as 100 % solutions was prepared in triplicates and the accuracy was indicated by % recovery.

Precision:

The precision of the proposed method was ascertained by actual determination of sex replicates of fixed concentration of the drug within the Beer's range and finding out the absorbance by the proposed method. From these absorbance's, Mean, Standard deviation, % RSD was calculated.

LOD and LOQ:

The LOD and LOQ were determined by measuring the standard deviation of the response and slope. The LOD and LOQ were calculated by using following formulae.

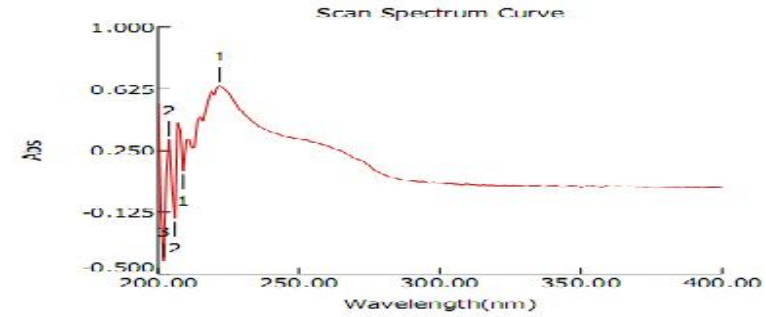
$$LOD = 3X\frac{SD}{Slope} \qquad LOQ = 10X\frac{SD}{Slope}$$

3. Results and Discussion

The solubility of Prasugrel Hydrochloride was determined by various trails. The number of polar and non-polar Solvents tried were distilled water, 0.1M hydrochloric acid, 0.1 N sodium hydroxide, 0.1 N hydrochloric acid, acetate buffer, methanol, ethanol, acetonitrile, benzene, acetone, n-hexane, glacial acetic acid, carbon tetra chloride, toluene, ethyl acetate. From the solubility studies, the drug is very soluble in 0.1 N HCl, methanol, ethanol, acetonitrile. It is sparingly soluble in dimethyl formamide, dichloromethane and it is practically insoluble in distilled water, acetone, benzene, diethyl ether toluene, pet ether, carbon tetrachloride, isopropyl alcohol. From the solubility data 0.1 N HCl was selected as a solvent for UV method because of its solubility, stability, economic and easy availability.

Determination of λ_{max}:

Prasugrel Hydrochloride was dissolved in 0.1 N HCl and made further dilution within the same to get the concentration of 25 µg /ml. The spectrum of Prasugrel Hydrochloride was recorded in a UV-Visible spectrophotometer and the wavelength maximum was found to be 222 nm. It was observed that Prasugrel Hydrochloride was stable for 3 hr and 45 mins. The representative spectrum was shown in figure 2.



No.	P/V	Wavelength(nm)	Abs	Comment
1	Peak	222.00	0.645	
2	Peak	204.00	0.316	
1	Valley	209.00	0.127	
2	Valley	206.00	-0.161	
3	Valley	202.00	-0.420	

Figure 2. Typical Spectrum of Prasugrel in 0.1 N HCl

Linearity:

The calibration curve for prasugrel was drawn by plotting the absorbance versus concentration yielded. A coefficient of regression r^2 = 0.9973 over a concentration range (5-50 µg/ml), the representative linear regression equation for prasugrel y = 0.018x + 0.0949 as shown in fig.3.

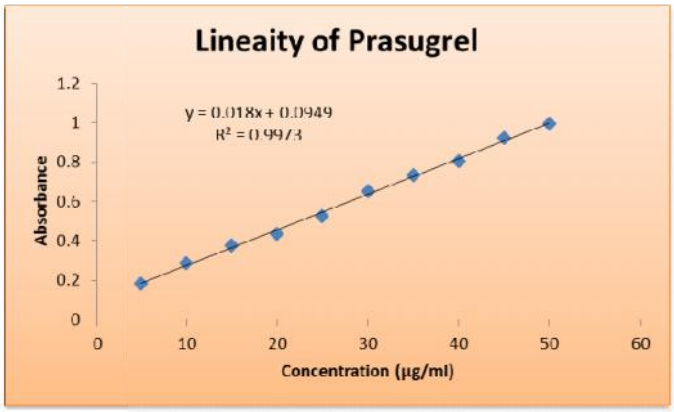


Figure 3. Linearity curve of Prasugrel

Accuracy

The accuracy of the proposed analytical method was determined by recovery experiments. The recovery studies were carried out at three different concentration levels in triplicate (50, 100, and 150%). The Mean % recovery was found to be 99-100 %. The % recovery results of the method were given in table 1.

Table 1. Results of Accuracy

Concentration (µ)	Abs	µg/ml added	µg/ml found	Recovery	% Mean Recovery	Mean Recovery
12.5 µg/ml	0.222	12.403	12.333	99.442	99.144	100.220
	0.217	12.403	12.056	97.203		
	0.221	12.403	12.278	98.994		
	0.224	12.403	12.444	100.338		
	0.223	12.403	12.389	99.890		
	0.221	12.403	12.278	98.994		
25 µg/ml	0.451	24.805	25.056	101.010	100.562	
	0.449	24.805	24.944	100.562		
	0.447	24.805	24.833	100.114		
45 µg/ml	0.674	37.127	37.444	100.856	100.955	
	0.678	37.127	37.667	101.454		
	0.677	37.127	37.611	101.305		
	0.674	37.127	37.444	100.856		
	0.672	37.127	37.333	100.556		
	0.673	37.127	37.389	100.706		

Precision:

The precision of the method was determined by six replicate samples of a standard drug solution. The relative standard deviation was less than 2%. The % RSD of inter and Intraday precision was found to be 1.46 and 1.40 respectively. The results shown in table 2, indicating that the developed UV method was found to be precise.

Table 2. Results of inter and intraday Precision

	Absorbance	
S. No.	Interday	Intraday
1	0.442	0.469
2	0.436	0.475
3	0.451	0.462
4	0.449	0.461
5	0.447	0.477
6	0.454	0.467
Mean	0.45	0.469
SD	0.01	0.007
%RSD	1.46	1.40

Limit of detection and limit of quantification

The LOD is the smallest concentration of the analyte that gives a measurable response (a signal-to-noise ratio of 3). The LOD for prasugrel was found to be 1.67 µg/ml. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (a signal-to-noise ratio of 10). The LOQ of prasugrel was found to be 5.56 µg/ml. It was concluded that the developed method is sensitive.

Assay:

Twenty tablets of marketed tablets of prasugrel was determined for its % purity and Content of the drug in each tablet. The mean % purity was found to be 99.22 %. The values are given in Table 3.

Table 3. Results of Assay

S. No	Abs	% Purity
1	0.442	98.22
2	0.436	96.89
3	0.451	100.22
4	0.449	99.78
5	0.447	99.33
6	0.454	100.89
Average		99.22
SD		1.45
%RSD		1.46

4. Conclusion

A convenient, economical and rapid UV-Spectrophotometric method has been developed for estimation of prasugrel in the tablet dosage form. The assay provides a linear response across a wide range of concentrations. Low intra-day and inter-day % RSD coupled with excellent recoveries. The proposed method was simple, fast, accurate and precise for the quantification of prasugrel in dosage form and in bulk drugs for routine analysis in quality control laboratories.

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