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Research Article

DIFFERENCE SPECTROPHOTOMETRIC ESTIMATION OF PRASUGREL HYDROCHLORIDE IN BULK AND TABLET DOSAGE FORM

Desai Darshali Satishkumar* Barmecha Bharati Subhash, Walode Sanjay Gomaji
Department of Pharmaceutical Chemistry, Sinhgad Institute of Pharmaceutical Sciences, Pune University, Lonavala (Pune), Maharashtra, India

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*Email: desaidarshali2@gmail.com

ABSTRACT
A simple, precise and accurate difference spectroscopic method has been developed for the estimation of prasugrel hydrochloride in bulk and in pharmaceutical dosage form. The proposed method is based on the principle that prasugrel hydrochloride exhibits in two different chemical forms that differs in the absorption spectra in basic and acidic medium. The absorbances were measured in acidic and basic solution separately against reagent blank. Prasugrel hydrochloride has exhibited maximum absorbance at 254 nm and 239 nm in acidic and basic solution respectively. Difference in absorbances between these two maxima was calculated to find out the amplitude. The amplitude plotted against concentration, showed linear response in the concentration range of 5-30 µg/ml with the linear regression value 0.997. The proposed method was applied to pharmaceutical formulations and the common excipients present in the formulation did not interfere in the analysis of the drug. The method was validated as per ICH guidelines and statistical results of analysis were found to be satisfactory.
Key Words: Prasugrel hydrochloride, Difference spectrophotometry, ICH guidelines, Validation.

INTRODUCTION

Prasugrel hydrochloride (PRL), 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 member of third generation thienopyridine class of ADP receptor inhibitors which gets metabolized to an irreversible $P2Y_{12}$ receptor inhibitor. 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]

Literature survey reveals that a few analytical methods like LC-MS[4], HPTLC[5] HPLC[6-7] and some spectrophotometric methods[8-9] have been reported for the estimation of PRL. As per our knowledge there was no any difference spectroscopic method was found to be reported in literature. Hence the main objective of this study was to develop a simple and accurate method for estimation of PRL in bulk and tablet dosage form.

MATERIALS AND METHODS

Chemicals and reagents

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.

Freshly prepared 0.1N sodium hydroxide, 0.1N hydrochloric acid and distilled water were used in the present analysis.

Instruments

A Jasco V-530 UV-Visible double beam Spectrophotometer with 1 cm matched pair quartz cell was used for all spectral measurements. The absorption spectrum was recorded in the wavelength range of 200-400nm throughout the experimental work.

Difference spectroscopy

Selectivity and accuracy of spectrophotometric analysis of sample containing absorbing interference may be markedly improved by the technique like difference spectrophotometry. The essential feature of difference spectrophotometric assay is that the measured value is the difference in absorbance (A) between two equimolar solutions of the analyte in different chemical forms which exhibit different spectral characteristics. This is simplest and most commonly employed technique for altering the spectral properties of an analyte by the adjustment of pH by means of aqueous solution of acid, alkali or buffer.

Preparation of standard stock solution

Standard stock solution was prepared by dissolving the 10 mg of PRL in 10 ml methanol. 2.5 ml of resultant solution was pipette out and diluted to 25 ml with methanol to get final concentration of about 0.1mg/ml.

Preparation of working standard solution

Working standard solution was prepared by series of dilutions of 0.5, 1, 1.5, 2, 2.5 and 3 ml of standard stock solution to 10 ml of 0.1N HCl and 0.1N NaOH separately to get concentrations of 5, 10, 15, 20, 25, 30µg/ml for PRL. These solutions were used to determine absorption maxima, beer's law and linearity.

Determination of λ max

The above prepared solutions were scanned over the range of 400 nm to 200 nm against reagent blank. From the spectrum obtained, the λmax was found to be 254 nm (Figure 3) and 239 nm (Figure 4) in acidic and basic solutions respectively. Difference in absorbances between these two maxima was calculated to find out the amplitude.

METHOD VALIDATION

The method was validated for different parameters like linearity, accuracy and precision according to the ICH guidelines[10].

Linearity

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, series of dilutions were made to 10 ml with 0.1 N HCl and 0.1 N NaOH separately to get concentrations of 5, 10, 15, 20, 25, 30µg/ml for PRL. The absorbances were measured at 254 nm and 239 nm in acidic and basic solutions respectively against reagent blank. Calibration curve was

prepared by plotting concentration versus difference in absorbance and found to be linear in the concentration range of 5-30 µg/ml (Figure 2).

Precision

Precision of the method was determined by performing interday variation, intraday variation and repeatability studies and expressed in forms of %RSD. In interday variation, the absorbances of working standard solutions of PRL (5-30 µg/ml) were measured on three consecutive days. In intraday variation the absorbances were measured three times a day. In repeatability study, six determinations of the fixed concentration of both acidic and basic solutions of the drug were analyzed separately. The results of precision data are given in Table 2.

LOD and LOQ

In this study, 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 results of validation parameters are shown in Table 1.

Analysis of tablet formulation

Twenty tablets were accurately weighed and triturate thoroughly to get fine powder. The powder equivalent to 25 mg of PRL was weighed and transferred into 25 ml volumetric flask. The contents of the flask were dissolved in the 10 ml of the methanol with the aid of ultrasonication for 10 mins. The solution was filtered through whatmann filter paper no. 41 and volume was made up to 25 ml with methanol. From the resultant solution, further dilutions were prepared with 0.1 N HCl and 0.1 N NaOH separately to get final concentration of PRL. The absorbances were measured at selected wavelengths and the concentration of each analyte was determined with the equation obtained from calibration curve.

Accuracy (Recovery studies)

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% in which the amount of marketed formulation was kept constant (10 µg/ml) and the amount of pure drug was varied that is 2 µg/ml, 5 µg/ml and 8 µg/ml for 80%, 100% and 120% respectively. The amount of PRL recovered was estimated by applying obtained values to the regression line equation. The results of % recovery are given in Table 3.

RESULTS AND DISCUSSION

Prasugrel HCl obeys Beer's law in the concentration range of 5-30µg/ml with the linear regression value 0.997. The % RSD for intraday, interday precision and repeatability study, less than 2% and recovery study with SD less than 2%, indicates that the method is precise and accurate. Further it was concluded that, there was no any interference of excipients in analysis of marketed formulation. Therefore the present method can be employed for the estimation of prasugrel in pharmaceutical formulations.

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REFERENCES

1. Jakubowski JA, Brandt JT. Platelet inhibitory activity and pharmacokinetics of prasugrel (CS-747), a novel thienopyridine $P2Y_{12}$ inhibitor: A single ascending dose study in healthy humans. Platelets 2006; 17(4): 209–217.
2. Wiviott SD, McCabe CH. Prasugrel versus clopidogrel in patients with acute coronary syndromes. N Engl J Med 2007; 357 (20): 2001–2015.
3. Martin MT, Spinler SA, Nutescu EA. Emerging antiplatelet therapies in percutaneous coronary intervention: A focus on prasugrel. Clin Ther 2011; 33(4): 425-442.
4. Wickremsinhe ER, Ruterbories KJ, Verburg EM. Stereoselective metabolism of prasugrel in humans using a novel chiral liquid chromatography-tandem mass spectrometry method. Drug Metab Dispos 2007; 35(6): 917-921.
5. Borole TC, Damle MC, Bothara KG. Development and validation of stability indicating HPTLC method for determination of prasugrel. J Chem Pharm Res 2010; 2(4): 907-913.
6. Rani GU, Devanna N. Analytical method development and validation of prasugrel in bulk and its pharmaceutical formulation using HPLC. J Appl Pharm Sci 2011; 01(07): 172-175.
7. Pulla RP, Sastry BS, Prasad YR. Estimation of prasugrel in tablet dosage form by RP- HPLC. Int J Chem Res 2011; 2(3): 34-36.
8. Kumar AA, Laxmiram M, Kumar BB, Sankar DG. Spectrometric determination of prasugrel in bulk and in its pharmaceutical formulation by UV method. Int J Adv Pharm Sci 2011; 2 (1): 55-57.
9. Viriyala RK, Annapurna MM, Bisht SP, Jena FM, Kumar BR. Validated new spectrophotometric methods for the estimation of prasugrel in bulk and pharmaceutical dosage forms. Int J comp pharm 2011; 2(06): 1-3.
10. ICH, Q2 (R1), Validation of Analytical Procedures: Text and Methodology International conference on Harmonization, Geneva, 2005, 1-13.

TABLE 1: Validation parameters of prasugrel HCl

Parameters	Prasugrel hydrochloride
λmax (nm)	254nm (0.1 N HCl) 239nm (0.1N NaOH)
Linearity range	5-30µg/ml
Correlation coefficient	0.997
Regression equation (Y = mx + c)	y = 0.007x - 0.029
Slope (m)	0.007
Intercept (c)	0.029
LOD	0.676µg/ml
LOQ	2.02µg/ml

TABLE 2: Precision data

	Fortified amount (mg)	Amount found (mg)	% RSD
Intraday (n = 3)	10	9.96	0.64
	15	14.89	0.54
	25	24.90	1.09
Interday (n = 3)	10	9.87	0.79
	15	14.93	0.68
	25	24.82	0.53
Repeatability (n = 6)	15	14.87	0.73

TABLE 3: Results of % recovery in tablet formulation

Formulation	Estimation of prasugrel in tablet formulation			%Recovery of prasugrel				
	Label claim (mg)	Amount found (mg)	% RSD	% of drug added	Concentration (µg/ml)		% of drug recovered	% RSD
					Pure drug	Formulation		
Tab. Prasusafe	5	4.91	0.6	80%	2	10	97.5	0.87
	5	4.89		100%	5	10	100.4	0.62
	5	4.85		120%	8	10	104.25	0.43

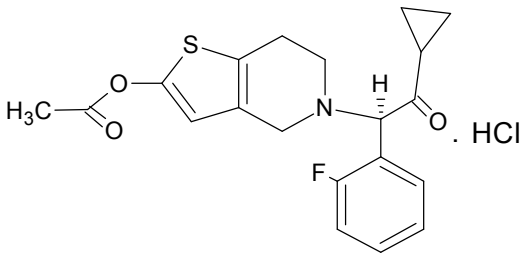

Figure 1: Chemical structure of prasugrel hydrochloride

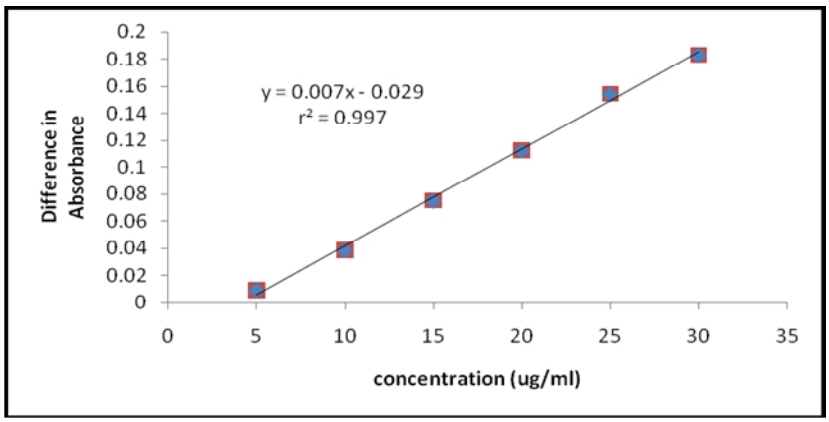

Figure 2: Calibration curve of prasugrel hydrochloride

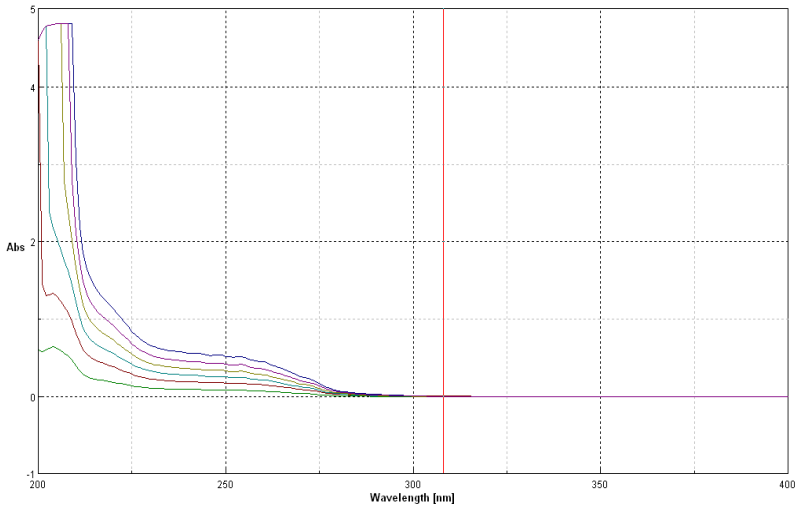

Figure 3: Overlay spectra of PRL with 0.1N HCl at 254nm

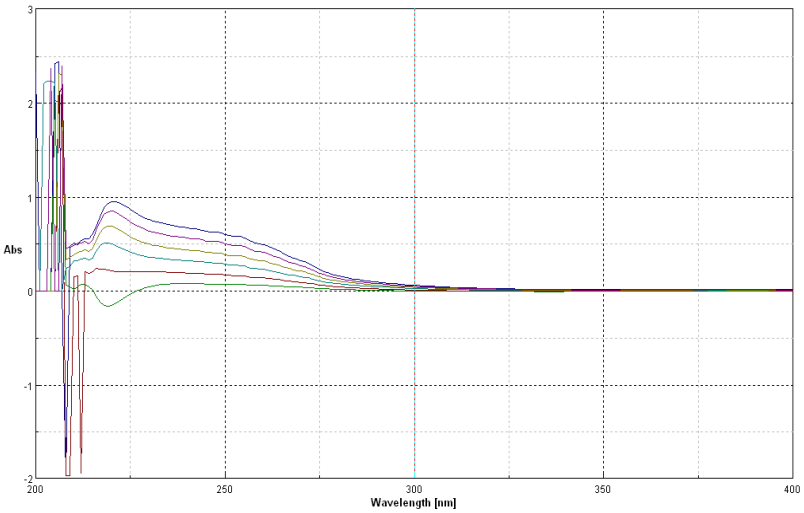

Figure 4: Overlay spectra of PRL with 0.1N NaOH at 239nm

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