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DEVELOPMENT AND VALIDATION OF COLORIMETRIC METHOD FOR THE DETERMINATION OF PRASUGREL HCL IN BULK AND DOSAGE FORM

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Abstract

Simple, precise and accurate visible spectrophotometric method was developed for the estimation of Prasugrel Hcl in bulk drug and in pharmaceutical formulation. The proposed method was based on the reduction of Fe^{3+} to Fe^{2+} by Prasugrel Hcl, the resulting Fe^{2+} reacts with 1,10-phenanthroline to give a soluble yellowish-orange complex in neutral medium, which shown the maximal absorption is at the wavelength 510 nm. The linear relationship between the absorbance and the concentration of prasugrel Hcl was in the range of 50-300µg/ml with a correlation coefficient is r^2=0.999. This new method has offered a determination of prasugrel Hcl drug without any interference with excipients indirectly with a high accuracy for the analytical results. The method was found to be simple, economical, accurate and reproducible and can be used for routine analysis of prasugrel Hcl in bulk and in pharmaceutical formulation.

Keywords: Prasugrel Hcl, $Fecl_3$, 1,10-phenanthroline, Colorimetry.

Introduction

Prasugrel hydrochloride chemically is 5-[2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-4,5,6,7-tetrahydrothieno [3,2-c] pyridine-2-yl acetate hydrochloride. 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 Hcl 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. Literature review revealed that few analytical methods have been reported like UV & HPTLC, HPLC, LC for its analysis of pure drug. The purpose of this study was to develop simple, accurate, economical and precise visible method for the estimation of the drug in pure and in pharmaceutical dosage form. The method validated by evalution of linearity, precision, accuracy as per ICH guidelines.

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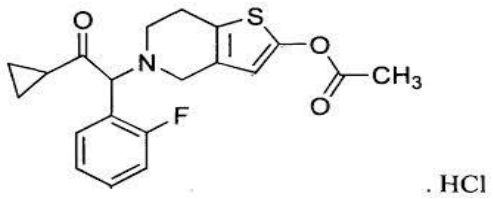


Fig. No. 01: Chemical Structure of Prasugrel Hydrochloride (PRA)

Materials and methods

Apparatus

Shimadzu UV-1800 UV-Visible spectrophotometer was employed with spectral bandwidth of 1nm attach with computer loaded with shimadzu UV PC software (UV Probe) version 2.31 and using a pair of 10mm matched glass cells.

Drug and Chemicals

Pharmaceutical grade Prasugrel Hcl was procured from Hetero pharmaceuticals, Hyderabad, India. It was reported to be 99.99% pure and was used without further purification. 1,10-phenanthroline A.R, Qualigens. $Fecl_3$ A.R, Fischer. Orthophosphoric acid A.R, Merk and double distilled water was used through the study. Tablets used for analysis was PRASUDOC manufactured by LUPIN Laboratories limited, Mumbai, India containing Prasugrel hydrochloride 10 mg per tablet.

Reagents and Standards

1, 10-phenanthroline solution (0.198% w/v, 1.0 x 10^{-2}M): Prepared by dissolving 198 mg of 1,10-phenanthroline in 100ml of 0.1N hydrochloride.

$Fecl_3$ stock solution (0.162% w/v, 1M) : Weigh accurately 162 mg of anhydrous ferric chloride and dissolved in 100 ml of distilled water. 33.3 ml of above stock solution was further diluted to 100ml with distilled water.

Orthophosphoric acid solution (2 x 10^{-1}M): 1.3 ml of orthophosphoric acid was diluted 100ml with distilled water.

Standard stock solution of Prasugrel Hcl

A stock solution of Prasugrel Hcl was prepared by accurately weighed 100 mg of pure drug and transfer into 100 ml volumetric flask and dissolved in10 ml methanol and final volume made to 100 ml with methanol (1mg/ml). From this 100 µg/ml solution was prepared in distilled water.

Procedure for Method Development

Aliquots of standard solution containing 50-300 µg/ml were transferred into a series of 10ml volumetric flask and 1 ml of 0.333 M ferric chloride was added to each flask. Then 2 ml of PTL was added to all flasks and the volume was equalized with distilled water, boiled for 30 min and cooled to room temperature and 2 ml of OPA was added to all and final volume was made upto 10ml with distilled water. The absorbance was measured at 510 nm against corresponding reagent blank. The amount of prasugrel hydrochloride in sample was estimated from corresponding calibration graph.

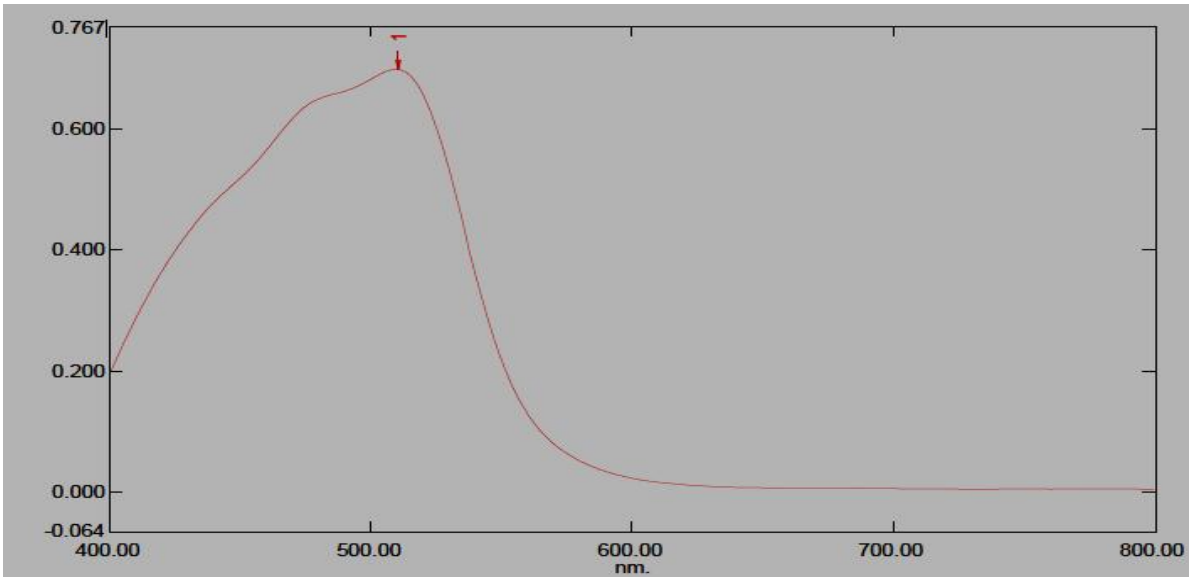


Fig. No. 02: Determination of λmax with PTL

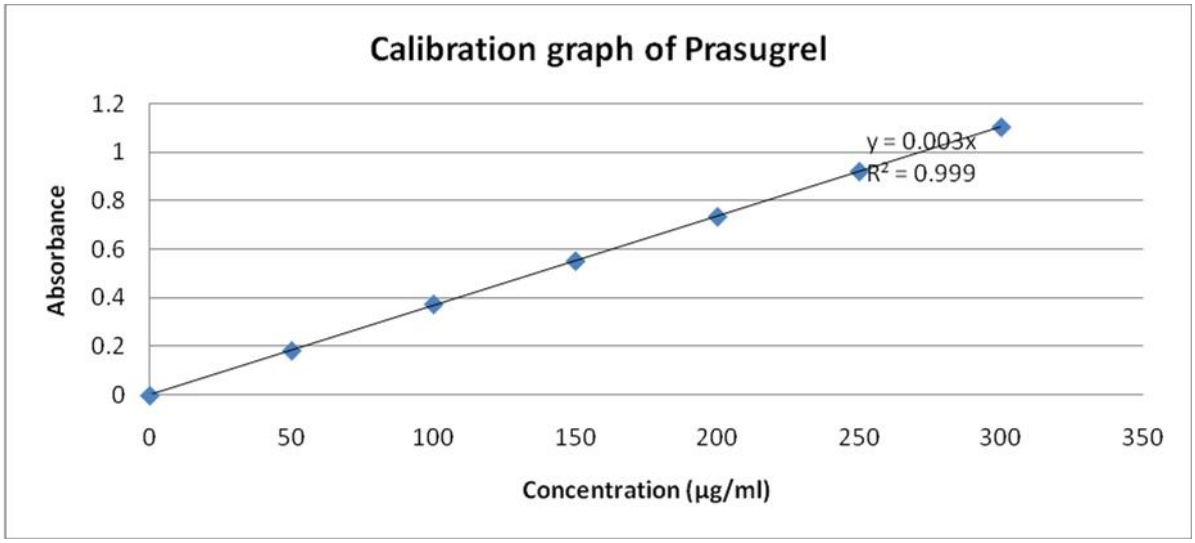


Fig. No. 03

Estimation of Pharmaceutical Formulation

The formulation containing 10 mg and 5 mg of Prasugrel Hcl tablets was analysed. In this procedure, 20 tablets were accurately weighed and powedered. The powder equivalent to 10 mg of Prasugrel was transferred to 10 ml volumetric flask and 10 ml of methanol is added to it. This mixture was then filtered through whatmann filter paper. Appropriate volumes of this filtered solution were taken according to the procedures described earlier for method development. Procedure is repeated as mentioned above for the formulation containing 5 mg of prasugrel Hcl.

Table No. 01: Result of Marketed Formulation Analysis

S. No	Label claim (mg)	Mean (n=6)	Std. deviation	%RSD
1	10 mg	99.10	0.4081	0.0684
2	5 mg	99.05	0.0408	0.0068

Validation

The methods were validated according to ICH guidelines to study linearity, accuracy and precision.

Linearity

In order to find out the linearity range of the proposed Visible method, studies were carried out by plotting absorbances of analyte against concentrations of the analyte. A good linear relationship (r=0.999) was observed between the concentrations of Prasugrel HCl and the corresponding absorbance. The regression of Prasugrel Hcl concentration over its absorbance was found to be y=0.003x+C (where y is the absorbance and x is the concentration of Prasugrel HCl). The slope, intercept and the correlation coefficient of the drug were shown in table no.2.

Table No. 02: Linearity studies of the proposed methods

S. No	Parameter	Value
1	Linearity (µg/ml)	50-300
2	Linearity Equation	Y= 0.003X + 0.003667
3	Slope ± SD	0.003±0.000733
4	Intercept ± SD	0.003667±0.000066
5	Correlation coefficient	0.999

Precision

Precision is the level of repeatability of results as reported between samples analyzed on the same day (Intra- day) and samples run on three different days (Inter- day). To check the intra- day and inter- day variation of the method, solution containing 100 µg/ml Prasugrel HCl were subjected to the proposed visible method of analysis and the recoveries obtained were noted. The precision of the proposed method i.e. the intra and inter –day

variations in the absorbance of the drug solutions was calculated in terms of % RSD and the results were presented in the table. Statistical evaluation revealed that relative standard deviation of drugs at different concentration levels for six times was less than 2.0. (intra day -0.48, inter day- 0.473).

Table No. 03: Intraday and Interday data of pharmaceutical formulation

S.No	Label claim mg/ml	Intraday precision %RSD (n=6)	Interday precision %RSD (n=6)
			Day I
1	10	1.88	1.64
2	5	1.75	1.59

Accuracy

Accuracy is expressed as the closeness of the results from standard samples to that of the actual known amounts to determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (2.5mg, 5mg, 7.5 mg) of bulk sample to the pre-analyzed formulation. The solutions were suitably diluted in the range and then each of the dilution was observed six times. The percentage recovery of the drug was calculated. The results were shown in the Table No.4.

Table No. 04: Accuracy studies of the proposed method

S.No	Labeled amount (mg)	Spiked level	Amount of standard drug added to pre-analyzed formulation(mg)	Amount recovered mean ± s.d ((n=6) (mg)	% recovery
1.	10	50	2.5	2.46±0.02036	99.475
2.	10	100	5	4.97±0.024218	99.737
3.	10	150	7.5	7.48±0.024468	99.312

Results and discussion

The main purpose of this study was to establish simple spectrophotometric methods for the determination of Prasugrel Hcl in pure form and in its pharmaceutical dosage form. Prasugrel Hcl contains a oxomethyl group, which could be oxidized with $Fecl_3$ in neutral medium. When treated with known excess of oxidant, Prasugrel Hcl undergoes oxidation, giving products of oxidation (inclusive of reduced form of oxidant, Fe^{2+} from Fe^{3+}), besides unreacted oxidant. It is possible to estimate the drug content colorimetrically, which is equivalent to either the reacted oxidant or reduced form of oxidant formed. The reduced form of Fe^{3+} has a tendency to give colored comp[lex on treatment with PTL. The first step in the method mentioned above is the oxidation of Prasugrel Hcl with the oxidant,

$C_{20}H_{20}FNO_3S$ + Fe^{3+} → $C_{20}H_{20}FNO_2S$ + Fe^{2+} + Fe^{3+}
+OH (Excess) oxidation products (Reduced form of oxidant) (unreacted)

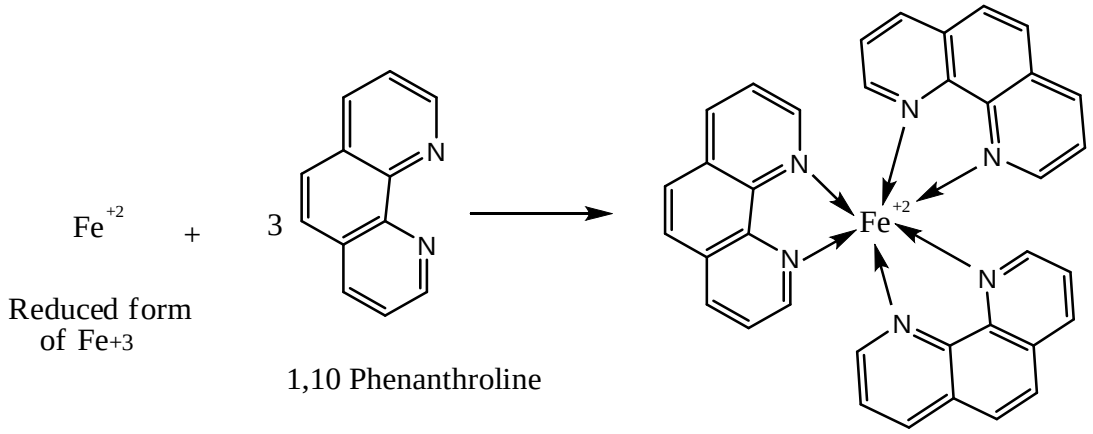
In this method, as Fe^{3+} interferes, even though to a little extent in the determination of Fe^{2+}, the reactivity of the interfering entity has to be made insignificant by complexing it with o-phosphoric acid.

Fe^{3+} + o-phosphoric acid → Complex
(Unreactive)

The second step concerns with the estimation of the reduced form of oxidant with appropriate chromogenic agent i.e. 1,10-phenanthroline and gives yellowish-orange colored complex.

Conclusion

The sensitive and accurate visible spectro - photometric method for the quantitation of Prasugrel Hcl have been developed and validated based on current ICH guidelines. The procedure is based on well established and characterized redox and complex formation reactions and use cheaper and readily available chemicals. The method has been demonstrated to be free from rigid experimental conditions. These merits besides, the use of simple and inexpensive chemicals and instruments, recommend the use of the methods in routine quality control laboratories.



(Fe+2 - 1,10 Phenanthroline)complex

Reaction of Fe^{3+} with of PTL

Table No. 05: Optical characteristics of Prasugrel

S.No	Parameter	Value
1	Wave length (λ) (nm)	510
2	Beer's law limits (µg/ml)	50-300
3	Sandell's sensitivity (µg cm^{-2} / 0.001 absorbance unit)	30 µg/ml
4	Regression equation (Y = mx+c)	0.003x+ 0.003667
5	Slope ± SD	0.003± 0.000733
6	Intercept ± SD	0.003667± 0.000066
7	Correlation coefficient (r^2)	0.999
8	Color stability	60 minutes
9	Molar extinsion coefficient (L/Mol. cm)	272.10

Acknowledgments

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