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International Journal of Current Research
Vol. 3, Issue, 11, pp.418-420, October, 2011

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

COLORIMETRIC ESTIMATION OF PRASUGREL IN BULK AND PHARMACEUTICAL FORMULATIONS

[1]Paramdeep Bagga, [1]Mohammed Salman Khan, [2]Mohammed Haris Siddiqui, [1]Tariq Mahmood and [1]Hefazat Hussain Siddiqui*

[1]Azad Institute of Pharmacy and Research, Lucknow-226002
[2]Department of Biotechnology, Integral University, Lucknow-226026. India

ARTICLE INFO

Article History:
Received 27th June, 2011
Received in revised form
25th August, 2011
Accepted 29th September, 2011
Published online 30th October, 2011

Key words:
Prasugrel, Colorimetry,
Absorbance, Validation,
Folin-Ciocalteu reagent,
Detection Limit.

ABSTRACT

A simple and cost effective colorimetric method is described for the determination of prasugrel in pure form and in pharmaceutical formulations. The determination of the drug in pharmaceutical formulations is based on the drugs redox reaction with Folin-Ciocalteu reagent (Phenol reagent). The linearity range for prasugrel of blue chromogen produced at wavelength of detection 725 nm was obtained as 10–50 µg/ml. The linear regression equation obtained by least square regression method, were Y =0.0173.X – 0.1671, where Y is the absorbance and X is the concentration (in µg/ml) of pure drug solution. The absorbance was found to increase linearly with increasing concentration of prasugrel, which is corroborated by the calculated correlation coefficient value of 0.999. The limit of detection and limit of quantification was found to be 1.6145µg/ml & 4.8925 µg/ml respectively. The validity of the described procedure was assessed. Statistical analysis of the result has been carried out revealing high accuracy and good precision. The proposed method was successfully applied to the determination of prasugrel in pharmaceutical formulations without any interference from common recipients.

INTRODUCTION

Prasugrel chemically is 5-[2-cyclopropyl-1-(2- fluorophenyl)-2-oxoethyl]-4, 5, 6, 7-tetra hydrothieno [3, 2- c] pyridin-2-yl acetate (Figure 1). It is a member of the thienopyridine class of ADP receptor inhibitors, like ticlopidine and 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 [(1, 2, 3)]. Literature survey revealed that some analytical methods like UV [(4)], HPLC [(5)], HPTLC [(6)] & LC-MS [(7, 8)] have been reported for the estimation of prasugrel. The aim of the study is to develop a simple, sensitive, cost effective, accurate and precise colorimetric method for determination of prasugrel in pharmaceutical formulations and bulk drugs. In the present investigation we developed colorimetric method for the determination of the drug in pharmaceutical formulations based on the drugs redox reaction with Folin-Ciocalteu reagent (Phenol reagent). [(9, 10)]

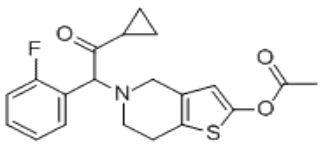

Fig. 1: Structure of Prasugrel

***Corresponding author:** hefazats@hotmail.com

EXPERIMENTAL

Instruments

A Shimadzu UV-Visible Spectrophotometer (UV-1700) with a matched pair of 10 mm quartz cells were used forexperimental purpose. Shimadzu AUX-220 balance was used for weighing the samples.

Chemicals and Reagents

Solvents & Reagants	Manufacturers
Methanol(AR Grade)	Rankem
Water (Milli Q grade)	Millipore water filter
Prasugrel Hydrochloride	Torrent Pharmaceuticals, Ahemdabad India
Phenol Reagent	Fisher Scientific, Mumbai.
Sodium Hydroxide	Fisher Scientific, Mumbai.

Apparatus/Instruments

Name	Model/Code	Manufacturer/Supplier
UV-Visible Spectrophotometer	UV-1700	Shimadzu,Japan
Micropipettes	FAA-1000	Microlit,Lucknow
Micro-pipette tips	521020	Tarsons
Centifuge Machine	DB-5586	Decibel

MATERIALS AND METHODS

Preparation of 1N NaOH

4 gm of NaOH was accurately weighed in a 100ml volumetric flask, dissolved in 10 ml of double distilled water & final volume was made to 100ml.

Preparation of 1N Phenol reagent

10 ml of standard phenol reagent was taken and diluted to 20ml with double distilled water.

Preparation of Standard Stock Solutions

Standard stock solution (primary) was prepared by dissolving 50 mg of prasugrel in 10 ml of methanol and diluted to make the final volume of 50ml (1000µg/ml) and was stored at +4ºC during the study.

Preparation of calibration standard solutions & QC samples

Suitable aliquots of the drug solution (0.1to 0.5ml) were taken in a series of 10 ml volumetric flasks. To each flask was added 0.8ml of FCR solution and 6 ml of double distilled water. All flasks were shaken well for at least 5 min., followed by addition of 1 ml of NaOH solution. Finally volume was made up to the mark with purified water to get standard solutions of 10-50µg/ml. The volumetric flasks were stored for 45min in dark. The absorbance of blue coloured chromogen was measured at 725 nm against reagent blank.

Procedure for formulatons

Twenty tablets of prasugrel were accurately weighed, finely powdered and mixed. A portion of the powder equivalent to 10mg of prasugrel was transferred into a 10 ml volumetric flask and 3mL of methanol was added. The content of the flask was sonicated for 15 min and diluted to volume with methanol. This solution was centrifuged for 15 min at 5000 rpm to separate out the insoluble excipients. Suitable aliquots of the clear supernatant was taken in triplicate and treated in the similar manner as that of standard solutions. The absorbance of blue coloured chromogen was measured at 725 nm against reagent blank. The amount of prasugrel per tablet was calculated using the calibration curve.

RESULTS AND DISCUSSION

VALIDATION

Linearity range

Under the experimental conditions, the linearity range was found to be 10–50 µg/ml for prasugrel at 725 nm The statistical analysis (ICH guidelines 1996; USP 2000) of data obtained for the estimation of prasugrel in pure solution indicated high level of accuracy for the proposed methods as evidenced by the low values of standard deviation and coefficient of variation (Table 1). The linear regression equation obtained was Y =0.0173.X + 0.1671, where Y is the absorbance and X is the concentration (in µg/ml) of pure drug solution (Figure2). Linearity of the regression equation and negligible scatter of points for the two drugs by the proposed methods were demonstrated from the highly significant ($p > 0.05$) correlation coefficient value. The reported slope values without intercept on the ordinate, at 95% confidence limits, suggested that the calibration lines of prasugrel solutions in methanol did not deviate from the origin as the above-obtained values fall within the confidence limits. (Table 2).

Table 1: Linearity table of prasugrel in Working Standard

Conc.(µg/ml)	Mean Abs*	Std. Error	%CV
10	0.0058±0.0001	0.0001	1.9795
15	0.0835±0.0016	0.0009	1.9011
20	0.1775±0.0024	0.0014	1.3415
25	0.2795±0.0030	0.0017	1.0751
50	0.6939±0.0037	0.0021	0.5342

*Average of three determinations with standard deviations
CV – Coefficient of variation.

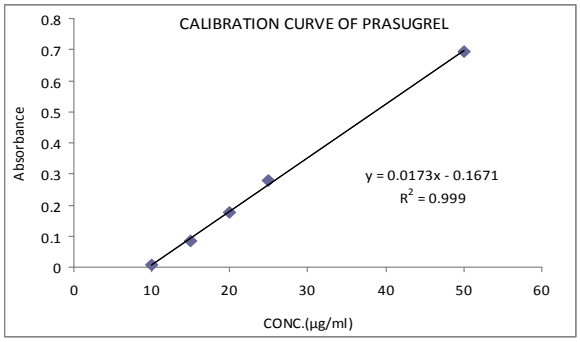

Fig. 2: Linearity curve of Prasugrel

Table 2: Regression analysis of data for the estimation of prasugrel from standard solution

Statistical Parameters	Prasugrel
Regression equation	Y=0.0173X-0.1671
Correlation coefficient	0.999
Molar absorptivity, L mol–1 cm–1	2.807×10^{3}
Standard error of slope	3.211×10^{-4}
Standard error of intercept on ordinate	8.910×10^{-3}
Standard error of estimate	1.00×10^{-2}
95% confidence interval of slope	1.63×10^{-2},1.83×10^{-2}
95% confidence interval of intercept	-1.95×10^{-1},-1.39×10^{-1}

Accuracy

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of bulk samples of prasugrel within the linearity range were taken and added to the pre-analyzed formulation of concentration 20 µg/ml. From that percentage recovery values were calculated. The results were shown in Table (3).

Precision

The precision of the proposed method was ascertained by actual determination of six replicates of fixed concentration of the drug within the Beer's range and finding out the absorbance by the proposed method. From this absorbance, mean, standard deviation and % RSD was calculated.

Detection Limit & Quantitation Limit

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The LOD and LOQ were calculated by using the relation $3.3\sigma/S$ and $10\ \sigma\ /S$ respectively, where σ is the

standard error of estimate and S is the slope. Calculated values of limit of detection (LOD) and quantitation (LOQ) for prasugrel were found to be 1.6145 and 4.8925 µg/ml respectively.

Table 3: Accuracy Studies

Sample ID	Concentration (µg/ml)		% Recovery	Statistical analysis
	Pure drug	Formulation		
S1:80%	16	20	99.8552	Mean=100.1854
S2:80%	16	20	100.0807	
S3:80%	16	20	99.4847	S.D=0.4698
S4:80%	16	20	100.5639	
S5:80%	16	20	100.7410	
S6:80%	16	20	100.3867	%RSD=0.4689
S7:100%	20	20	101.5096	Mean=100.9395
S8:100%	20	20	101.6256	
S9:100%	20	20	101.6546	S.D=0.8507
S10:100%	20	20	100.3500	
S11:100%	20	20	99.5382	
S12:100%	20	20	100.9588	%RSD=0.8428
S13:120%	24	20	100.0432	Mean=100.8295
S14:120%	24	20	100.8734	
S15:120%	24	20	100.6362	S.D=0.4430
S16:120%	24	20	101.1633	
S17:120%	24	20	101.2556	
S18:120%	24	20	101.0052	%RSD=0.4394

Table 4: Precision Readings

Concentration (µg/ml)	Optical Density	Statistical Analysis
30	0.3518	Mean=0.3498
30	0.3546	
30	0.3511	S.D=.0033
30	0.3478	
30	0.3467	
30	0.3465	%RSD=.9328

Table 6: Analysis of pharmaceutical formulation

		UV Spectrophotometric Method		
	Labelled	Amount	% Drug	%RSD
Formulation	Amount(mg)	Recovered* (mg)	Recovered	
Tablet	5mg	4.9943±0.0583	99.8867	1.1678

*Mean of six determinations

Analysis of pharmaceutical formulations

The optimized spectrophotometric method was applied to the direct determination of Prasugrel in tablet using calibration curve method without any sample extraction or filtration. From the absorbance value, the drug content per tablet (on an average weight basis) was calculated. The results are shown in Table5.

CONCLUSION

The proposed method is economic, simple, sensitive, reproducible, accurate &no interference is observed due to common excipients of tablet. Thus the proposed method can be used for the routine analysis of prasugrel in bulk as well as in its pharmaceutical preparations.

ACKNOWLEDGEMENT

Authors are thankful to Prof S.W.Akhtar, Honourable Vice Chancellor Integral University, Lucknow for providing necessary facilities for the work. The authors also thank Torrent Pharmaceuticals, Ahemdabad, India for providing sample of Prasugrel as gift.

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