DEVELOPMENT, ESTIMATION AND VALIDATION OF PRASUGREL IN BULK AND IN ITS PHARMACEUTICAL FORMULATION BY UV-VIS SPECTROSCOPIC METHOD.

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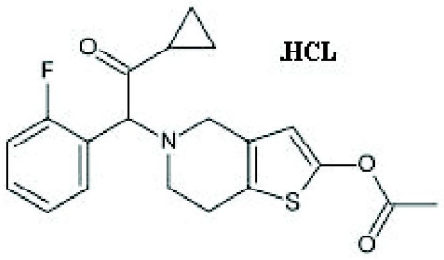
ABSTRACT

The present study describes a simple, accurate, precise, specific and highly sensitive method for the determination of bosentan present in pharmaceutical dosage forms. The method is validated for the determination of prasugrel in bulk and tablet dosage form. Prasugrel is a agent which reduces the aggregation (“clumping”) of platelets by irreversibly binding to P2Y12 receptors. The solvent used is 0.1N Hcl and the ëmax or the absorption maxima of the drug was found to be 249nm. A linear response was observed in the range of 1-50¼g/ml with a regression coefficient of 0.9993. The method was then validated for different parameters as per the ICH (International Conference for Harmonization) guidelines. This method can be used for the determination of Prasugrel in quality control of formulation without interference of the excipients.

INTRODUCTION

Prasugrel [1] chemically is 5-[2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-4,5,6,7-tetra hydrothieno [3,2-c] pyridin-2-yl acetate. It is a member of the thienopyridine class of ADP receptor inhibitors, like ticlopidine and clopidogrel.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 disease [2, 3, 4].

Literature survey revealed that some analytical methods like LC-MS [5,6] have been reported for the estimation of Prasugrel and also HPTLC method was reported for its analysis [7] and no spectrophotometric method was reported . Hence the objective was to develop a simple, sensitive, accurate and precise method for determination of Prasaugrel by uv-visible spectrophotometric method in the pure form and its tablet formulation as per ICH guide lines [8].



5-[2-cyclopropyl-1-(2-fluorophenyl)-2-oxoethyl]-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl acetate Hydrochloride

MATERIALS AND METHODS

The instrument used for the study was ELICO UV -Visible spectrophotometer with 1cm matched pair quartz cells. The solvent used was 0.1N Hcl

METHOD DEVELOPMENT

Solubility Test: Solubility test for the drug prasugrel was performed by using various Solvents. The solvents include Water, Methanol, Ethanol, Acetonitrile, and 0.1N Hydrochloric Acid (HCl), 0.1 N Sodium Hydroxide (NaOH) and Chloroform. However, 0.1N Hydrochloric Acid (HCl) was chosen as a solvent for developing the method.

Determination of ëmax

Preparation of Stock Solution

Standard stock solution of prasugrel was prepared by dissolving 10mg of Prasugrel in 10ml of 0.1N HCl to produce a concentration of 1000¼g/ml. 1ml of this stock solution was taken and then diluted up to 10ml by using 0.1N Hcl to produce a concentration of 100¼g/ml which is the standard stock solution.

Preparation of Working Standard Solution

From the above stock solution, Working standard solutions are prepared from 10 to 100 ppm and the solutions are scanned in spectrophotometer from 200 nm to 400 nm.All the solutions are having the ëmax at 249 nm (fig.1).

Preparation of Calibration Curve

1ml of the 100¼g/ml solution was diluted to 10ml by using 0.1N Hcl to produce 10¼g/ml solution. 2ml, 3ml, 4ml and 5ml of 100¼g/ml solution were diluted to 10ml using 0.1N Hcl to produce20¼g/ml, 30¼g/ml, 40¼g/ml, and 50¼g/ml solutions respectively. Then the construction of calibration curve was done by taking the above prepared solutions of different concentration ranging from 10-50¼g/ml. Then, the calibration curve was plotted by taking concentration on x-axis and absorbance on y-axis (in fig.2). The curve showed

linearity in the concentration range of 10-50¼g/ml. The correlation coefficient (r^2) was found to be 0.9993.

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ASSAY OF PRASITA TABLETS (5 MG)

A quantity of powder equivalent to 50mg of prasugrel was taken in a 50ml volumetric Flask and it was dissolved and diluted up to the mark with 0.1N Hcl. The resultant solution was ultrasonicated for 5 minutes. The solution was then filtered using Whatmann filter paper No.40. From the filtrate, appropriate dilutions were made in 0.1N Hcl to obtain the desired concentration (50¼g/ml). This solution was then analysed in UV and the result was indicated by % recovery given in table 2.

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, Accuracy and Precision.

Linearity: Various aliquots were prepared form the stock solution (100¼g/ml) ranging From 10-120¼g/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 1-50¼g/ml (table 1).

Accuracy

The accuracy of the method was determined by preparing solutions of Different concentrations that is 80%, 100% and 120% in which the amount of marketed formulation was kept constant (10mg) and the amount of pure drug was varied that is 8mg, 10mg and 12mg for 80%, 100% and 120% respectively. The solutions were prepared in triplicates and the accuracy was indicated by % recovery (table 4).

Precision

The precision of the proposed method was ascertained by actual determination of eight replicates of fixed concentration of the drug within the Beer's range and finding out the absorbance by the proposed method. From these absorbances, Mean, Standard deviation, % RSD was calculated. The readings were shown in Table 3**.**

RESULTS AND DISCUSSIONS

From the optical characteristics of the proposed method, it was found that Prasugrel obeys linearity within the concentration range of 1-50 mg/ml. From the results shown in Table 3, it was found that the % RSD is less than 2, which indicates that the method has good eproducibility. From the results shown in accuracy Table 4, it was found that the percentage recovery values of pure drug from the pre analyzed solution of formulation were in between 99.9-100.5 which indicates that the proposed method is accurate and also reveals that the commonly used excipients and additives in the pharmaceutical formulations were not interfering in the proposed method.

Table 1: Linearity table of Prasugrel in Working Standard

Concentration (mg/ml)	Absorbance
0	0
1	0.030
5	0.061
10	0.123
15	0.190
20	0.248
30	0.371
40	0.496
50	0.620

Table 2: Amount of Prasugrel in tablets

Formulation	Labelled amount(mg)	Amount found	%sRecovery
Brand I	5 mg	4.965	99.3 ± 0.385

●Each value is average of three determinations ± standard deviation.

Table 3. Precision readings

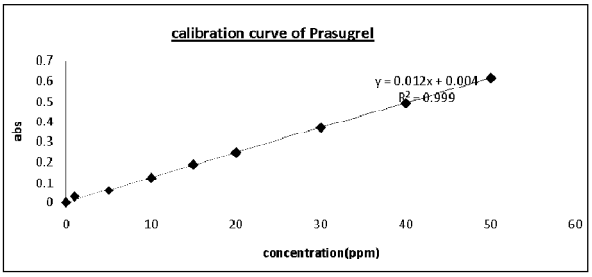
Concentrations (mg/ml)	Absorbance	Statistical Analysis
50	0.615	
50	0.621	
50	0.619	Mean = 0.621
50	0.621	
50	0.619	SD = 0.0029
50	0.623	
50	0.624	%RSD = 0.466
50	0.618	

Table 4: Accuracy Readings

Sample ID	Concentration (mg/ml)		%Recovery of Pure drug
	Pure drug	Formulation	
S_1:80%	8	10	99.9
S_2:80%	8	10	98.34
S_3:80%	8	10	101.5
S_4:100%	10	10	99.95
S_5:100%	10	10	99.95
S_6:100%	10	10	99.26
S_7:120%	12	10	99.3
S_8:120%	12	10	99.7
S_9:120%	12	10	99.9

TABLE 5: OPTICAL CHARACTERISTICS

Parameters	*VALUE (0.1 N Hcl)*
Absorbance maximum ($ë_{max}$)in nm	249
Beers law limit (¼g/ml)	1-50
Molar Absorptivity (L/Mol/cm)	4.314
Slope	0.0123
Intercept	0.0045
Correlation coefficient	0.9993
LOD(¼g/ml)	1.892
LOQ(¼g/ml)	6.872



Calibration curve of prasugrel.

CONCLUSION

The proposed method was simple, sensitive and reliable with good precision and accuracy. The proposed method is specific while estimating the commercial formulations without interference of excipients and other additives. Hence, this method can be used for the routine determination of Prasugrel in pure samples and pharmaceutical formulation.

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