ISSN: 0975-7538
Research Article

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Development and validation methods for the estimation of Clopidogrel in bulk and pharmaceutical dosage forms

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ABSTRACT

The present research work discussed Development and validation of HPLC method for the estimation of clopidogrel in bulk and pharmaceutical dosage forms, which is based on the measurement of absorption maxima at 217 nm. Double beam U.V visible spectrophotometers, Shimadzu, Kyoto, Japan. Model UV1800 with 1cm matched quartz cells and de-ionized water as solvent were used. Developed methods obeyed the Beer's law in the concentration range of 16 to 24µg/ml having lined equation y = 0.015x + 0.251 with the correlation coefficient of 0.999. Method was validated statistically. Percentage recovery of the drug for the proposed method ranged from (98.76%) indicating no interference of the excipients. The developed method was validated with respect to linearity, precision, accuracy (recovery), limit of detection (LOD) and limit of quantitation (LOQ).

Keywords: Deionised Water; Absorbance Maxima; Limit of Detection; Limit of Quantitation

INTRODUCTION

Plavix (clopidogrel bisulfate) is an inhibitor of ADP-induced platelet aggregation acting by direct inhibition of adenosine diphosphate (ADP) binding to its receptor and of the subsequent ADP-mediated activation of the glycoprotein, GPIIb/IIIa complex. Chemically, it is methyl (+)-(S)-α-(2-chlorophenyl)-6,7-dihydrothieno [3,2-c]pyridine-5(4H)-acetate sulfate (1:1). The empirical formula of clopidogrel bisulfate is $C_{16}H_{16}ClNO_2S \bullet H_2SO_4$ and its molecular weight is 419.9. (Anupama B. et al, 2011).

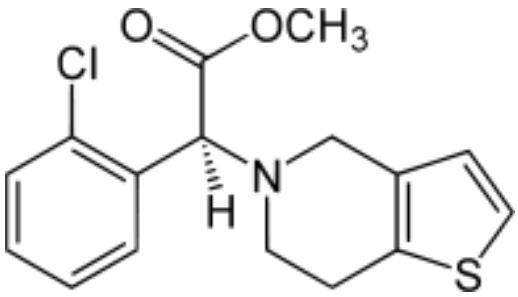

Figure 1: Molecular Structure of Clopidogrel

Clopidogrel bisulfate is a white to off-white powder. It is practically insoluble in water at neutral pH but freely soluble in pH 1. It also dissolves freely in methanol, dissolves sparingly in methylene chloride, and is practically insoluble in ethyl ether. (Rajput S J et al, 2008). It has a specific optical rotation of about +56°. Plavix for oral administration is provided as pink, round, biconvex, debossed film-coated tablets containing 97.875 mg of clopidogrel bisulfate, which is the molar equivalent of 75 mg of clopidogrel base. (Sonu S. Singh et al, 2005)

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Received on: 18-09-2011
Revised on: 26-02-2012
Accepted on: 02-03-2012

MATERIALS & METHODS

Reagents

Water (Triple distilled water), Methanol HPLC grade (MERCK Ltd.), Acetonitrile (MERCK Ltd.), Tetra butyl ammonium hydrogen sulphate (TBHS) (Himedia Laboratories Pvt. Ltd).

Instrumentation

a. Quantitative HPLC was performed on a binary gradient HPLC with Shimadzu LC-10AT and LC-10AT Vp series HPLC pumps, with a 20µl sample loop (manual), and SPD 10A UV-Visible absorbance detector. The output signal was monitored and integrated by using Shimadzu CLASS-VP Version 6.12 SP1 software. Shinwa Ultron ES-OVM L-57 (150x4.6mm, Packed with 5 microns) column was used for the separation.

b. Afcoset ER 200A electronic balance was used for weighing samples.

c. Ultra Sonicator (ENERTECH)

d. Triple Quartz Distillation Unit (BOROSIL)

e. HPLC Injecting Syringe (50 µl) (HAMILTON)

Preparation of Buffer

1.36 gm of potassium dihydrogen orthophosphate (KH_2PO_4) in 1000 ml of Triplele distilled water and sonicate for 10min to dissolve completely.

Preparation of mobile phase

750 ml of Buffer was taken in a 1000 ml bottle, and the final volume was made up to 1000 ml with Acetonitrile (250ml). Then the total content was sonicated for 30 minutes for degassing purpose. Then the bottle kept for sometimes, so that its temperature will come down to room temperature, then filtered through 0.45 µm (pore diameter) Whattman filter paper. Out of the 1000 ml, 500 ml was taken for HPLC run and the rest was kept for dilution purpose for analysis of Clopidogrel.

Preparation of the Stock solutions

Accurately weighted 100mg of Clopidogrel bisulphate working standard transferred to a 100ml volumetric flask containing 75 ml methanol sonicated for 10 minutes, diluted with methanol up to the mark and filtered through 0.45 µ membranes to get this stock solution (1 mg/ml).

Preparation of the Working standard solutions

The stock solution equivalent to 5 µg to 3000 µg was transferred into a series of 10 ml volumetric flask, Volume was made up to 10 ml with methanol, sonicated and filtered through 0.45 µ membrane.

Procedure for Calibration curve

The column was equilibrated with the set chromatographic conditions for 30 minutes, then 10 µl each of the working standard solutions in method was injected thrice and average retention time and peak area ratio of drug to internal standard were noted, and a graph was plotted by taking concentration on X-axis and peak area ratio on Y-axis, the results of which are shown in table-10.2 and figure-10.3.

Preparation of sample drug solution from formulations

The Tablet formulations having **Batch no. PBK** (689)79-40/75-1M-HDPE was taken and made it to powder form. The powder equivalent to 75 mg of Clopidogrel was dissolved in 100 ml of methanol to get a stock solution and then sonicated for 5min and then stirr for 30 min. From this solution, 5ml was taken and further diluted with methanol up to 50ml stock, which is within the linearity range. This solution was filtered through a 0.45µ nylon filter paper and was used for analysis.

RESULTS AND DISCUSSION

Linearity

The linear fit of the system was illustrated graphically. The least square regression analysis was carried out for the slope, intercept and correlation coefficient. The results are presented in Table-10.2 and Fig-10.3 & 10.4 for Clopidogrel.

Precision

The precision of each method was ascertained separately from the peak area ratios obtained by actual determination of eight replicates of a fixed amount of drug and internal standard. The precision of the assay was also determined in terms of intra-and inter-day variation in the peak areas for a set of drug solutions on three different days. The intra-and inter-day variation in the peak area ratio of the drug solution to that of internal standard was calculated in terms of % RSD and the results are presented in the Table 2.

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 drug to the pre-analyzed formulation solution of concentration 10 µg/ml containing internal standard. Then the percentage recovery values were calculated. The results were shown in Table 10.5 for Clopidogrel.

System suitability parameters for Method

System suitability parameters can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually developed after method development, and validation has been completed. (or) The USP (2000) defines parameters that can be used to determine system suitability prior to analysis. The system suitability parameters like Theoretical plates (N), Resolution (R), LOD (µg/ml) and LOQ (µg/ml) were calculated and compared with the standard values to ascertain whether the proposed RP-HPLC method for the estimation of Clopidogrel in pharmaceutical formulations was validated or not. The results were shown in Table 10.8 for both Clopidogrel.

Table 1: Result from Validation and system suitability Studies of the method

Method Characteristics	Clopidogrel
Theoretical plate	9765.06
Resolution between drug & IS	22.721
% RSD	0.6
Accuracy (RSD%)*	0.1243 0.3860 0.3051
Precesion (RSD%)**	1.0
LOD (µg/ml)	0.17482
LOQ (µg/ml)	0.5827

* Each value is represented as Mean ± SD, Where n = 3 and ** Each value is represented as Mean ± SD, Where n = 6.

Table 2: Accuracy study of Clopidogrel

Sl. No	Drug	Conc. Of pure drug (µg /ml)	Conc. Of Formulation (µg /ml)	% Recovery* of pure drug	% RSD
1	Clopidogrel	40	50	98.29± 0.1222	0.1243
		50	50	99.393± 0.3837	0.3860
		60	50	101.55± 0.3089	0.3051

* Each value is represented as Mean ± SD, Where n = 3

From the linearity tables, it was found that these drugs obey linearity within the concentration range of 1-200 µg/ml for Clopidogrel. From the results shown in precision table, it was found that % RSD is less than 2%; which indicates that the proposed methods have good reproducibility. From the results shown in accuracy tables, it was found that the percentage recovery values of pure drug from the pre-analyzed solutions of formulations were in between 98 – 102 %, which indicates that the method was accurate and also reveals that the commonly used excipients and additives present in the pharmaceutical formulations were not interfering in the proposed method. The system suitability parameters also reveal that the values were within the specified limits for the proposed method.

CONCLUSION

The proposed method was found to be simple**,** precise**,** accurate and rapid for determination of Clopidogrel from pure and its dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims, and they suggested non-interference of formulation excipients in the estimation. Hence, these methods can be easily and conveniently adopted for routine analysis of these drugs in pure form and its dosage forms and can also be used for dissolution or similar studies.

ACKNOWLEDGEMENT

Authors would like to give special thanks to Dr. M.L. Kori principal Vedica college of pharmacy, Bhopal, Dr. Rakesh Sagar HOD Department of Pharmacognosy, Laboratory and instrumental facility for this research work. Mr. Anuj asati Asst. professor and Mr. F.A. Toppo Asst Professor Vedica college of pharmacy, Bhopal, for guidance and support.

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