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STABILITY INDICATING SPECTOPHOTOMETRIC METHOD FOR DETERMINATION AND VALIDATION OF CLOPIDOGREL BISULFATE IN TABLET DOSAGE FORM

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ABSTRACT
A simple, sensitive, accurate, rapid and reliable visible spectophotometric method has been developed for determination of clopidogrel bisulfate in tablet dosage forms. The quantitative determination of the drug was carried out using Linear Least Squares Equation method values measured at 220.0 nm and Calibration graph constructed at 220.0 nm was linear in concentration rage of 40-65 µg/ml with correlation coefficient 0.9977. The method was validated as per ICH guidelines and can be used for determination of clopidogrel bisulfate in tablet dosage forms.

KEYWORDS: Spectrophotometric, Clopidogrel bisulfate, Acetonitrile, Tablet, Validation.

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INTRODUCTION

Clopidogrel bisulfate, chemically (+)-(*S*)-α-(2-chlorophenyl)-6,7-dihydrothieno [3,2-*c*]pyridine-5(4H)-acetic acid methyl ester sulphate is a potent oral antiplatelet agent often used in the treatment of coronary artery disease, peripheral vascular disease and cerebrovascular disease. It is marketed by Bristol-Myers Squibb and Sanofi-Aventis under the trade name Plavix which is the world's second highest selling pharmaceutical with sales of US$5.9 billion. The mechanism of action of clopidogrel is irreversible blockade of the adenosine diphosphate (ADP) receptor P2Y12 and is important in platelet aggregation, the cross-linking of platelets by fibrin. The blockade of this receptor inhibits platelet aggregation by blocking activation of the glycoprotein IIb/IIIa pathway. [1] It is not in any pharmacopoeia. Literature survey reveals the estimation of Clopidogrel bisulfate in pharmaceutical formulations by various chemometric[2], HPLC[3-6], HPTLC[7-8], TLC[9], and an LC-ESI-MS-MS[10-11] method was developed. However, no Spectophotometric method is reported for the determination of Clopidogrel bisulfate. The aim of present work is to develop a simple, rapid, reproducible, inexpensive and efficient Linear Least Squares Equation[12-13] Spectophotometric method for estimation of Clopidogrel bisulfate in tablet formulation.

MATERIAL AND METHODS

Instrument

1) PC Based Double Beam UV-VIS Spectrophotometer (SYSTRONICS 2201) Mumbai, India.
    a) Spectral bandwidth of 2.0 nm.
    b) Wavelength accuracy of ± 1 nm.
2) Matched quartz cells of 10 mm optical path length.

Clopidogrel bisulfate drug powder procured from Cadila Pharmaceutical Ltd. Ahmedabad, was used in this study.

Solvent

Acetonitrile (AR grade)

Preparation of standard Drug solution (CDB)

An accurately weighed 10 mg of Clopidogrel bisulfate was transferred to 100 ml volumetric flask. It was dissolved in acetonitrile and volume was adjusted to 100 ml with acetonitrile to obtain stock solution of drug of concentration of 100 µg/ml. Working standard solution of Clopidogrel bisulfate were prepared by diluting different volumes of stock solution (100 µg /ml) in a 10 ml volumetric flask to give solution in range of 40 to 65 µg /ml using acetonitrile. Solutions were scanned in the UV range of 200 – 400 nm **(Fig. 1)** and calibration curve was constructed by absorbance values at 220.0 nm against concentrations. The calibration curve was found to be linear in the concentration range of 40 to 65 µg/ml. Aliquot portion of this solution were further diluted to get the final concentration 50 µg/ml. Absorbence of resultant solutions were measured at 220.0 nm and the concentration of sample solution were calculated by using formula.

Preparation of Sample solution (CDB)

Twenty tablets were weighed; emptied and average weight was calculated. Accurately weighed quantities of tablet powder equivalent to 10 mg of Clopidogrel bisulfate was transferred to 100.0 ml volumetric flask. Shake of the flask with 50 ml in acetonitril for 20 min. and volumes were made upto the mark. Filtered the solution through whatman filter paper No.1. Further suitable dilutions were made to obtain six replicates of 50 µg/ml solutions. These solutions were analyzed and amount of Clopidogrel bisulfate was determined. The results are summarized in (**Table 3**).

VALIDATION OF PROPOSED METHOD

Linearity Study

A calibration curve was constructed at optimum experimental conditions using absorbance values at 220.0 nm versus concentration in the range of 40 to 65 µg/ml. It has shown linear data in (**Table 1**). High value of the correlation coefficient (r=0.9947) indicates a good linearity and adherence of the method to Beer's law[14,15].

Recovery Studies

To study validity and reproducibility of the proposed method, recovery studies were carried out by adding known amount of drug to preanalysed sample at four different levels and the percentage recoveries were calculated (**Table 3**).

Repeatability

Repeatability is performed by intraday and inter day precision. Intraday precision was determined by analyzing the three different concentration of drug for three times in same day. Inter day precision was determined by analyzing three different concentration of the drug for three day in a week.

Specificity

Accurately weighed six quantities of tablet powder equivalent to about 10 mg of Clopidogrel bisulfate were taken six different 100.0 ml of volumetric flask. Each weight was stored for 48 hours under the following different degradation conditions in room temperature (Normal), 0.1N HCl (Acid), 0.1N NaOH (Alkali), 10% H_2O_2 (Oxide) at 55° C, in UV-Chamber at 265 nm (UV), at 60° (Heat). After 48 hour the content in flasks were shaken with acetonitrile for 10 min. and volume was made upto 100.0 ml. The solution was filtered through whatman filter paper No.1 and the filtrate further diluted to get required concentration 50µg/ml and measured the absorbance at 220.0 nm. respectively. Degradation study shows in the assay value of Clopidogrel bisulfate when exposed to acid, alkali, oxidation, uv light, heat, that show that the graph of Clopidogrel bisulfate is spectrally homogenous i.e. no change in absorption value at 220.0 nm providing stability indicating nature of the method. **(Table 2)**

RESULT AND DISCUSSION

The method was validated in terms of accuracy, precision and reproducibility and results were given in (**Table 3**). Two formulations were analyzed and amount of drug in each was determined by proposed method. The accuracy of proposed methods was proved by performing the recovery study in the commercially available formulations. The reproducibility of the proposed method determine by, recovery studies were carried out by adding known amount of drug to preanalysed sample at four different levels and the percentage recoveries were calculated. For all the degraded samples, Clopidogrel bisulfate passed the purity testing, leading to a conclusion that the specificity of the method was confirmed and proves that the method is stability indicating. Optical characteristics, such as Beer's Law limit, molar absorptivity were given in (**Table 1**). The amount of drug found in formulations is well agreed with label claim.

The proposed method, thus, found to be simple, precise, accurate, reproducible and sensitive and can be utilized as a quality tool for estimation of Clopidogrel bisulfate in pure and pharmaceutical dosage form.

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Table 1: Statistical Data of Standard Calibration Curve

Parameter	Value
λ max	220.0 nm
Beers Law Limits (mcg/mL)	40-65 (µg/ml)
Molar Absorbtivity	3.202×10^2
Correlaton coefficient (r)	0.9977
Slope (β)	0.01275
Y- Intercept (α)	0.9583
Limit of Detection	0.4 µg/ml
Limit of Quantitation	2.0 µg/ml

$Y = \alpha + \beta x$ were x is the concentration of the drug in µg/ml, Y is amplitude at the specified wavelength, β is slope and α is Y-intercept.[12-13]

Table 2: Results of specificity study (CDB)

Sr. No.	Parameters	Absorbance at 220.0 nm	Percentage Drug Estimated
1	Normal	1.598	99.30
2	0.1 N HCL (2 mL) (50°c)	1.584	98.14
3	0.1 N NaOH (2 mL) (50°c)	1.590	99.09
4	10 %H_2O_2 (2mL) (50°c)	1.580	97.52
5	UV – light	1.581	99.24
6	Heat (60°c)	1.588	98.77

Table 3: Summary of Results for the Estimation of CDB according to their method

Formulation (Brand Name)	Label Claim	Obtained Amount of Label Claim ±S.D.	%of Label claim ±S.D.	% C.V.	% Recovery ± S.D.
CAPLOR*	75 mg	74.71± 0.169	99.62±0 .224	0.225	99.64±0.236
CLASS*	75mg	74.82± 0.840	99.76±1.121	1.123	99.54±0.237

* Average of six estimations

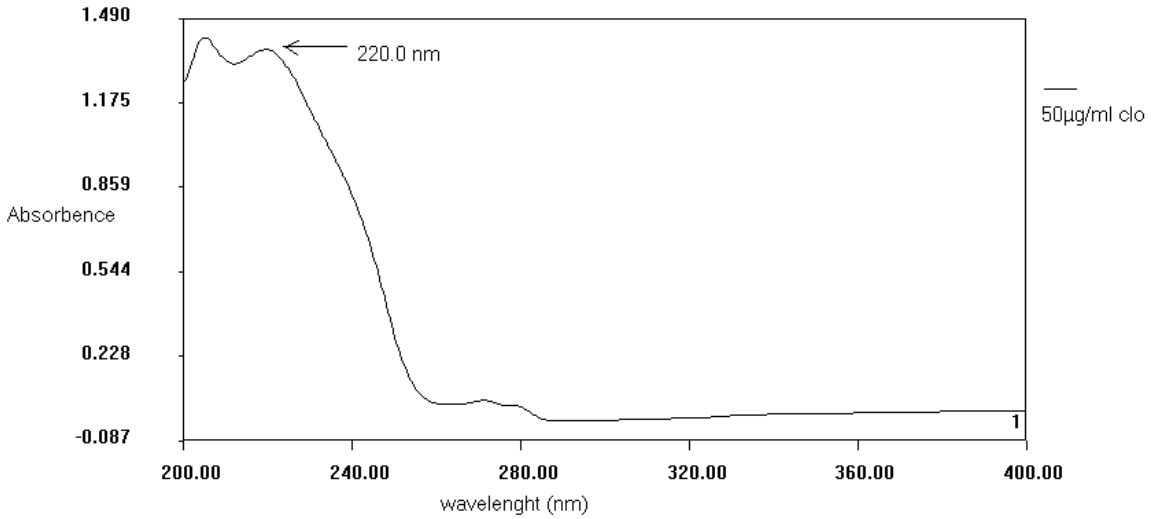

Fig 1: UV spectra of CDB.

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