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Original Article

Method Development and Validation for the Quantification of Clopidogrel Bisulphate in Bulk and its Dosage form

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ABSTRACT

Objective: The main objective of present research work simple, accurate and precise UV Spectroscopic method was developed for the determination of purity of Clopidogrel Bisulphate. **Experimental approach:** The method was developed using Shimadzu 1800 UV-Visible double beam spectrophotometer with UV probe software with Ethanol as a solvent. The wavelength is monitored at 218 nm. The developed method was validated as per ICH guidelines. **Results:** The method produced linear response over the wide concentration range of 2-12 µg/mL, with an average accuracy of 99.06%, as well as average intra- and inter-day variations of 0.528 and 0.624 %, respectively.
Conclusion: The developed method was found to be precise, robust and accurate. This method can be suggest to routine quality control analysis of Clopidogrel bisulphate in its pure and pharmaceutical formulation.
Key words: Clopidogrel Bisulphate, ethanol, ICH guidelines, Linear

1. INTRODUCTION

Clopidogrel bisulphateis an antiplatelet agent and used to inhibit blood clots in a variety of conditions such as peripheral vascular disease, coronary artery disease, and cerebrovascular disease, the drug is an irreversible inhibitor of the p_2y_{12}adenosine diphosphate receptor found on the membranes of platelets cells.Clopidogrel[1] use is associated with several serious adverse drug reactions such as severe

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neutropenia, various forms of hemorrhage, and cardiovascular edema[2].

A literature survey revealed that liquid chromatography techniques have been reported for the determination of Clopidogrel bisulphate in pure drug, pharmaceutical dosage forms and biological samples[3-6]. Hence, the authors have attempted to develop a simple, rapid, precise and accurate method for the estimation of these drugs in tablet dosage forms. Confirmation of the applicability of the developed method was validated according to the International Conference on Harmonization (ICH) for the determination of Clopidogrel bisulphate in bulk and in tablet dosage forms.

2. MATERIALS AND METHODS

Chemicals and reagents:

An analytically pure sample of Clopidogrel bisulphate was procured as a gift sample from MSN laboratories (Hyderabad, India). Tablet formulation (Clopitab), manufactured by Acme Formulation Pvt. Ltd. India was procured from a local pharmacy with labeled amount75mg/ tablet.

Instrument used:

UV/VIS double beam spectrophotometer Shimadzu 1800 enabled with UV probe software, having deuterium lamp was used.

Methodology

Selection of solvent:

The selection of solvent was done based upon the drug solubility, stability and absorbance maxima of the compound in the particular solvent. 10 mg of Clopidogrel bisulphate was weighed and solubility of this sample was checked in the 0.1N Hydrochloric acid, 0.01N Sodium hydroxide, Methanol, Ethanol, Phosphate buffer pH6.8 and distilled water. From the reported studies ethanol50% was not used for the determination of Clopidogrel bisulphate. Hence the current method was developed in 50%v/v ethanol.

Preparation of standard stock solution:

Clopidogrel bisulphate pure 10 mg was weighed and transferred to a 10 ml volumetric flask and dissolved in ethanol. It was dissolved properly and diluted up to the mark with diluent to obtain final concentration of 1000 µg/ml. 5µg/ml solution was prepared from the stock solution was prepared using distilled water, which was used as working standard .

Method Validation

Linearity and Range:

Calibration standards of Clopidogrel bisulphate covering the range 2- 12µg/ml were prepared with the suitable dilution made from stock solution. The calibration curves were obtained by plotting the intensity of absorbance against concentration. The slope and intercept of the calibration line were determined by linear regression using the least squares method.

Precision:

The precision of an analytical method is the degree of agreement among individual test results, when the method is applied repeatedly to multiple samplings of homogenous samples. The intra & inter-day precision was evaluated by analyzing six sample solutions (n = 6), at the final concentration of analyses (8µg/ml) of Clopidogrel bisulphate. The Clopidogrel bisulphate concentrations were determined and the relative standard deviations (RSD) were calculated. % RSD was calculated.

Accuracy:

The accuracy of the proposed method was tested by recovery studies at different replicate levels in triplets for 50%, 100% and 150%.The sample solutions were prepared by adding a known amount of pure drug to the pre-analysed formulation. The mean percent recovery was calculated and was reported in the clopitab.

Limit of detection (LOD):

Limit of detection is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected. From the standard stock solution 0.1ml was pipette out into 10 ml volumetric flask and the volume was made up to the mark with distilled water. The Limit of detection was found to be 0.20359 µg/ml.

Limit of quantitation (LOQ):

Based on the LOD strength (0.01 mcg / ml, standard solution), the LOQ values were calculated by multiplication with three times. From the standard stock solution 0.15 ml was pipette out was placed into 10 ml volumetric flask and volume was made up to the mark with distilled water. The Limit of quantitation was found to be 0.61695 µg/ml.

3. RESULTS AND DISCUSSION

Clopidogrel bisulphate exhibits maximum absorbance at 218 nm. So determination of Clopidogrel bisulphate by UV spectrophotometric method was thus attempted. Beer's law was obeyed in the concentration range of 2 to 12µg/ml. Interday and intraday studies showed high degree of repeatability of an analytical method under normal operating conditions. The accuracy of the method was determined by investigating the recovery of the drugs using spiked concentrations of the standard drug. Precision was determined by analysis of Tablet containing Clopidogrel bisulphate. The results were tabulated in the following tables.

Table 1: Results of calibration curve at 218 nm for Clopidogrel Bisulphate

S.No	Concentration (µg/ml)	Absorbance
1	2	0.267
2	4	0.379
3	6	0.477
4	8	0.574
5	10	0.671
6	12	0.765

Table 2: Precision results of Clopidogrel Bisulphate

S.No	Precision Studies	
	Intra Day	Inter Day
1.	0.574	0.578
2.	0.568	0.582
3.	0.572	0.576
4.	0.569	0.583
5.	0.543	0.587
6.	0.577	0.579
Mean	0.57217	0.58083
Std Dev	0.00302	0.00362
% RSD	0.52835	0.62406

Table 3: Determination of Accuracy results for Clopidogrel bisulphate

S. No	Spike Level	Absorbance	µg/ml Added	µg/ml Found	% Recovery
1	50 %	0372	4.73563	4.75216	99.630228
2	100 %	0.574	9.42127	9.31034	99.935003
3	150 %	0.764	18.6268	18.3512	100.563978

Table 4: Total Summary of Method Development and Validation Parameters

S No.	Parameters	Results
1	Absorption Maxima (nm)	218
2	Beer's-Lambert's range (µg/ml)	2-12
3	Regression equation (y)*	Y = 0.049x +0.17
4	Slope (b)	0.049
5	Intercept (a)	0.17
6	Correlation coefficient (r^2)	0.999
7	Intraday precision (% RSD)**	0.52835
8	Interday precision (% RSD)**	0.62406
9	Accuracy (% mean recovery)	99.06-100.56
10	Limit of detection (µg / ml)	0.20359
11	Limit of quantification (µg / ml)	0.61695
12	Assay of tablets (%Purity)	100.084

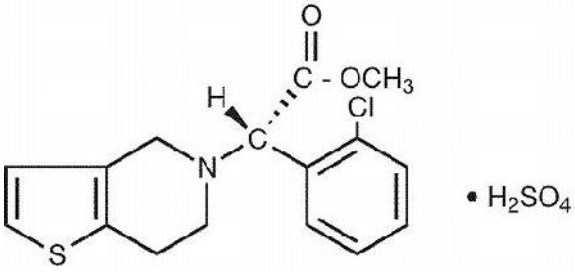

Fig 1: Structure of Clopidogrel bisulphate

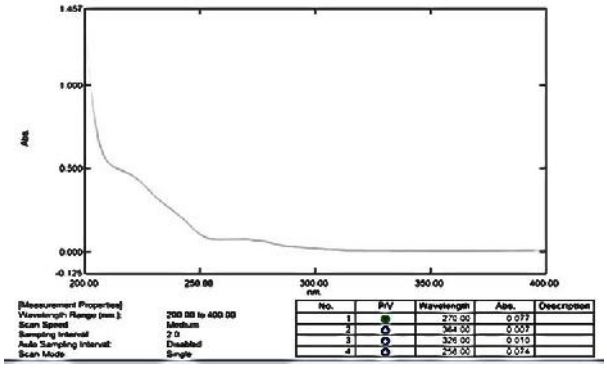

Fig 2: UV spectra of Clopidogrel Bisulphate showing absorbance at 218 nm

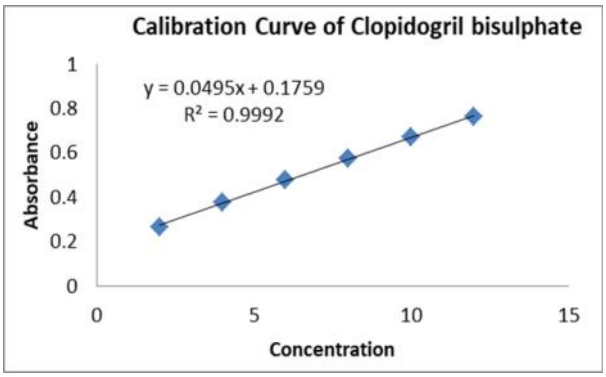

Fig 3: Calibration curve for Clopidogrel Bisulphate

4. CONCLUSION

A novel, simple and cost effective spectrophotometric method for the quantitative estimation of Clopidogrel bisulphate in bulk drug and pharmaceutical formulations has developed. The method was found to be precise, robust and accurate. The developed method can be successfully used for routine analysis of Clopidogrel bisulphate in its pure and pharmaceutical formulation.

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