1. INTRODUCTION

Clopidogrel bisulphate is 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 p2y12:adenosine diphosphate receptor found on the membranes of platelets cells. Clopidogrel use is associated with several serious adverse drug reactions such as severe
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 in and 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 (Clopitalab), manufactured by Acme Formulation Pvt. Ltd. India was procured from a local pharmacy with labeled amount 75 mg/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 pH 6.8 and distilled water. From the reported studies ethanol 50% 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 g/ml. 5 g/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 2-12 g/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 (8 g/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 clopitalab.

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 0.1 ml 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 g/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 12 g/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.

| S.No | Concentration (g/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 | 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 | g/ml Added | g/ml Found | % Recovery |
|-------|-------------|------------------|------------|------------|------------|
| 1     | 50 %        | 0.372            | 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 (g/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 (r²)        | 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 (g / ml)         | 0.20359       |
| 11    | Limit of quantification (g / ml)    | 0.61695       |
| 12    | Assay of tablets (%Purity)          | 100.084       |

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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