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Research Article

DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF CLOPIDOGREL BISULFATE IN PHARMACEUTICAL DOSAGE FORM BY REVERSE-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

The present work was focused on the development and validation of reversed-phase high-performance liquid chromatography (RP-HPLC) method which is simple, rapid, precise, accurate, sensitive, and economical for the quantification of clopidogrel bisulfate in bulk and tablet formulation. The separation was attained on reversed-phase Princeton (C18) column with dimensions (250×4.6 mm, 5µ) employing buffer which is a mixture of water (pH 3.0, adjusted with orthophosphoric acid) and methanol in the ratio (20:80) v/v as mobile phase, at flow rate 1.0 ml/min. and detection was carried out at wavelength 240 nm. The retention time under the optimized condition of clopidogrel bisulfate was found to be 4.388 minutes. The linearity of the method was demonstrated in the concentration range of 45-120 µg/ml for clopidogrel bisulfate with a correlation coefficient (r^2) of 0.9996 respectively. The percentage relative standard deviation was <2% and percentage recovery was found to be 99.72-101.09% for clopidogrel bisulfate. Assay of marketed tablet formulations was found to be 99.92%. The developed RP-HPLC method was found to be simple, specific, sensitive, rapid, linear, accurate, precise, and economical, and could be used for regular quality control of clopidogrel bisulfate in bulk and tablet formulations.

Keywords: Clopidogrel bisulfate, RP-HPLC, Method validation.

1. INTRODUCTION

Clopidogrel bisulfate (CLO), is chemically Methyl 2-(2-Chlorophenyl) -2-(6, 7-dihydro thieno [3,2-C] Pyridine-5(4H)-yl) Acetate sulphate (Fig. 1). It is an Anti-platelet agent as an ADP receptor blocker mainly to treat patients with the acute coronary syndrome, myocardial infarction (MI), peripheral vascular disease, and some stroke (Ischemic type) patients [1, 2].

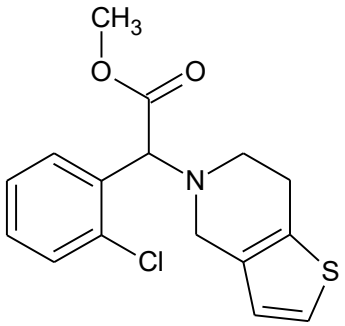

Fig. 1: Chemical structure of Clopidogrel

An extensive literature survey revealed that several HPLC methods were reported for the estimation of clopidogrel bisulfate in bulk and tablet formulation [3-16]. The proposed method is simple, accurate, reproducible, economical, and suitable for routine determination of clopidogrel bisulfate in bulk and tablet formulations. The method was validated in compliance with ICH guidelines.

2. MATERIAL AND METHODS

2.1. Chemicals and reagents and Instruments

Pharmaceutical grade Clopidogrel bisulfate was procured as a gift sample from Cadila Pharmaceuticals Ltd., Ahmedabad (India), Clopivas-75 a tablet formulation, obtained commercially. Methanol, orthophosphoric acid, hydrochloric acid, and sodium hydroxide analytical grade were used throughout the work. Shimadzu HPLC system and PDA detector with Lab Solution software were used. Based on the solubility study, methanol was selected as the solvent for dissolving CLO.

2.2. Chromatographic Conditions

Chromatographic separation was achieved on a reversed-phase Princeton (C18) column with dimensions (250× 4.6mm,5µ) at ambient temperature using a mobile phase consisting of a mixture of buffer (pH 3.0, adjusted with orthophosphoric acid) and methanol in the ratio of (20:80) v/v at a flow rate of 1.0 ml/min. Detection was carried out at 240nm. The pH of the mobile phase was set at 3.0, Injection volume was 10 µl. The optimized chromatographic condition is shown in Table 1.

Table 1: Optimized chromatographic condition.

Chromatographic condition	
Mobile phase	Water(pH adjusted to 3.0 with orthophosphoric acid):Methanol (20:80) v/v
Flow rate	1.0 ml/min.
Column	Princeton C18 (250×4.6mm, 5µ)
Detector wavelength	240nm
Column temperature	30°C
Injection volume	10 µl
Runtime	20 minutes
Diluent	Methanol
Retention time	4.388minutes

2.3. Preparation of standard solution of CLO:

For CLO, an accurately weighed 7.5 mg of CLO was transferred to a 10.0 ml volumetric flask and dissolved in 5.0 ml of methanol. The volume was completed to 10.0 ml with methanol. One milliliter of the resulting solution was pipetted in 10.0 ml volumetric flask and the volume was made up to 10.0 ml with methanol to furnish a solution of concentration 75 µg/ml of CLO.

2.4. Preparation of sample solution of CLO:

Twenty tablets were weighed and finely powdered. An accurately weighed amount of powder equivalent to 7.5 mg of CLO was transferred into a 10.0 ml volumetric flask. Then 5.0 ml of methanol was added in it. The flask contents were sonicated for 10 min to make the contents homogeneous. This solution was then diluted up to the mark with methanol. The resultant solution was filtered through Whatman Grade I filter paper. One milliliter of the filtrate was transferred to a 10 ml volumetric flask and then the volume was made up to the mark with methanol to furnish a sample solution containing 75 µg/ml of CLO. Six replicates of tablet powder equivalent to 7.5 mg of CLO were transferred into six 10.0 ml volumetric flask and homogenous sample solutions were prepared similarly.

2.5. Method Validation

The developed method was validated following ICH guidelines (ICH Q2R1) for accuracy, precision, specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), robustness [17].

2.5.1. Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a standard true value or an accepted reference value, and the value found. It was computed at three different levels, i.e., 80, 100, and 120% of the label claim. Standard addition and recovery experiments were conducted to determine the accuracy of CLO for the quantification of drugs in the sample.

2.5.2. Precision

The system precision was evaluated by measuring the area of six qualified working standards for CLO and calculating the percentage of relative standard deviation (RSD). The assay method precision was evaluated by conducting six independent assays of test samples of CLO against qualified working standards and calculating the percentage of relative standard deviation (RSD). The intermediate precision of the method was also verified using different analysts and different days.

2.5.3. Linearity

Linearity test solutions of CLO were prepared at concentration levels of 45-120 µg/ml respectively. Linearity test solutions were prepared by diluting the stock solution to the required concentrations. Linearity was established by the least-squares linear regression analysis of the calibration data. Peak areas were plotted against the respective concentrations and linear regression analysis performed on the resulting curves. The linear curve of clopidogrel bisulfate was shown in Fig. 2.

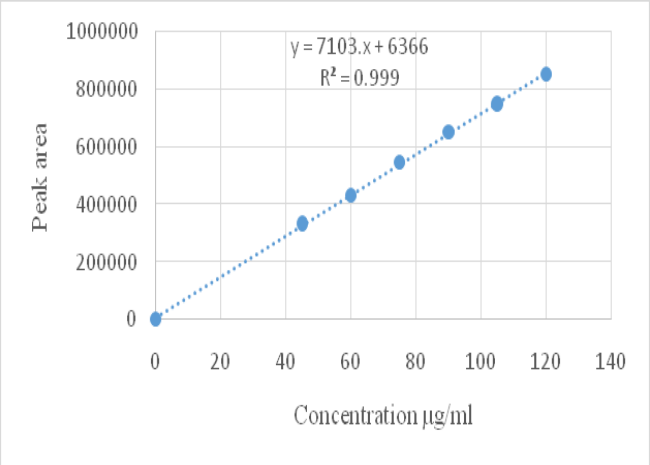

Fig. 2: Linear curve of clopidogrel bisulfate

2.5.4. Specificity

The specificity of the developed method was established by comparing the chromatograph of the standard and sample. It was found that there was no interference due to excipients and impurities at the retention time of the drug.

2.5.5. LOD and LOQ

The LOD is the lowest analyte concentration that can be detected. LOQ is the lowest analyte concentration that can be quantified with acceptable accuracy and precision. The limits of detection (LOD) and quantification (LOQ) were calculated from the standard deviation of the response and the slope of the calibration plot.

LOD and LOQ were established, under ICH definitions, by use of the equations LOD = 3.3σ/S and LOQ= 10σ/S, where σ is the standard deviation of the regression line and S is the slope of the calibration plot.

2.5.6. Robustness

To evaluate the robustness of the developed method, the chromatographic conditions were deliberately altered and the resolution for CLO was evaluated. To study the effect of wavelength on the estimation, the wavelength was altered by ±2 nm, *i.e.,* 238 and 242 nm from the actual wavelength, 240 nm. To study the effect of flow rate on estimation, the flow rate was altered by ±0.1 ml/min *i.e.,* 0.9 and 1.1 ml/min from the actual flow rate, 1.0 ml/min.

3. RESULTS AND DISCUSSION

3.1. HPLC method development and optimization.

Initially, pure drugs solution was chromatographed using a mobile phase consisting of a mixture of buffer (pH 3.0, adjusted with orthophosphoric acid) and methanol in the ratio of (20:80) v/v at a flow rate of 1.0 ml/min. gives well-resolved peaks of drugs as well. Detection was carried out at 240nm. The retention time under the optimized condition of clopidogrel bisulfate was found to be 4.388 minutes. The total run time of the chromatogram was about 20 minutes.A typical chromatograph of standard and sample of clopidogrel bisulfate is shown by Fig. 3 and Fig. 4.

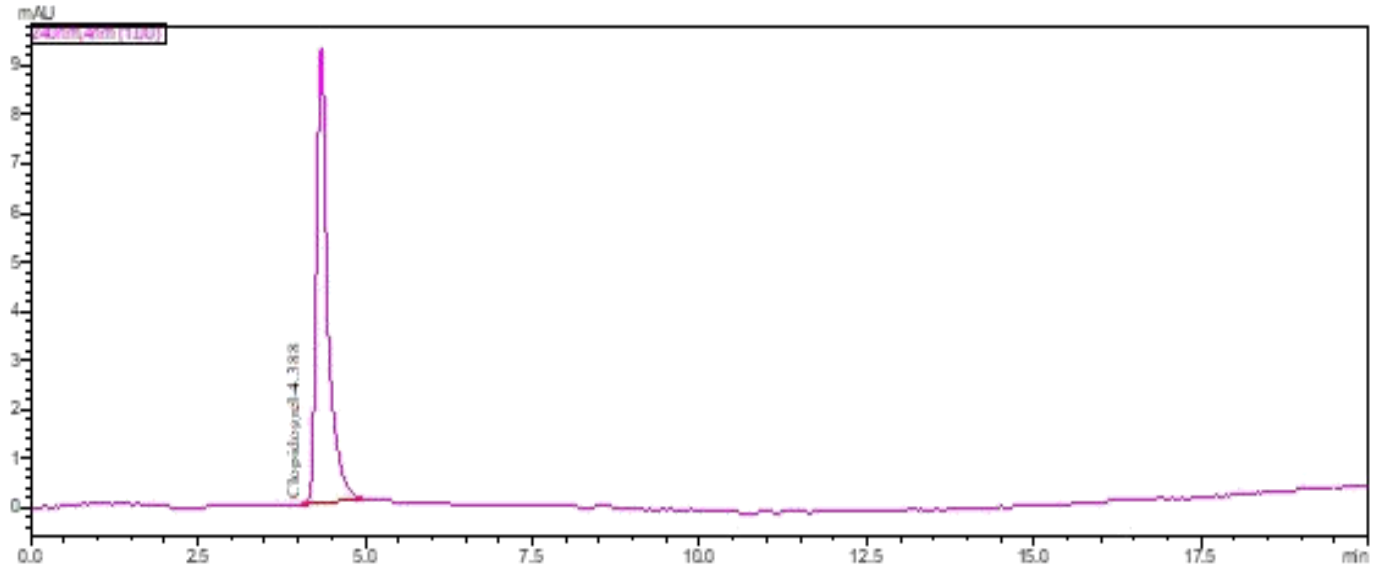

Fig. 3: A typical chromatograph of standard clopidogrel bisulfate (CLO)

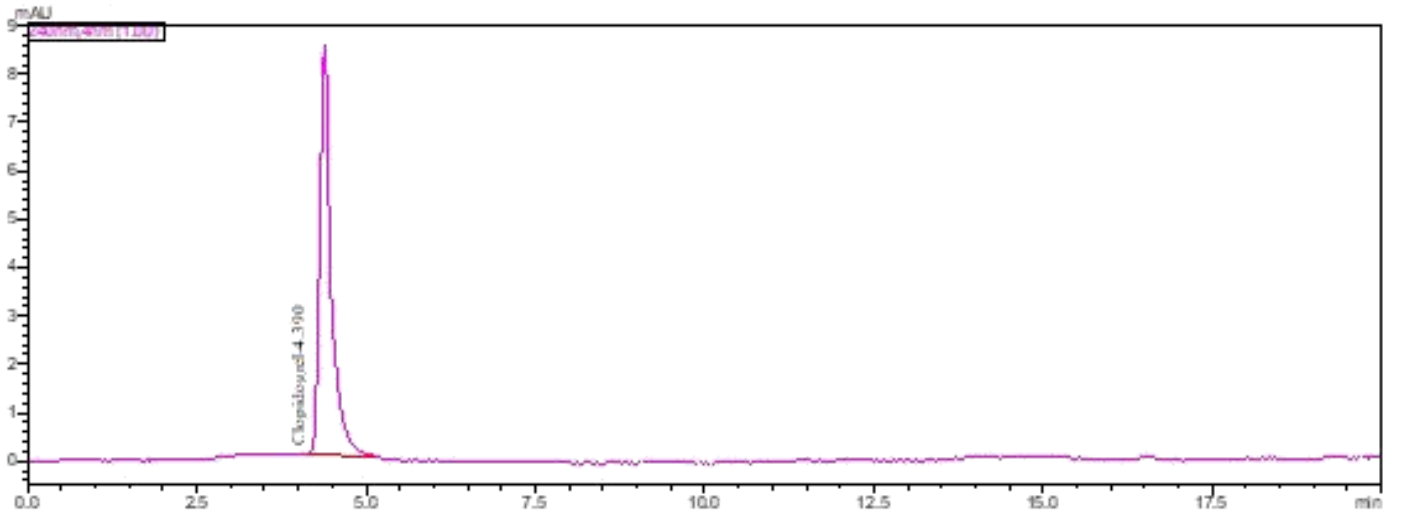

Fig. 4: A typical chromatograph of sample clopidogrel bisulfate (CLO)

3.2. Validation of the method

3.2.1. System Suitability

The suitability of the system was demonstrated by assessing various parameters. It was established by injecting six replicate injections of the standard solution. Theoretical plates were found to be 3811, tailing factor of 1.54, and %RSD of peak area was 0.8 for CLO respectively (Table 2). All the system suitability parameters were well within the limits, indicating that the system was well suitable for performing the analysis.

Table 2: System suitability results

Parameter	CLO
Theoretical Plate	3811
Retention Time (Rt)	4.388
Tailing factor	1.54
% RSD	0.8

Rt: Retention time, %RSD: Percentage relative standard deviation

3.2.2. Linearity

Linearity was established by the least-squares linear regression analysis of the calibration data. Calibration plots were linear over the concentration range 45-120µg/ml for CLO. Peak areas were plotted against the respective concentrations and linear regression analysis performed on the resulting curves.

Table 3: Linearity results

Parameter	CLO
Concentration Range (µg/ml)	45-120
Slope (m)	7103.4
Intercept	6366
Coefficient correlation (r^2)	0.9996

The equation for the calibration plots of CLO was Y=7103.4x+6366 with a correlation coefficient of 0.9996. The results of linearity are shown in Table 3.

3.2.3. Accuracy

The percentage recoveries were 99.72-101.09% for CLO. The %RSD value was found to be <2%. The results of recovery are shown in Table 4.

Table 4: Recovery results

Drug	Level (%)	Amount taken (µg/ml)	Amount found* (µg/ml)	% Recovery*
CLO	80	60	59.83	99.72
	100	75	75.81	101.09
	120	90	90.75	100.83

**Average of three determinations*

3.2.4. Precision

The result of intraday and interday for CLO was found to be 0.8 and 0.4 respectively. The percentage RSD of system, method, and intermediate precision study was well within ±2.0%, indicate that the method was precise.

3.2.5. LOD and LOQ

The LOD and LOQ of CLO were found to be 6.90 µg/ml and 20.91 µg/ml respectively.

3.2.6. Robustness

To evaluate the robustness of the developed method, the chromatographic conditions were deliberately

altered and the resolution for CLO was evaluated. The effect of wavelength and effect of flow rate on the estimation of CLO were studied. The results of robustness are shown in Table 5.

Table 5: Robustness results

Condition		CLO	
		Amount estimated* [%]	RSD [%]
Change in wavelength (240±2 nm)	**238 nm**	100.16	0.0900
	242 nm	100.08	0.1417
Change in flow rate (1.0±0.1 ml/min.)	**0.9 ml/min.**	99.37	0.2068
	1.1 ml/min.	99.56	0.2009

**Average of three determinations, %RSD: Percentage relative standard deviation*

3.2.7. Analysis of clopidogrel bisulfate from marketed tablets

The percentage assay of tablet formulation was found to be 99.92% for CLO. The stability of the drug solutions was observed for 24 h. % RSD of 0.8 indicates the stability of the method for 24 h. Hence, the method was found to be specific.

Table 6: Summary of validation parameter

Parameter	CLO
Calibration range (µg/ml)	45-120
Optimized wavelength (nm)	240
Retention Time	4.388
Regression equation (Y)	Y = 7103.4x+6366
Slope	7103.4
Intercept	6366
Coefficient correlation (r^2)	0.9996
Precision (% RSD)	
Intraday	0.8
Interday	0.4
% Assay*	99.92
LOD (µg/ml)	6.90
LOQ (µg/ml)	20.91

**Average of five determinations, LOD: Limit of detection, LOQ: Limit of quantification*

4. CONCLUSION

The method enables simple, rapid, accurate, precise, specific, economical, and sensitive analysis of clopidogrel bisulfate in bulk and tablet dosage form. This method was validated under ICH guidelines. The method can, therefore, be used for routine quality control analysis clopidogrel bisulfate in bulk and tablet dosage form.

5. ACKNOWLEDGEMENTS

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Conflict of interests

Authors declare that they have no conflict of interest exists in this investigation.

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