



[Research article]

## Method Development and Validation of Clopidogrel Bisulphate by Reverse Phase-HPLC in Bulk and Pharmaceutical Dosage Forms

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### ABSTRACT

A new, simple sensitive, rapid, accurate and precise RP-HPLC method was developed for the estimation of Clopidogrel bisulphate in bulk drug and pharmaceutical formulation. Clopidogrel bisulphate was chromatographed on a reverse phase C18 column (150 mm x 4.5 mm, i.d 5 $\mu$ m) in a mobile phase consisting of acetonitrile and phosphate buffer (pH: 3.0) in the ratio of 60:40 % v/v. The mobile phase was pumped at a flow rate of 1 ml/min with detection at 224 nm. The detector response was linear in the concentration of 50-150  $\mu$ g/ml. The limit of detection and limit of quantitation was found to be 1.3 and 4.2  $\mu$ g/ml, respectively. The intra and inter day variation was found to be less than 2%. The mean recovery of the drug from the solution was 99.79%. The proposed method is simple, fast, accurate, precise and reproducible hence, it can be applied for routine quality control analysis of Clopidogrel bisulphate in bulk drug and pharmaceutical formulation.

**Key words:** Clopidogrel bisulphate, RP-HPLC, Validation, Accuracy, Precision.

### INTRODUCTION

Clopidogrel is an oral, thienopyridine class antiplatelet agent used to inhibit blood clots in coronary artery disease, peripheral vascular disease, and cerebrovascular disease. Clopidogrel is a prodrug, the action of which may be related to an ADP receptor on platelet cell membranes. The drug specifically and irreversibly inhibits the P2Y<sub>12</sub> subtype of ADP receptor, which is important in activation of platelets and eventual cross-linking by the protein fibrin. The blockade of this receptor inhibits platelet aggregation by blocking activation of the glycoprotein IIb/IIIa pathway. The IIb/IIIa complex functions as a receptor, mainly for fibrinogen and vitronectin but also for fibronectin and von Willebrand factor. Activation of this

receptor complex is the "final common pathway" for platelet aggregation and is important in the cross-linking of platelets by fibrin.

Clopidogrel bisulphate is chemically (S)-(+)-Methyl 2 - (2-chlorophenyl) -2- (6,7-dihydro-4H-thieno [3,2-c] pyridin-5-yl) acetate hydrogen sulfate<sup>[1]</sup>. The molecular formula of Clopidogrel bisulphate is C<sub>16</sub>H<sub>16</sub>ClNO<sub>2</sub>S.H<sub>2</sub>SO<sub>4</sub>. The molecular mass of is Clopidogrel bisulphate 419.03 g/mol. It is an official drug in British Pharmacopoeia. It is completely soluble in water, methyl alcohol, grain alcohol and glacial acetic acid bit soluble in acetone or chloroform<sup>[2]</sup>. The structure of Clopidogrel was shown in fig 1.

Clopidogrel bisulphate was determined by HPLC in normal mode<sup>[5]</sup> and in reversed-phase ion pair mode<sup>[15]</sup>. Clopidogrel bisulphate was also determined in combination with other drugs like

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aspirin<sup>[11]</sup> and stability indicating HPLC of Clopidogrel bisulphate<sup>[13]</sup> and also by UV spectrophotometric techniques<sup>[10,12,9]</sup> and impurities present in Clopidogrel<sup>[7,14]</sup> and potentiometric technique<sup>[6]</sup>.

## MATERIALS AND METHODS

Quantitative HPLC was performed on a isocratic high pressure liquid chromatography (Waters model 2695) Equipped with a photodiode array detector capable of operating in the range of 190 nm to 400 nm Hypersil BDS C18 (150mm x 4.5mm, 5 $\mu$ m)

## REAGENTS AND CHEMICALS

Sodium di hydrogen phosphate, ortho phosphoric acid of AR grade, methanol of HPLC grade, acetonitrile of HPLC grade and water HPLC grade were obtained from Rankem Chemicals Ltd., Mumbai. Clopidogrel bisulphate was obtained as a gift sample from Sun Pharma, India. The commercially available Clopidogrel bisulphate tablets were procured from the local market.

## PREPARATION OF BUFFER SODIUM DIHYDROGEN PHOSPHATE BUFFER (PH-3.0)

Sodium dihydrogen phosphate buffer was prepared by dissolving 158 gm of disodium hydrogen Phosphate in 1000 ml of double distilled water and the pH was adjusted to 3.0 with ortho-phosphoric acid.

## CHROMATOGRAPHIC CONDITIONS

The mobile phase consisting of acetonitrile and sodium di hydrogen phosphate buffer (pH: 3.0) in the ratio of 60:40 % v/v was filtered through 0.45 $\mu$ m membrane filter before use, degassed and pumped from the solvent reservoir into the column at a flow rate of 1 ml/min. The detection was monitored at 224nm, and the run time was 20 minutes. The volume of the injection loop was 10  $\mu$ l and prior to the injection of the drug solution; the column was equilibrated for at least 30 minutes with the mobile phase flowing through the system. The column and the HPLC system were kept in 35 $^{\circ}$ c temperature. Stock standard solution of Clopidogrel bisulphate was prepared by dissolving a quantity of Clopidogrel bisulphate hydrochloride equivalent to 10.0 mg of Clopidogrel bisulphate in 10.0 mL of diluent to obtain a solution having a known concentration of 1.0 mg/mL Clopidogrel bisulphate. Nominal (working) standard solution

was prepared by diluting 1 mL of stock standard solution to 10 mL diluent to obtain a solution having a known concentration of 100 $\mu$ g/mL Clopidogrel bisulphate. Nominal solutions of the formulated Clopidogrel bisulphate tablet solution prepared by taking 75mg equivalent powder in 10 mL volumetric flask and dissolve with water from that take 1.5 mL of solution transferred into 10 mL volumetric flask and volume filled with water.

## RESULTS

### METHOD DEVELOPMENT

AC18 column (150mm x 4.5mm, 5 $\mu$ m) as a stationary phase with a mobile phase of acetonitrile and phosphate buffer pH3.0 (60:40, v/v) at a flow rate of 1.0mL/min and a detection wavelength of 224 nm afforded the best separation of Clopidogrel bisulphate. The standard solutions prepared as above were injected into the 10  $\mu$ l loop, and the chromatogram was recorded as shown in fig 2. The retention time of Clopidogrel was found to be 9.182 min. The calibration curve was constructed by plotting concentration versus peak area ratio. The amount of Clopidogrel present in the sample was calculated through the standard calibration curve.

### ASSAY

Twenty tablets each containing 75 mg were weighed accurately and powdered. A quantity equivalent to 10 mg of Clopidogrel was weighed accurately and transferred to 10 ml volumetric flask containing 3 ml of water. The contents were sonicated for 20 min. and made up to the mark with the water. The resulting solution is filtered through 13 mm  $\times$  0.45 $\mu$ m PVDF. 1.5mL of the above solution was pipette into 10mL volumetric flask and made up with water. The solution obtained was diluted with the water to obtain a concentration in the range of linearity previously determined for the pure drug. The 10 $\mu$ l sample solution was injected under the chromatographic conditions, and the chromatogram was recorded. The amount of Clopidogrel present in tablet formulation was determined by comparing the peak area from the standard. The results were furnished in Table 1.

### METHOD VALIDATION

The linearity, precision, accuracy, limit of detection, limit of quantitation, ruggedness and robustness has been validated for the determination of Clopidogrel.<sup>[3,4]</sup>

### LINEARITY AND RANGE

The linearity experiment was carried out in triplicate to ascertain accuracy and precision of the method. The standard curve was obtained in the concentration range of 50-150 µg/ml. The peak area ratios of the drug versus concentration were found to be linear, and the results are furnished in Table 2. The linearity was evaluated by linear regression analysis using the least square method. It was found that correlation coefficient and regression analysis are within the limits. The linearity graph was shown in fig 3.

### ACCURACY

Accuracy of the method was performed by preparing the placebo of the drug formulation according to the formulation procedure. To the required quantity of placebo, a known quantity of Clopidogrel with the same proportion as in the drug, formulation was added to get three concentrations (50, 100, 150 µg/mL of Clopidogrel). Results have shown that the recovery of Clopidogrel is within 98.0–102%, and the RSD is lower than 2.0%. The results are shown in Table 3.

### PRECISION

#### Repeatability

Repeatability of the method was evaluated by calculating the RSD of the peak areas of six

replicate injections for the standard concentration (100%) of Clopidogrel, which was found to be 0.43%. The results are furnished in Table 4.

#### Intermediate precision (ruggedness)

The Intermediate precision method was also evaluated by analyzing six samples of Clopidogrel by two analysts in the same laboratory using different HPLC systems. Results of this study showed that the RSD of the percentage of Clopidogrel in Clopidogrel tablets for the 12 samples (6 samples from each analyst) was 0.8% and 0.4% indicating a good intermediate precision of the method Table 5.

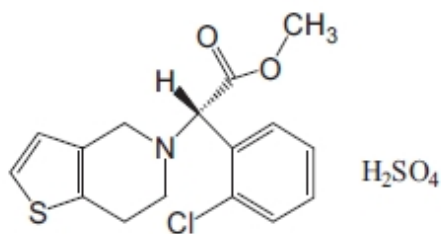
### LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTITATION (LOQ)

The LOD and LOQ for Clopidogrel were predicted basing on the parameters of standard error of estimate and slope, calculated from linearity of the response data of Clopidogrel Bisulphate. The results were shown in Table 6.

### ROBUSTNESS

The robustness was checked by changing the flow rate to 0.8 and 1.2 ml/min, the mobile phase pH 2.8 to 3.2, and column oven temperature 30°C to 40°C the method suits best, and the results are shown in Table 7.

Fig 1: Structure of Clopidogrel bisulphate



Clopidogrel hydrogen sulfate

Fig 2. Chromatogram of Clopidogrel Bisulphate standard solution

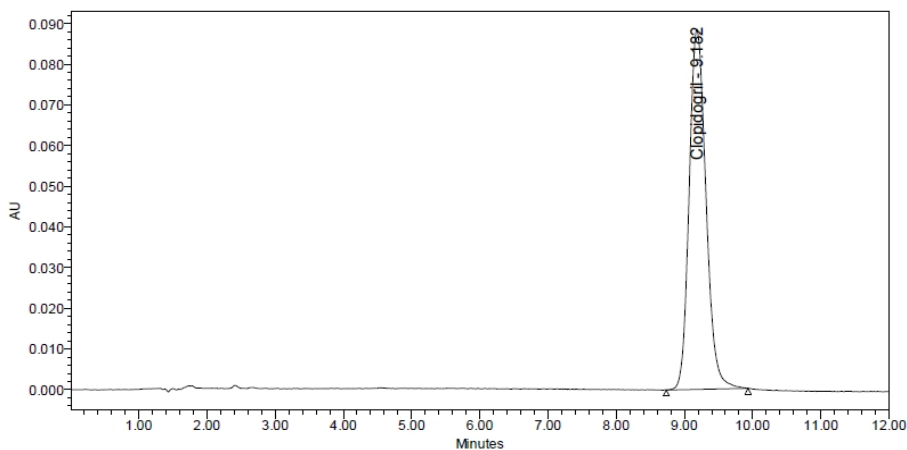


Fig 3: Linearity graph for Clopidogrel Bisulphate

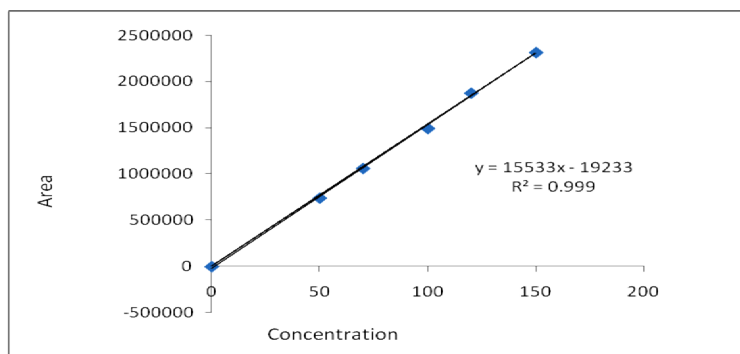


Table 1: Quantitative Estimation of Clopidogrel Bisulphate in tablet dosage form

S. NO.	Tablet Sample	Label Claim in mg/tablet	Peak Area		Amount found mg/tablet	Percentage content of Drug
			Test	Standard		
1	Clopidogrel bisulphate	75	1229063	1515350	74.9	99.86

Table 2: Linearity data of Clopidogrel bisulphate

Concentration( $\mu\text{g/ml}$ )	Area
50	842089
75	1063739
100	1495425
125	1788165
150	2417255

**Table 3: Accuracy data of Clopidogrel Bisulphate**

Sample	Mean area counts	Amt added (µg/ml)	Amt recovered (µg/ml)	%recovery	Mean
50%-Rec-1	604982	50	49.9	99.8	99.86
50%-Rec-2	833740	50	50.1	100.2	
50%-Rec-3	660589	50	49.8	99.6	
100%-Rec-1	1518091	100	99.9	99.9	99.93
100%-Rec-2	1519697	100	99.8	99.8	
100%-Rec-3	1525624	100	100.1	100.1	
150%-Rec-1	2502382	150	149.8	99.8	99.6
150%-Rec-2	2517458	150	149.5	99.6	
150%-Rec-3	2519699	150	149.2	99.4	

**Table 4: Repeatability data of Clopidogrel Bisulphate**

Injection number	Area of Clopidogrel bisulphate
1	1515163
2	1512512
3	1506778
4	1517826
5	1526474
6	1513348
Mean	1515350.17
SD	6564.30275
%RSD	0.433187186

**Table 5: Ruggedness of Clopidogrel Bisulphate**

S.No.	System suitability	Observed value		Acceptance criteria
		Analyst-1	Analyst-2	
1	%RSD for Clopidogrel bisulphate in standard solution	0.4	0.8	NMT 2.0%
2	The Tailing factor	1.18	1.21	NMT 2.0

**Table 6: LOD and LOQ data of Clopidogrel Bisulphate**

S.NO	Name	LOD Value (µg/ml)	LOQ Value (µg/ml)
1.	Clopidogrel bisulphate	1.3	4.2

**Table 7: Robustness data of Clopidogrel bisulphate**

Para meter	Tailing factor	%RSD
<b>Buffer pH</b>		
2.8	1.17	0.3
3	1.18	0.8
3.2	1.21	0.4
<b>Flow rate (ml/min)</b>		
0.8	1.22	1.1
1	1.18	0.7
1.2	1.25	0.2
<b>Temperature(°C)</b>		
30	1.23	0.2
35	1.21	0.4
40	1.2	0.1

## CONCLUSION

The developed method is cheap, easy, and it gives the sharp peak with high resolution. The developed method is applied for the determination of Clopidogrel bisulphate. The assay results are with the label claim of the formulation. The developed method is validated as per ICH guidelines using

parameters like Accuracy, Precision, Linearity, and Range, Specificity, Ruggedness, LOD, LOQ and Robustness. Hence the developed method is found to be satisfactory, and it complies with all validation parameters. So this developed method can be used for the routine analysis of Clopidogrel bisulphate in tablet dosage form.

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