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Development and validation of stability indicating RP-HPLC method for the determination of clopidogrel bisulphate in bulk and its dosage forms

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ABSTRACT

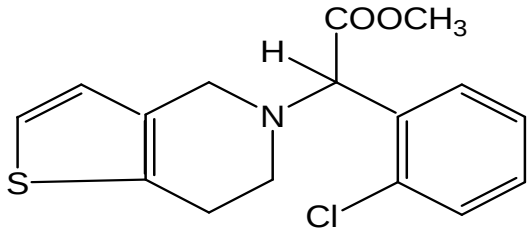
A rapid and sensitive stability indicating Reverse Phase High Performance Liquid Chromatographic [RP-HPLC] method was developed for the estimation of Clopidogrel bisulphate [CGB] in presence of its oxidative, acid, alkaline and thermal degradation products. The method was validated as per International Conference on Harmonization [ICH] guidelines. The mobile phase used in this study is a mixture of acetonitrile and tetrabutyl ammonium hydrogen sulphate buffer. Stationary phase was waters symmetry C_8 reverse phase column (150×3.9mm, 5µm) dimensions at ambient temperature. The analysis was performed with run time of 18.0 minutes at a flow rate of 1.00ml/min. The CGB was monitored at 225nm with UV detection and CGB was eluted at 4.59min. The method was linear (r2 =0.999) at concentration ranging from 100 to 600µg/ml, precise (intra-day relative standard deviation [RSD] and inter-day RSD values < 1.0%), accurate (mean recovery = 99.5%), specific and robust. Detection and quantification limits were 27.0µg/ml and 92.0µg/ml, estimated from linearity by regression analysis. The results showed that the proposed method was suitable for rapid determination of clopidogrel in bulk dosage forms.

Keywords: Clopidorgel bisulphate, RP-HPLC, Validation, Dosage forms.

INTRODUCTION

Clopidogrel bisulphate, 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 white to off-white crystalline solid, freely soluble in water and methanol. It is also acts as an antihypertensive agent. The mechanism of action of Clopidorgel 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].

The target and objective of this study is to develop a new, simple and fast analytical method by stability indicating RP-HPLC method to quantify CGB in bulk and its capsule dosage forms. Validation study is carried out as per ICH guidelines[2]. Analytical methods are essential to characterize drug substances and drug products composition during all stages of pharmaceutical development. For routine analytical purpose it is always necessary to establish methods capable of analyzing huge number of samples in a short time period with high accuracy and precision.



CLOPIDOGREL BISULPHATE

Fig: 1.01 Structure of Clopidorgel bisulphate

Very few analytical methods are reported for the quantification of Clopidorgel bisulphate in plasma by liquid chromatography, fluorescence detection, and UV detection. In the present investigation the authors propose a simple, sensitive and reproducible RP-HPLC method for the determination of Clopidorgel bisulphate. Comprehensive literature survey reveals the estimation of Clopidorgel bisulfate in pharmaceutical formulations by various chemometric[3]; HPLC[4-7], HPTLC[8-9], TLC[10], and an LC-ESI-MS-MS[11-12] method were developed.

The proposed method was validated with respect to selectivity, linearity, precision, and accuracy, limit of quantitation (LOQ) and limit of detection (LOD) according to ICH requirements[13-17] to show it could be used for determination of CGB in pharmaceutical formulations.

MATERIALS AND METHODS

Instrumentation:
Quantitative HPLC was performed on a high performance liquid chromatography equipped with 2695pump and 2996 photodiode array detector was used. The output of signal was monitored and integrated using Agilent EZ Chrome Elite software.

Reagents and chemicals:
HPLC grade acetonitrile and water as well as tetrabutyl ammonium hydrogen sulphate, A.R. grade were purchased from Fisher scientific, Mumbai, India. All other chemicals used were of HPLC grade.

Chromatographic conditions:
The mobile phase used in this study is a mixture of acetonitrile and tetrabutyl ammonium hydrogen sulphate buffer. Stationary phase was waters symmetry C_8 reverse phase column (150×3.9mm, 5µm) dimensions at ambient temperature. The contents of the mobile phase were filtered before use through a 0.45µ membrane. The mobile phase was pumped from the solvent reservoirs to the column at a flow rate of 1.0ml/min for 18.0min. The elution was monitored at 225nm using UV-detector. The retention time of the drug was found to be 4.59min.

Preparation of standard drug solution:
About 20mg of Clopidogrel bisulphate standard was weighed accurately and transferred into a 50mL volumetric flask and dissolved in methanol (used as diluent). The solution was sonicated for 15min and then the volume made up with a further quantity of the diluent to give 0.4mg/mL. 10uL of the solution was injected each time into the column for five times the corresponding chromatograms were obtained. From these chromatograms, the retention times and the areas under the peaks of the drug were noted.

Preparation of sample solution:
About 20mg of clopidogrel bisulphate sample was weighed accurately and transferred into a 50mL volumetric flask and dissolved in methanol (used as diluent). The solution was sonicated for 15min and then the volume made up with a further quantity of the diluent to give 0.4mg/mL. 10uL of the solution was injected each time into the column for five times the corresponding chromatograms were obtained. From these chromatograms, the retention times and the areas under the peaks of the drug were noted.

RESULTS AND DISCUSSION

HPLC method development and optimization:

The chromatographic method was optimized by changing various parameters, such as the mobile phase composition. Different mobile phases were tried, but satisfactory separation and good symmetrical peak were obtained with the mobile phases consisting of tetrabutyl ammonium hydrogen sulphate and acetonitrile ratio of 70:30% v/v. A typical chromatogram obtained by using the aforementioned mobile phase and 10µl of the injected assay preparation is illustrated in **Fig: 1.02.**

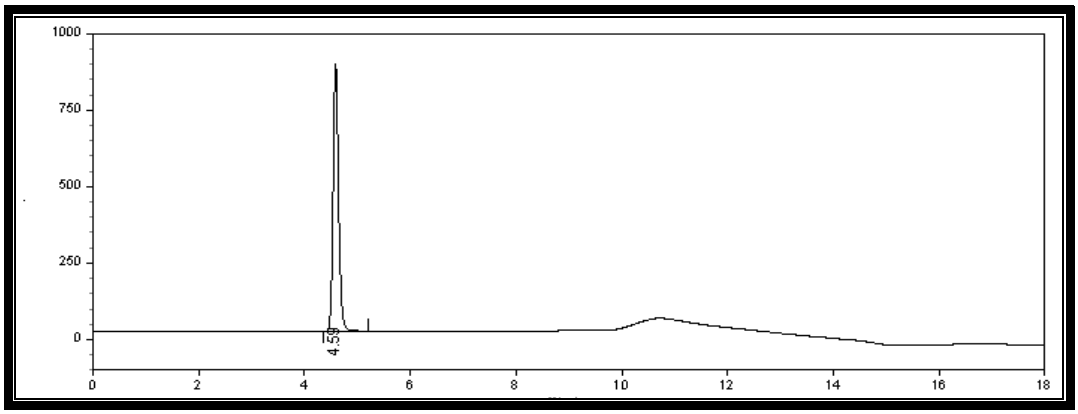


Fig: 1.02 - A typical chromatogram showing the peak of Clopidogrel

Method validation:

The objective of method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines Q2A and Q2B (2009). Method validation characteristics were tested in accordance with ICH guidelines. Method specificity was verified by comparing the chromatograms of sample of pharmaceutical preparation, standard solution and blank. Method precision, recovery in the range of 50% to 150% of label claim of the drug using the blend, Linearity was tested in the range 100–600µg/ml. Intra and inter-day instrumental system precision as well as repeatability and intermediate method precision were obtained using six replicates per day. Limits of detection [LOD] and limit of quantification [LOQ] were provided for CGB. Calculation was made by means of RSQ (Residual Square of regression).

Forced Degradation: Control Sample: 20 mg of Clopidogrel bisulphate was accurately weighed and transferred into a 100 ml volumetric flask To it 70 ml of diluent was added and sonicated for 30minutes with intermittent shaking at controlled temperature and the volume was diluted with diluent and mixed. Filtered the solution through 0.45 µm membrane Filter. Transferred 5.0 ml of the above solution into a 100 ml volumetric flask and diluted to volume with diluent**. (Figure 1.03.)**

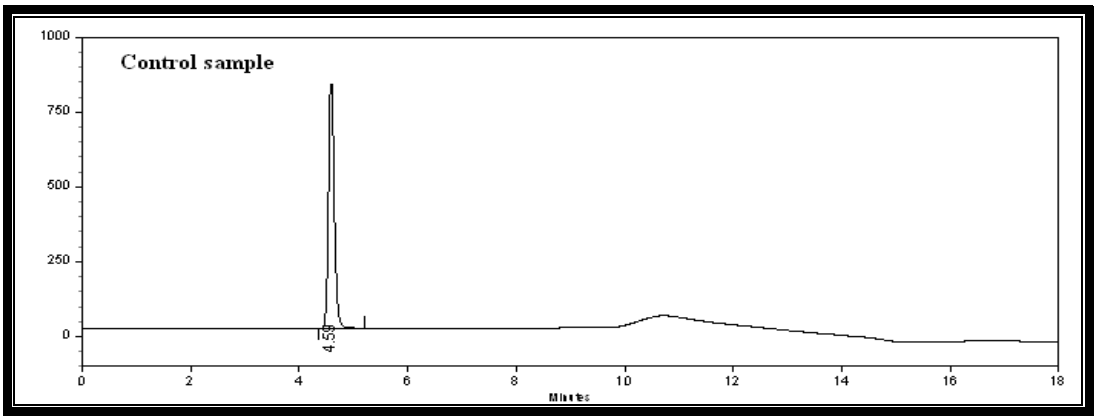


Fig: 1.03 - A typical chromatogram of clopidogrel control sample

Acid Degradation Sample: About 20mg of Clopidogrel bisulphate sample was weighed accurately and transferred into a 100 ml volumetric flask add about 70 ml of diluent, and sonicate for 30minutes with intermittent shaking at controlled temperature. Then 10ml of 5N acid, was added refluxed for 30min at 60°C, then cooled to room

temperature, neutralize with 5N NaOH and diluted to volume with diluent and mixed. The solution was filtered through 0.45 µm membrane Filter. 5.0 ml of the above solution was transferred into a 100 ml volumetric flask and dilute to volume with diluent. **(Figure 1.04)**

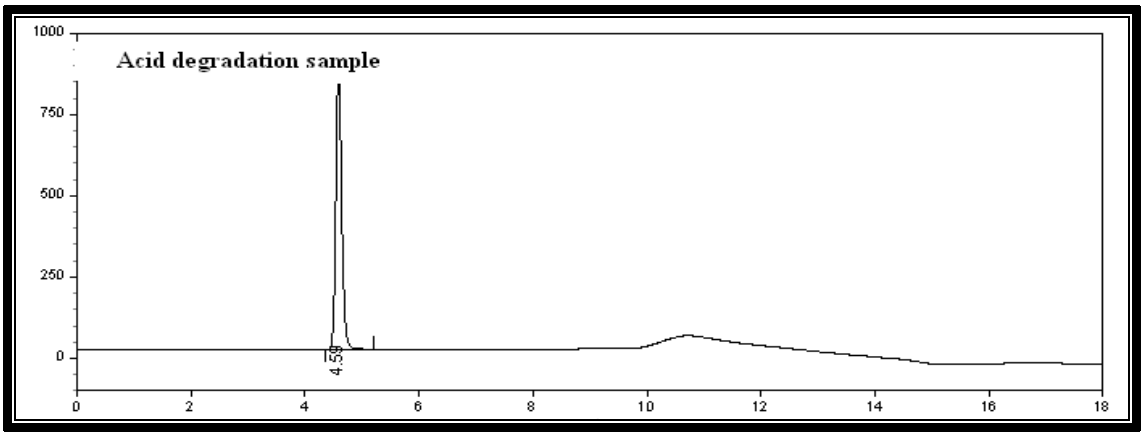


Fig: 1.04 - A typical chromatogram of clopidogrel acid degradation Sample

Base Degradation Sample:
About 20mg of Clopidogrel bisulphate sample was weighed accurately and transferred into a 100 ml volumetric flask add about 70 ml of diluent, and sonicate for 30minutes with intermittent shaking at controlled temperature. Then 10ml of 5N Base (NaOH) was added, refluxed for 30min at 60°C, then cooled to room temperature, neutralized with 5N Acid (HCl) and diluted to volume with diluent and mix. The solution was filtered through 0.45 µm membrane Filter. Transferred 5.0 ml of the above solution into a 100 ml volumetric flask and dilute to volume with diluent. **(Figure 1.05)**

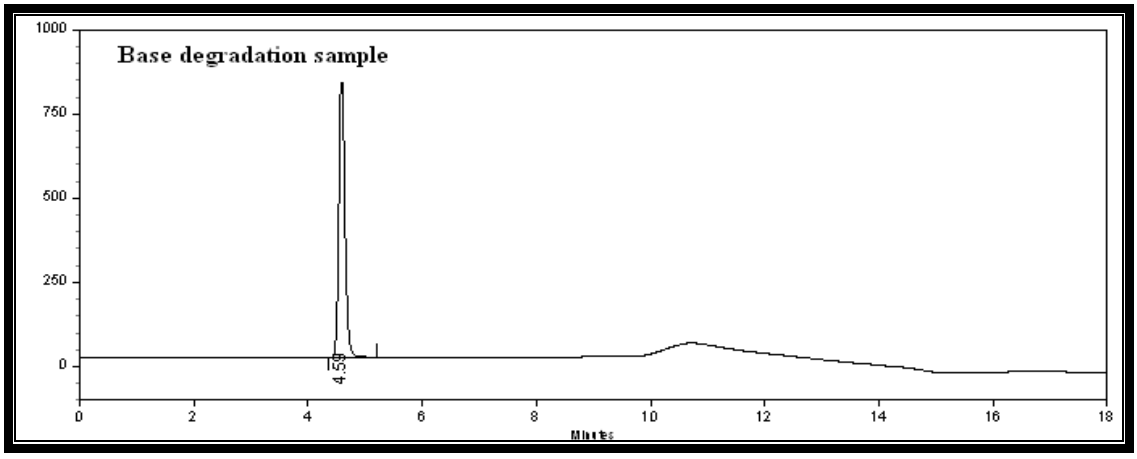


Fig: 1.05 - A typical chromatogram of clopidogrel base degradation Sample

Peroxide Degradation Sample: About 20mg of Clopidogrel bisulphate sample was weighed accurately and transferred into a 100 ml volumetric flask add about 70 ml of diluent, and sonicate for 30minutes with intermittent shaking at controlled temperature. Then 2ml of 30% Peroxide, was added, refluxed for 30min at 60°C, then cooled to room temperature and diluted to volume with diluent and mixed. Filter the solution through 0.45 µm membrane Filter. 5.0 ml of the above solution was transferred into a 100 ml volumetric flask and diluted to volume with diluent. **(Figure 1.06)**

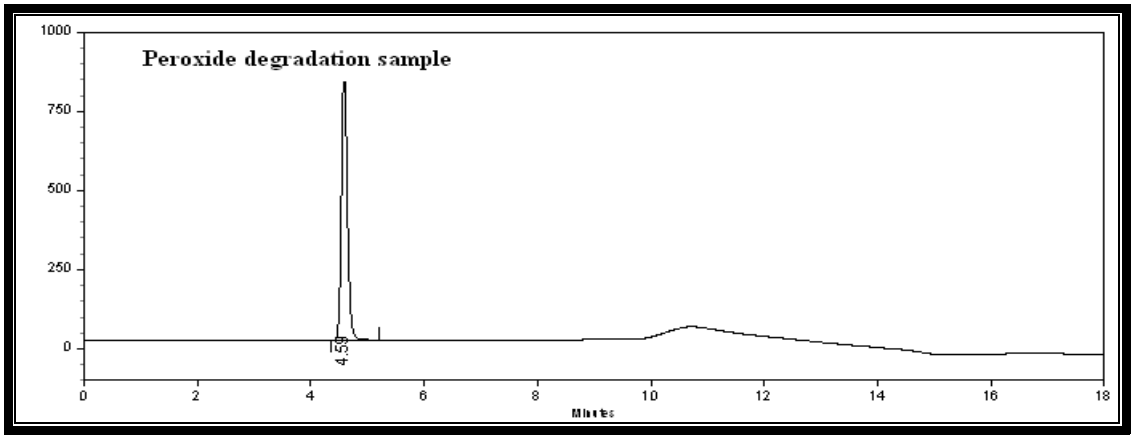


Fig: 1.06 - A typical chromatogram of clopidogrel peroxide degradation Sample

Thermal Degradation Sample: Clopidogrel bisulphate sample exposed to heat at 105°C for about 5days. Then about 20mg of Clopidogrel bisulphate sample was weighed accurately and transferred into a 100 ml volumetric flask. To it 70 ml of diluent was added and sonicate for 30minutes with intermittent shaking at controlled temperature and diluted to volume with diluent and mixed. The solution was filtered through 0.45 µm membrane Filter. Transferred 5.0 ml of the above solution into a 100 ml volumetric flask and dilute to volume with diluent. **(Figure 1.07)**

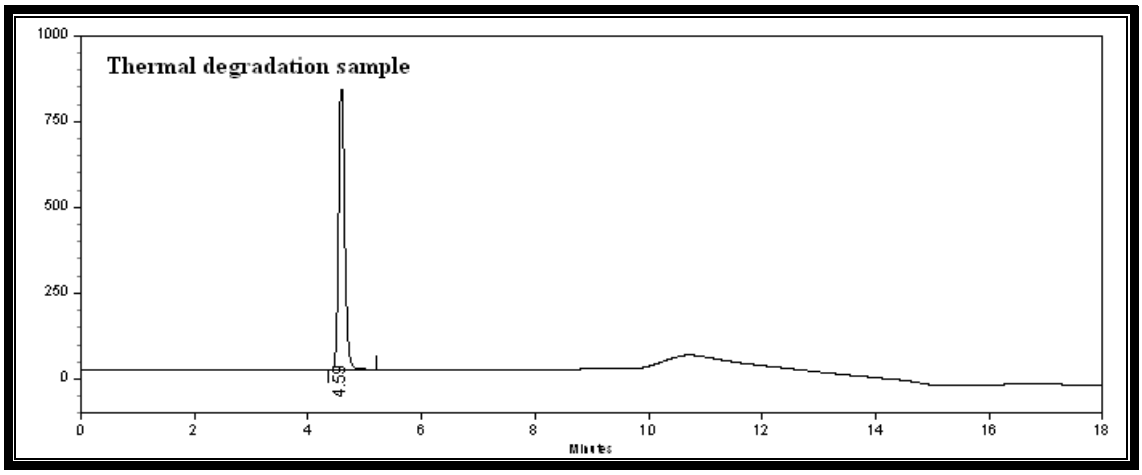


Fig: 1.07 - A typical chromatogram of clopidogrel thermal degradation Sample

System suitability:
For system suitability, five replicates of standard solution were injected and studied the parameters like theoretical plates, tailing factor. The represented data was shown in **Table: 1.01.**

Table: 1.01 - System suitability parameters for clopidogrel by proposed method

Name of the Compound	Theoretical plate	Tailing factor
Clopidogrel bisulphate	9117	1.53

Table: 1.02 Specificity parameters for clopidogrel standard by proposed method

CGB standard	Inj-1	Inj-2	Inj-3	Avg	%RSD
RT	4.62	4.58	4.66	4.59	0.34
Area	113261425	113899914	114047140	113736160	0.31

Specificity:
The HPLC chromatograms recorded for the placebo showed almost no peaks at the retention time of CGB. The peak for CGB is clearly separated from other excipients of the formulations. As there is no blank interference is observed at the retention time of CGB, the HPLC method presented in this study is specific for CGB. Standard solution and test solution was prepared as per the proposed analytical method. The results are listed in **Table 1.02 and 1.03.**

Table: 1.03 Specificity parameters for clopidogrel sample by proposed method

CGB sample	Inj-1	Inj-2	Inj-3	Avg	%RSD
RT	4.56	4.54	4.55	4.55	0.12
Area	110414935	110554929	110026295	110332053	0.21

Precision:

In the study of the instrumental system precision study for six standard preparations showed a %RSD of retention time 0.13% was obtained, % RSD 0.37% for the area obtained respectively. In the study of the instrumental system precision study for six sample preparations showed a %RSD of retention time 0.19% was obtained, % RSD 0.10% for the area obtained respectively. The results are listed in **Table 1.04** and **1.05.**

Table: 1.04 System precision for clopidogrel standard by proposed method

CGB	RT	AREA
Inj-1	4.55	110086772
Inj-2	4.55	110322816
Inj-3	4.57	110529436
Inj-4	4.55	110913698
Inj-5	4.58	111423608
Inj-6	4.56	110949829
AVG	4.56	110704360
%RSD	0.13	0.37

Table: 1.05 System precision for clopidogrel sample by proposed method

CGB	RT	AREA
Inj-1	4.55	110357244
Inj-2	4.57	110401964
Inj-3	4.55	110220924
Inj-4	4.54	110449023
Inj-5	4.52	110629555
Inj-6	4.52	110451834
AVG	4.54	110418424
%RSD	0.19	0.10

In the present study of method precision six standard preparations showed a %RSD of retention time 0.20% was obtained, %RSD 0.96% for the area obtained respectively. The study of method precision six sample preparations showed a %RSD of retention time 0.07% was obtained, %RSD 1.25% for the area obtained respectively. The results are listed in **Table 1.06** and **Table 1.07.**

Table: 1.06 Method precision for clopidogrel standard by proposed method

CGB	RT	AREA
Inj-1	4.53	110046246
Inj-2	4.55	109681033
Inj-3	4.53	109015165
Inj-4	4.54	109017902
Inj-5	4.57	112187272
Inj-6	4.51	111053666
AVG	4.54	110166881
%RSD	0.20	0.96

Table: 1.07 Method precision for clopidogrel sample by proposed method

CGB	RT	AREA
Inj-1	4.51	115233509
Inj-2	4.52	111071345
Inj-3	4.52	113195050
Inj-4	4.50	110752324
Inj-5	4.51	113402735
Inj-6	4.51	112240136
AVG	4.51	112649183
%RSD	0.07	1.25

Accuracy:

The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations on 10 mg, 20mg, and 30mg of CGB and analyzed as per the

proposed method. The percentage recoveries were found in the range of 99.6 to 100.5 with an overall %RSD of 0.19%. The results are given in **Table 1.08 to 1.13.**

Table: 1.08: Authentic level clopidogrel working standard areas

CGB	AREA
Inj-1	113922965
Inj-2	114086659
Inj-3	114112650
Inj-4	114341797
Inj-5	114175567
Inj-6	114737464
Mean Area	114229517

Table: 1.09: Authentic level clopidogrel sample areas

CGB	AREA
Inj-1	111035764
Inj-2	110933403
Inj-3	111813023
Inj-4	111055990
Inj-5	111218276
Inj-6	111522187
Mean Area	111263107

Table: 1.10 Assay preparations (50%) for clopidogrel sample by proposed method

CGB	RT	AREA
Inj-1	4.50	56282960
Inj-2	4.50	56505399
Inj-3	4.54	56339299
AVG	4.52	56375886
%RSD	0.22	0.17

Table: 1.11 Assay Preparation (100%) for clopidogrel Sample by proposed method

CGB	RT	AREA
Inj-1	4.56	112279622
Inj-2	4.57	112559113
Inj-3	4.51	112781766
AVG	4.55	112540167
%RSD	0.31	0.19

Table: 1.12 Assay Preparation (150%) for clopidogrel Sample by proposed method

CGB	RT	AREA
Inj-1	4.59	169121033
Inj-2	4.58	169439078
Inj-3	4.58	169931659
AVG	4.59	169497257
%RSD	0.08	0.22

Table: 1.13 % Recovery of Clopidogrel bisulphate

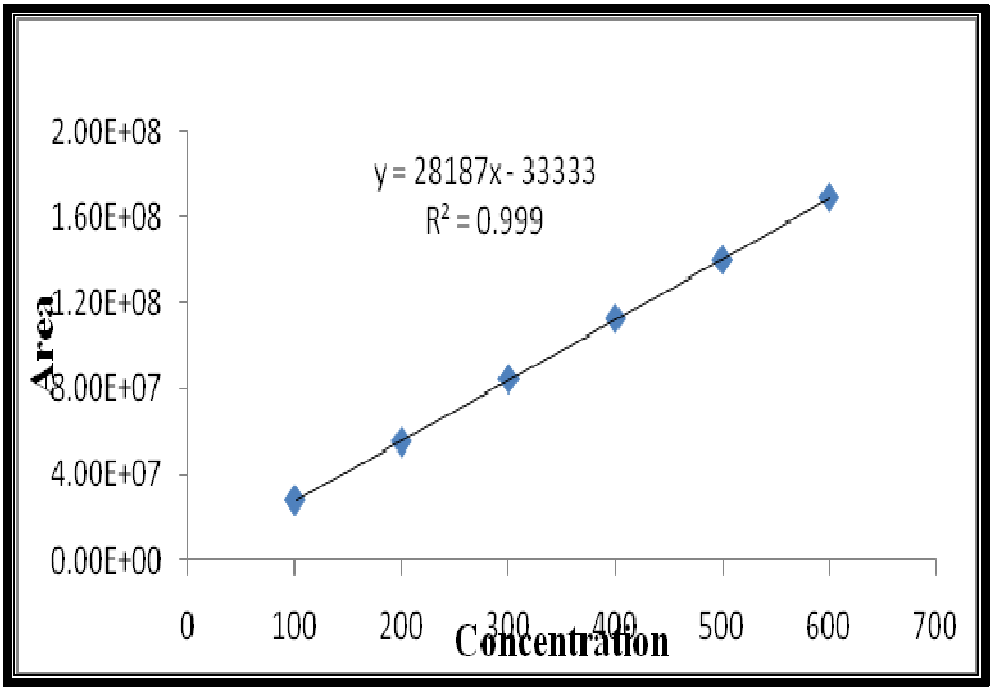
Name	Level – 1 (50%)	Level –2 (100%)	Level –3 (150%)
CGB	99.9	99.98	99.88

Table: 1.14 Linearity of Response for clopidogrel by proposed method

Linearity of Response for Clopidogrel		
% Level (Approx.)	**Concentration (µg/ml)**	**Average Area**
25	100	28187943
50	200	55375886
75	300	84563829
100	400	112751772
125	500	139939715
150	600	169127658
Intercept		28187
CC		0.9990

Linearity:
The standard curve was obtained in the concentration range of 100-600µg/ml. The linearity of the proposed method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r2] of standard curve were calculated and given in **Fig 1.08** and **Table 1.14.**

Fig: 1.08 - Linearity curve for Clopidogrel



LOD and LOQ:
Limit of detection was found to be 27µg/ml and Limit of quantification was found to be 92µg/ml.The details are given in Table **1.15 and Table 1.16.**

Table: 1.15 Limit of Detection for clopidogrel by proposed method

	LOD	Clopidogrel		
	RT	Area	Height	S/N
	4.56	8788	813	3.25
	4.55	8442	909	3.42
	4.57	8964	839	3.30
AVG	4.56	8731	854	3.32
%RSD	0.09	3.04	5.82	2.63

Table: 1.16 Limit of Quantification for clopidogrel by proposed method

	LOQ	Clopidogrel		
	RT	Area	Height	S/N
	4.57	32807	2550	10.38
	4.59	33310	2504	9.78
	4.56	31371	2501	9.97
	4.55	35666	2595	10.07
	4.55	33300	2495	9.98
	4.56	33761	2564	10.27
AVG	4.56	33369	2529	10.04
%RSD	0.18	4.18	1.7	2.19

CONCLUSION

The proposed stability indicating RP-HPLC was found to be simple and reliable analytical method for determination of clopidogrel bisulphate in pharmaceutical preparation using HPLC with UV detection. An analytical run takes about 4.59min. Separation of compounds is very fast with good reproducibility and peak asymmetry. Validation of this method was accomplished and the results obtained meet all requirements. The method is also found to be highly

reproducible with a good accuracy and precision. The proposed method allows reliably for the analysis of clopidegrel bisulphate in bulk and its pharmaceutical dosage forms.

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